TAXONOMIC REVIEW OF THE GENUS *HISTOTYLENCHUS* SIDDIQI, 1971 (NEMATODA: BELONOLAIMIDAE) IN SOUTH AFRICA.

by

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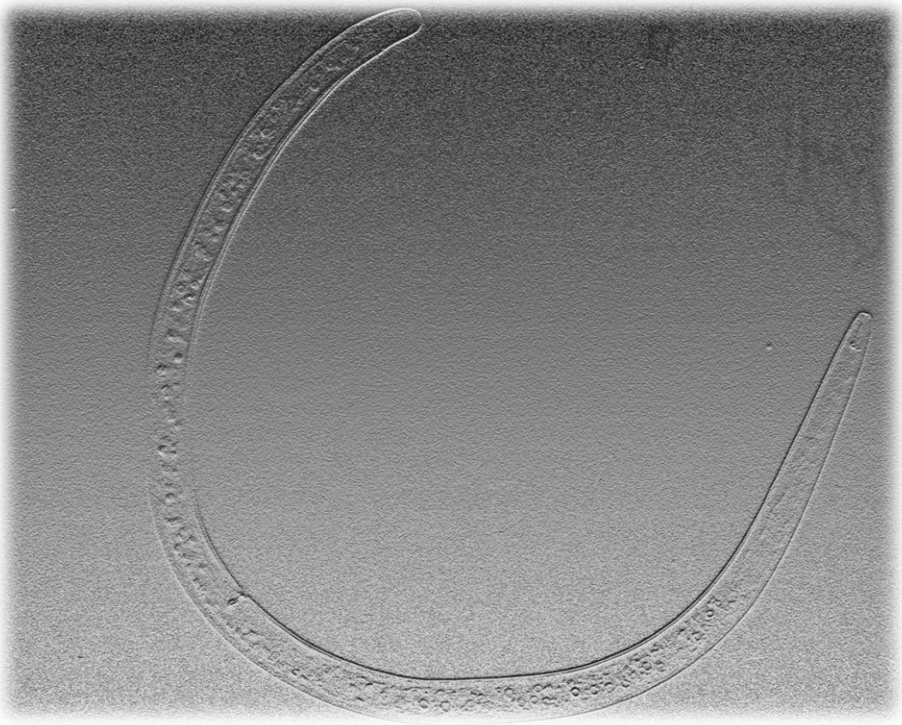


Introduction

1.1 Early classification of nematodes

Although living organisms were classified to some degree, albeit by simple means, it was not until Linnaeus' Systema Naturae in 1758 that classification started its evolution to the specialised science it is today (Hopwood, 1959). Linnaeus, in 1758, divided animals into six classes namely, Mammalia (mammals), Aves (birds), Amphibia (frogs), Pisces (fish), Insecta (arthropods and crustaceans) and Vermes (all the other invertebrates not belonging to Insecta). Subsequently, he divided the class Vermes into five orders (Intestina, Mollusca, Testacea, Lithophyta and Zoophyta). In his 1758 publication of Systema Naturae, Linnaeus recognised six species of worms (which included nematodes) infecting humans, which according to Grove (1900) he placed into the orders Intestina and Zoophyta. According to Ferris (2009), in 1767, Linneaus placed nematodes (together with protists and fungi) in the genus *Chaos*.

Through the rest of the 18th century other naturalists also contributed greatly to the further understanding and classification of worms. Grove (1900) noted of particular importance are the work of Goeze in 1782, the works of Gmelin in 1788 – 1793, and the work of Cuvier during 1798. Cuvier divided the worms he studied into two groups namely "cavitaires" (with a distinct digestive cavity and anus) and "parenchymateux" (lacking or with an incomplete digestive cavity and no anus). During 1800–1803, Zeder made the first notable distinction between (what is known today as) roundworms (human and animal parasitic nematodes), trematodes and cestodes, although he did not name the different groups as such. Between 1808 and 1810, Rudolphi renamed the groups used by Zeder. The group roundworms became Entozoa Nematoidea; the hookworms were renamed to Entozoa Acantocephalia; sucking worms were renamed Entozoa Trematoda; the tape worms became Entozoa Cestoidea and the cystic worms were renamed Entozoa Cystica (Grove, 1900).

1.2 Advancement of plant parasitic nematology

Although Needham recognised the first plant parasitic nematode, *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935 during 1743, plant parasitic nematodes were not recognised as a separate group for a long period of time. This is evident from

Rudolphi's classification in 1809, which did not differentiate between different groups of nematodes (Maggenti *et al.,* 1987). Probably, one of the most significant contributions to this was the fact that they were not parasitic to man and, therefore, not considered important. By the 19th century this view was altered as the focus of science broadened to include more than just human physiology (the key focus area of research during preceding centuries). Evidence of this is provided by the discovery of root-knot nematodes and the damage they caused to cultivated crops in London during 1855 and the discovery of cyst nematodes in Germany during 1859 (Siddiqi, 2000).

Since that time, various nematologists contributed to the successful description and classification of plant parasitic nematodes. Consider the monograph on Anguillulidae by Bastian in 1865; comprehensive illustrations and descriptions of various free-living nematodes by Bütschli along with the family Tylenchida that he first proposed; the study of soil-, plant-, and freshwater nematodes by de Man, as well as his ground-breaking formulation of the ratio's a, b and c (which is still being used in nematode morphometrics); and Filip'ev's recognition of the importance of including embryology and physiology as part of nematode science. Filip'ev also made various contributions to the classification of numerous plant parasitic nematodes (Siddiqi, 2000).

The 20th century witnessed the establishment of nematology as a scientific discipline in its own right, as well as the introduction of nematology to farmers by Steiner and Goodey (Hooper, 1994; Siddiqi, 2000). Cobb made significant contributions to the discipline of nematology. During his career, this renowned nematologist (as scientists/biologists in this field came to be known) described more than 1 000 new nematode species. Maybe more importantly, he studied the morphology, physiology, host relationships and habitat of nematodes, as well as inventing certain methods important to the advancement of the field, such as the "Cobb- slide" which is still in use today (Huettel & Golden, 1991).

1.3 Nematology in sub- Saharan Africa

Nematodes play a very important role as bio-indicators of soil health (Wilson & Kakouli-Duarte, 2009) and many are also considered important around the globe, because of their negative impact on crops, animals and humans (Blaxter *et al*., 1998). Some species are even considered of more importance by the poor under-developed countries of sub-Saharan Africa (Kusiluka & Kambarage, 1996). According to Grove (1900), human parasitism by nematodes in sub-Saharan Africa has long been known, with LaPin (1992) and Greenaway (2004), indicating that *Dracunculus medinensis* Linnaeus, 1758 (guinea worm) is the most common human infection. *Wuchereria bancrofti* Cobbold, 1877 (causing elephantiasis), *Loa loa* Cobbold, 1864 (African eye worm) and various gastrointestinal nematodes such as *Ascaris lumbricoides* Linnaeus, 1758 (hookworm) and *Trichuris trichiura* Linnaeus, 1758 (whipworm), also cause serious health problems in poor rural areas where basic hygiene practices are insufficient or lacking (Carbonez *et al*., 2002; Steketee, 2003; Negrão-Corrêa & Teixeira, 2006; Mitreva *et al*., 2007). Apart from human parasitism, nematodes also have an indirect impact on human well-being as a result of livestock parasitism (Waller, 2006). Numerous parasitic gastrointestinal nematodes are associated with livestock in sub-Saharan Africa, especially with small ruminants (Regassa *et al*., 2006). Livestock are negatively affected to such an extent that milk production, productivity and work capacity are reduced and even high mortality amongst ruminants can occur (Kusiluka & Kambarage, 1996; Regrassa *et al.*, 2006; Abebe *et al*., 2010).

Plant parasitic nematodes pose a threat to crops around the globe. To name but a few examples; *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Rotylenchulus reniformis* Linford & Oliveira, 1940 are economically important plant parasitic nematodes in cotton production areas in Brazil, China, India, the Middle East and the United States of America (Robinson, 2008). *Globodera* (Skarbilovich, 1959) Behrens, 1975 species, especially *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and *Globodera pallida* (Stone, 1973) Behrens, 1975, are serious quarantine pests in amongst others Australia, Canada and the United States of America (Hodda & Cook, 2009). Species of the genera *Hemicriconemoides* Chitwood &

Birchfield, 1957, *Helicotylenchus* Steiner, 1945, *Radopholus* Thorne, 1949, *Rotylenchulus* Linford & Oliveira, 1940 and *Xiphinema* Cobb, 1913 are damaging to coffee in all production regions around the globe (Souza, 2008) and *Ditylenchus dipsaci* (Kuhn, 1857) Filip'ev, 1936 is an important pest in all the temperate regions of the world (Subbotin *et al.*, 2005).

The main economically important plant parasitic nematode species in sub-Saharan Africa belong to the genera *Meloidogyne* Göldi, 1887 (Talwana *et al*., 2008), *Xiphinema*, *Trichodorus* Cobb, 1913, *Paratrichodorus* Siddiqi, 1974, *Nanidorus* Siddiqi, 1974, *Pratylenchus* Filip'ev, 1936, L*ongidorus* Micoletzky, 1922, and *Ditylenchus* Filip'ev, 1936. *Scutellonema bradys* (Steiner & LeHew, 1933) Andrássy, 1958, *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 and *Radopholus similis* (Cobb, 1893) Thorne, 1949 are also serious agricultural pests in the region (M. Marais, pers. comm.[1]).

1.4 Nematology in South Africa

South Africa has a diverse and abundant nematode fauna and according to Marais (2006), more than 500 (12.5%) of the 4 000 known species of plant parasitic nematodes are currently collected from the country. The important plant parasitic nematodes in South Africa belong to the same genera as those in the rest of sub-Saharan Africa. These include *Criconemoides* Taylor, 1936 on stone fruit and vineyards (Kleynhans *et al*., 1996), *Ditylenchus* on peanuts (De Waele *et al*., 1989; Wendt *et al*., 1995), *Meloidogyne* on e.g., sugarcane (Spaull, 1984), maize (De Waele & Jordaan, 1988a), sorghum (De Waele & Jordaan, 1988b), hemp (Van Biljon, 2003) and tomatoes (Kleynhans, 1991), *Paratrichodorus* on sugarcane (Spaull *et al*., 1990), *Pratylenchus* and *Radopholus* on bananas (Bridge *et al*., 1997; Gowen *et al*., 2005) and *Xiphinema* on grapevine (Smith, 1982).

[1]Dr. M. Marais. Nematology Unit, Biosystematics Division, ARC- Plant Protection Research Institute, Private Bag X134, Queenswood, Pretoria, 0121, South Africa.

1.4.1 Pioneers in the field of nematology in South Africa

George C. Martin (1912-1978) and Dr. W.J. van der Linde (1903-1978) can be seen as the earliest pioneers in the field of nematology in South Africa (Fig. 1). Martin focused on nematodes associated with tobacco for the main part of
his career (1947-1976) and published 120 papers during this time. Martin was among the first nematologists to develop the use of perineal pattern identification in root-knot nematodes. He is also credited for 580 reports of plant parasitic nematodes in Malawi, Zambia and Zimbabwe. The Nematological Society of Southern Africa (NSSA) honours this nematologist through the George C. Martin Memorial Scholarship, a scholarship available to assist any person wishing to study nematology. The first award of this scholarship was made to Mrs. M.S. Greef in 1982, with a total value of R600.00[2].

Another early pioneer honoured by the society is Dr. W.J. van der Linde, who completed his PhD in 1935. Dr. Van der Linde not only published many articles in the field of nematology, but he also developed many techniques for the extraction of nematodes from the soil. The control of root-knot nematodes by other means than chemical control and the identification of these nematodes through their host plants fascinated him. The W.J. van der Linde Memorial Medallion is awarded by NSSA to the best paper presented at the bi-annual NSSA symposium. The first medallion was awarded to Dr. A.J. Meyer in 1983. Dr. Meyer went on to become a prominent nematologist in South Africa until his death in 2010[2]. Since 2007 this award is also awarded to the best student paper presentation.

Nematologists who paved the way for biosystematics in the field include Dr. Victoria Coetzee, Prof. Juan Heyns, Dr. Esther van der Berg and Dr. Kent P. N. Kleynhans. Dr. Coetzee specialised in the taxonomy of the genus *Meloidogyne* and described the first *Meloidogyne* species (*Meloidogyne acronea*) from South Africa (Coetzee, 1956). Prof. Heyns (1929-2001) was a taxonomist at heart and throughout his career in nematology compiled an excellent nematode collection that is now housed at the National Collection of Nematodes, Pretoria, South Africa. He published more than 220 papers, several

[2] NSSA Archive maintained by N.H. Buckley, ARC-PPRI

monographs on various genera, described more than 325 species, 28 new genera, two new families, together with various re-descriptions of species (Coomans, 2002). Prof. Heyns was a fellow of various nematological societies including NSSA. Prof. Heyns also published various books,
including Heyns, (1971) and Keetch & Heyns (1982). Currently Keetch & Heyns (1982) is under revision (M. Marais, pers. comm.[1]). Prof. Heyns truly opened the door for nematology, especially nematode taxonomy, in South Africa. Several of his students (including Drs. Van den Berg and Kleynhans) went on to become specialist taxonomists.

Dr. Van den Berg received the first doctoral degree (1974) in nematology in South Africa, is a founding member and fellow of NSSA, has published more than 155 articles, received the Rhone-Poulenc award for achievement in nematology in 1997 and is still active in the field of nematology. Dr. Kleynhans (1935-2006) specialised in taxonomy of Belonolaimidae, Dolichoderidae and Heteroderidae. He submitted the first Afrikaans doctoral degree in nematology in South Africa (1982) and he made the first translations of nematological terms from English to Afrikaans and in 1991 published: “The root-knot nematodes of South Africa”. Together with his colleagues at the Plant Protection Research Institute, Pretoria, South Africa, he was the senior author in the 1996 publication: “Plant nematology in South Africa” for which he received the Rhone-Poulenc award for achievement in nematology in 1997 (Kleynhans, 1991; Kleynhans *et al*., 1996; Van den Berg, 2006).

1.4.2 The Nematological Society of Southern Africa

As a consequence to the presence of various plant parasitic nematodes, and their crop damaging potential in southern Africa, the Nematological Society of Southern Africa (NSSA) was established in 1973 (Keetch, 1989). To date, the NSSA has a total of 117 members, 87 of which are from South Africa (S. Steenkamp, pers. comm.[3]). During the 1983 symposium it was decided that a short technical course in nematology be

[3]Dr. S. Steenkamp, ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa

presented at the then University of Potchefstroom. The Nematology Short Course is currently still being presented at the North West University and many of South Africa's nematologists have attended the course[4]. The society has been and is a big part of many nematology careers in South Africa.

[4] NSSA Archive maintained by N.H. Buckley, ARC-PPRI

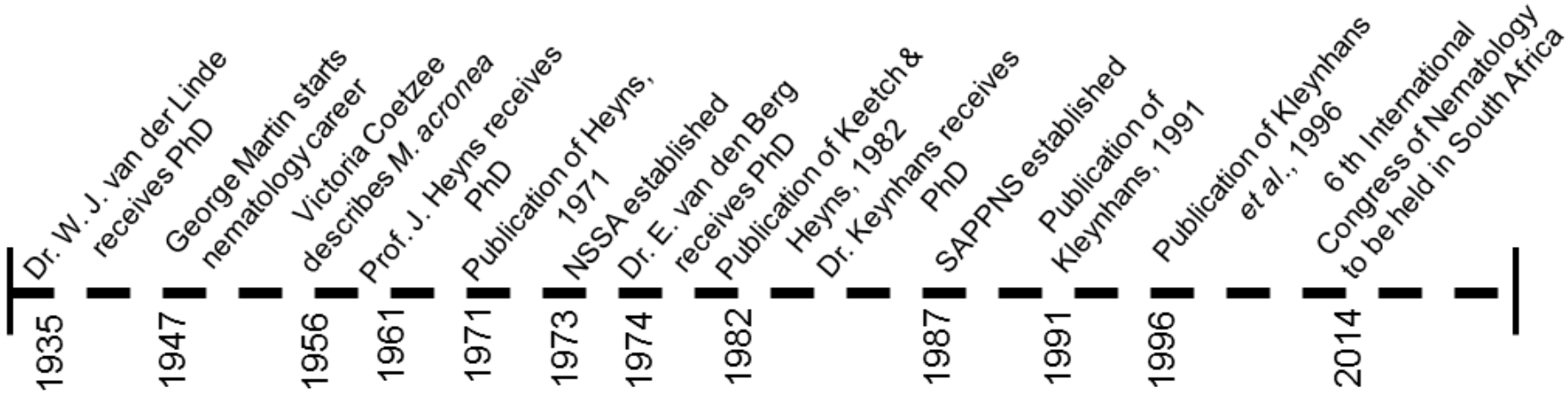


Figure 1: Condensed timeline of pioneers in the field of nematology and nematological events in South Africa (Coetzee, 1956; Heyns, 1971; Keetch & Heyns, 1982; Keetch, 1989; Kleynhans, 1991; Kleynhans *et al*., 1996; Coomans, 2002; Van den Berg, 2006; NSSA Archive maintained by N. H. Buckley, ARC-PPRI).

1.4.3. South African Plant- Parasitic Nematode Survey

Furthermore, mainly as a result of the rich nematode fauna in the region, the South African Plant- Parasitic Nematode Survey (SAPPNS) was established in 1987 with some of the aims to study the incidence and biogeography of plant parasitic nematodes (Marais, 2006).

Within the SAPPNS database, members of the genus *Histotylenchus* Siddiqi, 1971 are reported several times from various localities. *Histotylenchus* representatives are currently not regarded as economically important plant parasitic nematodes. However, as recorded in the SAPPNS, these are regularly associated with various other economic important nematodes such as *Longidorus pisi* Edward, Misra & Singh, 1964, *Pratylenchus zeae* Graham, 1951, *Rotylenchus* spp., *Scutellonema* Andrássy, 1958 spp., and *Xiphinema* spp. Unfortunately, available information for the genus *Histotylenchus* is limited (Jairajpuri & Baqri, 1968; Netscher & Germani, 1969; Kleynhans, 1975; Siddiqi, 1977; Kleynhans & Heyns, 1984; Kleynhans, 1992) and since the passing away of Kleynhans in 2006, no taxonomic work on this genus has been undertaken.

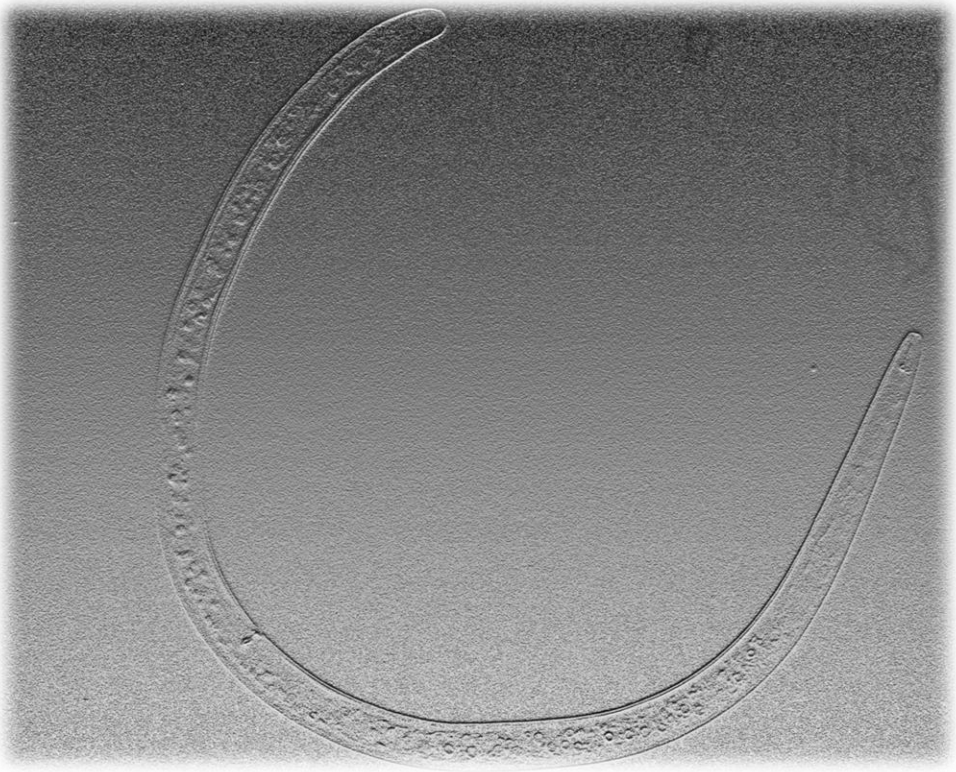
Against this background, the main aims of this study were to:

1. Review all existing literature concerning the genus *Histotylenchus* in Africa.
2. Investigate all the material of the genus collected in South Africa and deposited in the National Collection of Nematodes in South Africa.
3. Re-describe the genus *Histotylenchus*.

On completion of this short introduction (Chapter 1) this dissertation will provide a complete literature review and distribution of the genus *Histotylenchus* (Chapter 2). In Chapter 3 materials and methods used in this study are described and include the preparation of material for both light (section 3.1.2) and scanning electron (section 3.1.4) microscopy. Effects of preparation methods and age of permanent microscope slides on taxonomic characters (section 3.2) are also highlighted in this chapter. Chapter 4 provides the diagnosis and distribution for the genus *Histotylenchus* Siddiqi,

1971, and description of a new species, *Histotylenchus niveus* sp. n., two possible new species and the re-description of *Histotylenchus hedys* Kleynhans, 1975, *Histotylenchus histoides* Siddiqi, 1971 and *Histotylenchus mohalei* Kleynhans, 1992. A brief discussion and concluding remarks follows (Chapter 5). The literature cited for the purpose of this study will be provided (Chapter 6). Appendix I contains a compendium with diagnostic species measurements and morphometric data of the genus *Histotylenchus*. Appendix II different diagnostic characters used in the description of species and Appendix III contains information regarding the locality data of all examined material. Lastly acknowledgements and abstracts will be provided.

CHAPTER 2



Literature review

2.1 The Phylum Nematoda

The phylum Nematoda (Rudolphi, 1808) Lankester, 1877 is comprised of a large, ecologically and geographically diverse group of organisms, varying greatly in their taxonomy (Hugot *et al*., 2001; Parkinson *et al*., 2004; Hodda, 2007; Yin *et al*., 2009). According to Decreamer & Hunt (2006) nematodes are the most numerous Metazoa on earth, with the main groups being either free-living or parasitic in form. The phylum Nematoda is recognised by most taxonomists (Maggenti *et al*., 1987). However, currently two phylum names for these organisms exist, namely Nemata Cobb, 1919 and Nematoda. Much debate over the correct term has sprung from this (Chitwood, 1957; Andrássy, 1974; Maggenti, 1981; Inglis, 1983; Maggenti *et al*., 1987; Decraemer, 2000; Anonymous, 2006).

Maggenti *et al.* (1987) argued that Nemata should be recognised as the correct use of the phylum, because using Nematoda "does not indicate an author's commitment to nematodes as a class or phylum because the name has been used at both levels". It is, however, important to keep in mind when reading this statement of when Maggenti *et al*. (1987) wrote this article; the higher classification of nematodes has since changed, but nevertheless the word Nemata is still used by some authors. Decreamer (2000) argued that the use of Nemata is both confusing for nematologists (specifically when working on non-plant parasitic nematode genera) and non- nematologists. Decreamer (2000) further argued that the use of Nematoda is found in most textbooks concerning general zoology, and is the more commonly used term of the two and thus urges the use of Nematoda as the acceptable name for this group. In this dissertation the phylum Nematoda will be used.

Decraemer & Hunt (2006) stated that the higher classification of nematodes is currently in a state of flux as a result of molecular phylogenies. As a result of this, the higher classification is at present filled with proposals for Infraorders, revision of several phylogenetic relationships, development of complete genome sequences, etc. (Blaxter *et al*., 1998; De Ley & Blaxter, 2002; Bik, 2010). The role of morphology in the phylogeny of nematodes is, according to Ragsdale & Baldwin (2010), intensely debated,

but still remains vital because it is the principal interface of an organism with its environment. As a result of the current state of flux, for this dissertation the higher classification is based on Maggenti *et al.* (1988), because of the stability based on morphological systems.

2.2 Taxonomic review of the genus *Histotylenchus* Siddiqi, 1971

The genus *Histotylenchus* was described by Siddiqi in 1971 with *Histotylenchus histoides* as the type species of the genus. Siddiqi (1971) also transferred *Telotylenchus historicus* Jairajpuri & Barqi, 1968 and *Telotylenchus baoulensis* Netscher & Germani, 1969 to the genus *Histotylenchus* as these species showed an asymmetrical stylet conus and areolated lateral fields which Siddiqi considered as characteristic for the genus *Histotylenchus*. According to Loof, 1987, Siddiqi placed the genus *Histotylenchus* in the subfamily Telotylenchinae Siddiqi, 1960, together with the genera *Telotylenchus* Siddiqi, 1960 and *Pseudhalenchus* Siddiqi, 1971. In 1970, Siddiqi included the subfamilies Belonolaiminae Whitehead, 1960, Telotylenchinae, and Aphasmatylenchinae Sher, 1965 to the family Belonolaimidae (Whitehead, 1960) Siddiqi, 1970. Siddiqi (1970) also provided dichotomous keys to the subfamilies and genera of the families Dolichodoridae (Chitwood & Chitwood, 1950) Skarbilovich, 1959 and Belonolaimidae. Siddiqi (1971) separated the families Belonolaimidae and Dolichodoridae stating that the representatives of Belonolaimidae having overlapping oesophageal glands with one or more nuclei behind the oesophago-intestinal valve and the dorsal gland often being enlarged, while representatives of the Dolichodoridae have a cellular cardia. Siddiqi (1971) additionally added that there is also a clear difference in the labial framework sclerotisations of these two families. Kleynhans & Heyns (1984) published a dichotomous key for the families Belonolaimidae, Tylenchorhynchidae Eliava, 1964 and Dolichodoridae, placing the genus *Histotylenchus* in the family Belonolaimidae.

During the late 1980's Fortuner & Luc (1987) redefined the family Belonolaimidae to include the belonolaimids (previously differentiated by the overlapping oesophageal glands) and the tylenchorhynchids (previously differentiated by abutting, bulb-shaped,

oesophageal glands) and related groups. Fortuner & Luc (1987) also suggested that the families Telotylenchidae Siddiqi, 1960 and Tylenchorhynchidae be made junior synonyms of the family Belonolaimidae. It was further added by Fortuner & Luc (1987) that the subfamily Telotylenchinae, containing the genus *Paratrophurus* Arias, 1970 (with *Histotylenchus* a juniore synonym of *Paratrophurus*), was only included in the family because of "the many characteristics shared by belonolaimids and tylenchorhynchids". This resulted in the subfamilies Tylenchorhynchinae, Trophurinae Paramonov, 1967 and Merliniinae Siddiqi, 1971, being proposed as synonyms for the subfamily Telotylenchinae. Maggenti *et al*. (1988) accepted the classification as proposed by Fortuner & Luc (1987) and therefore considered the family Belonolaimidae to include the subfamilies Belonolaiminae and Telotylenchinae.

According to Siddiqi (1971) and Kleynhans & Heyns (1984), the asymmetrical stylet conus of *Histotylenchus* may be used as a differentiating characteristic for the genus. Siddiqi (1971) stated that although *Histotylenchus* and *Telotylenchus* are closely related, *Histotylenchus* clearly differs from *Telotylenchus* in having a rectangular head, areolated lateral field and males that have a recurved gubernaculum. Siddiqi (1977) published a dichotomous key for the genus based on female characteristics, including all five (of the then known) species. According to Kleynhans & Heyns (1984) the genus *Histotylenchus* is characterised by an angular lumen, the lip region being virtually continuous with the neck, a thick-walled vestibule, the female tail being sub-cylindrical with a broadly rounded terminus and in males the gubernaculum can bear a posterior projection and is often proximally recurved. Other characteristics for the genus described by Kleynhans & Heyns (1984) are the four lateral lines within areolated lateral field.

Fortuner & Luc (1987) proposed *Histotylenchus* and *Telotylenchoides* Siddiqi, 1971 as synonyms of *Paratrophurus*. This proposal was made because, according to the authors upon describing the genus, Siddiqi (1971) did not compare *Histotylenchus* with *Paratrophurus*. Fortuner & Luc (1987) did not recognise lateral field areolations, the difference in overlapping gland position and differences in the distance between dorsal

nucleus and intestine junction as diagnostic characteristics for genus differentiation. The slight difference in measurements of the distance between the dorsal nucleus and the junction in *Histotylenchus* and *Telotylenchus*, recorded by Siddiqi (1971), was not accepted by Fortuner & Luc (1987) as a diagnostic characteristic. Fortuner & Luc (1987) further argued that in order to observe the asymmetrical stylet conus in *Histotylenchus* special preparation techniques had to be followed, making this an undesired genus characteristic. In spite of these minor differences between the two genera, Fortuner & Luc (1987) also noted that there were also great similarities in the general appearance and scanning electron micrographs of the *en face* view of these two genera.

Castillo *et al.* (1989) rejected the synonymy of *Histotylenchus* and *Telotylenchoides* with *Paratrophurus* made by Fortuner & Luc (1987), arguing that the stylet conus of *Histotylenchus* is asymmetrical (clearly visible when the anterior end of specimens are in lateral position) and that the stylet conus of *Paratrophurus* appears solid in its distal half, and consequently reinstated *Histotylenchus* as a valid genus. In this process Castillo *et al*. (1989) proposed *Paratrophurus siddiqi* Fortuner & Luc, 1984 as a junior objective synonym of *Histotylenchus sudanensis* Siddiqi, 1977. The most recent description was made by Kleynhans (1992) upon describing *Histotylenchus mohalei* Kleynhans, 1992.

Siddiqi (2000) recognised that *Paratrophurus*, *Telotylenchoides* and *Histotylenchus* all showed diminished protoplasmic content of the tail, but stressed that this characteristic was not strong enough to unite the genera. Siddiqi (2000) recognised the subfamily Belonolaiminae as the only subfamily in Belonolaimidae and he placed the subfamily Telotylenchinae (= Tylenchorhynchinae, Trophurinae and Dolichorhynchinae Fotedar & Handoo, 1978), containing the genus *Histotylenchus* in the family Telotylenchidae. As a result of molecular work (De Ley & Blaxter, 2002) done on various families, including Telotylenchidae, Decraemer & Hunt (2006) placed both the subfamilies Belonolaiminae and Telotylenchinae (containing the genus *Histotylenchus*) in the family Dolichodoridae. Luc & Fortuner (1987) stated that representatives of Dolichodoridae have strongly

sclerotised labial framework, with the basal plate exceptionally thickened, whereas representatives of Belonolaimidae's labial framework is thin, slightly sclerotised, with the basal plate extending posteriorly in a very thin annular extension close to the cuticle (Fortuner & Luc, 1987).

Currently the genus *Histotylenchus* is accepted by various authors worldwide including Kleynhans (1975); Kleynhans & Heyns (1984); Kleynhans (1992); Kleynhans *et al*. (1996), Siddiqi (2000) and Decraemer & Hunt (2006).

2.3 Classification of the genus *Histotylenchus* Siddiqi, 1971

Classification of the genus *Histotylenchus* followed here is based on Maggenti *et al.* (1988) and that given in Kleynhans *et al*. (1996).

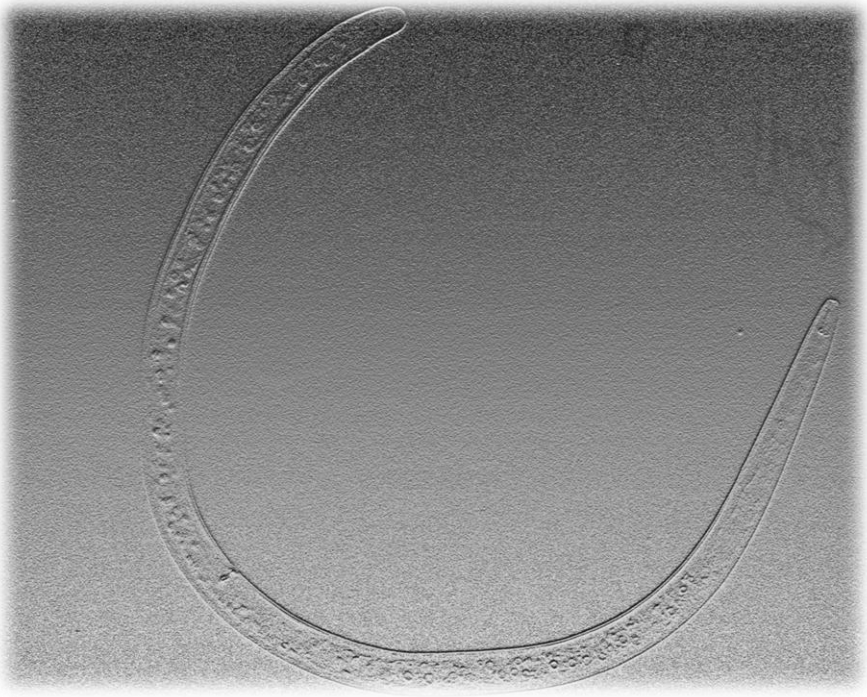
PHYLUM Nematoda (Rudolphi, 1808) Lankester, 1877
CLASS Secernentea Von Linstow, 1905
SUBCLASS Diplogasteria Maggenti, 1982
ORDER Tylenchida Thorne, 1949
SUBORDER Tylenchina Chitwood, 1950
SUPERFAMILY Tylenchoidea Örley, 1880
FAMILY Belonolaimidae Whitehead, 1960
SUBFAMILY Telotylenchinae Siddiqi, 1960
GENUS *Histotylenchus* Siddiqi, 1971

Histotylenchus Siddiqi, 1971
= *Telotylenchoides* Siddiqi, 1971

Etymology

Derived from the Greek *histo* meaning tissue or cellular (for areolated lateral fields), *tylos* meaning knob and *enchos* meaning spear.

CHAPTER 3



Materials and methods

3.1 Material

3.1.1 Fixed material (Type)

Material used in this study was obtained from:

1. The National Collection of Nematodes (NCN), Biosystematics Programme, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (*Histotylenchus hedys*, *Histotylenchus mohalei, Histotylenchus niveus* sp. n, *Histotylenchus* sp. 1 and *Histotylenchus* sp. 2).
2. The Rothamsted Nematode Collection housed at the Food and Environment Research Agency (Fera), York, United Kingdom (*Histotylenchus histoides*).
3. CABI Bioscience, Egham, United Kingdom (*Histotylenchus histoides*).

Collection areas are summarised in Table 3.1 below.

Table 3.1: Collection areas of species from present study.

Species	**Collection Areas**
Histotylenchus sp. 1	Limpopo Province, South Africa
Histotylenchus sp. 2	Eastern Cape Province, South Africa
Histotylenchus hedys	KwaZulu-Natal Province, South Africa
Histotylenchus histoides	Limbe, Malawi
Histotylenchus mohalei	Free State, Limpopo, Gauteng & Mpumalanga Provinces, South Africa
Histotylenchus niveus sp. n.	Northern Cape Province, South Africa

***Remark*:** Appendix III provides detailed locality data of all material examined.

3.1.2 Preparation of material for light microscopy

All fixed specimens received from the National Collection of Nematodes, Biosystematics Programme, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa were extracted from the soil using the sugar centrifugal-flotation method (Kleynhans, 1997). Specimens were killed in water by gradual application of heat, fixed and preserved (Table 3.2) in either FAA (40% formalin, acetic acid, 96%

ethanol and distilled water), TAF (triethanolamine, 40 % formalin, and distilled water) or FPG (40% formalin, propionic acid, glycerol and distilled water) (Netscher & Seinhorst, 1969). Fixed specimens were mounted on Cobb slides (Cobb, 1917) in anhydrous glycerine and sealed with 'glyceel' (Kleynhans, 1997).

3.1.3 Morphological observations and measurements

Material was drawn and measured using a Nikon Eclipse 80i light microscope equipped with a drawing tube; micrographs were taken using a Nikon DMX1200F mounted on a Zeiss Axiophot light microscope. Body length, as well as curved structures, was measured along the median line and straight structures were measured along the median axis. All measurements are given in micrometers (µm) unless noted otherwise. The terminology used for stoma and spicule morphology follows the proposals of Heyns (1971) and Fortuner (1984).

***Remark*:** Measurements were rounded off to the nearest decimal, including those obtained from literature.

Table 3.2: Specimens received from the National Collection of Nematodes, Biosystematics Programme, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa with corresponding fixative used.

Locality number	TAF	FAA	FPG
KP190		x	
KP337		x	
KP506		x	
KP808		x	
KP822		x	
KP946		x	
KP2039	x		
N125		x	
N208		x	
N222		x	
N260		x	
N289		x	
N291		x	
N315		x	
N321		x	
N356		x	
N365		x	
N375		x	
N380		x	
N436		x	
N437		x	
N414		x	
N445		x	
N569	x		
N620	x	x	
OVS30		Unknown	
OVS131		x	
OVS161		x	
OVS195		x	
OVS206		x	
OVS227		x	
OVS252		x	
OVS266		x	
OVS269		x	
TVL191		x	
TVL745		x	
TVL925		x	
TVL926		x	
TVL927		x	
TVL932		x	
TVL936		x	
TVL941		x	
TVL942		x	
TVL944		x	
TVL1063		x	
TVL1960			x
TVL2005			x
TVL2026			x

3.1.4 Preparation of material for scanning electron microscopy (SEM)

Material used for scanning electron microscopy was prepared by a method developed by Buckley and Tiedt (M. Marais, pers. comm[5]) as follows: Glycerin fixed specimens were removed fixed from slides and were hydrated in a graded series of glycerin-thinning medium after being removed from slides and placed in 100 % glycerin. The thinning medium consisted of 30 % absolute ethanol mixed with 70 % distilled water and the percentage glycerin in the thinning medium in the graded series was 85 %; 65 %; 45 %; 25 %; 5 % and 0 %. In 15-30 minute intervals the thinning medium was replaced by the next concentration of thinning medium in the series, with the last step (specimens washed with thinning medium containing 0 % glycerin) being repeated three times.

The specimens were then hydrated by placing the specimens for 15-30 minutes in 15 % ethanol, followed by 5 % ethanol and then 100 % water, with the last step being repeated three times. Specimens were then transferred to a capsule containing 30 % ethanol, and in 20 minute intervals were transferred to 50 %, 75 %, 95 % and 100 % ethanol, with the last step being repeated twice. After this specimens were transported in 100 % ethanol to the Laboratory for Electron Microscopy, North West University, Potchefstroom Campus, Potchefstroom, South Africa. Following conventional critical-point drying and gold palladium coating (21 or 25 nm), specimens were viewed and micrographs were taken by Dr. L.R. Tiedt with a FEI Quanta 200 scanning electron microscope at 10kV.

3.2 Effects of preparation methods and age of permanent microscope slides on taxonomic characters of *Histotylenchus* Siddiqi, 1971.

During this study it was noted that a total of 72 out of a total of 163 microscope slides were not suitable for taxonomic study as the cuticle had separated from the body in these specimens (Fig. 3.1). In particular it was the older material (prepared between 1963 and 1985) that presented this problem. Material prepared after 1985 did not show the separation of the cuticle from the body. All material examined that showed a separated cuticle was killed by slowly heating the specimens and was then fixed in FAA

[5] Dr. M. Marais. Nematology Unit Biosystematics Programme, ARC- Plant Protection Research Institute, Private Bag X134, Queenswood, Pretoria, 0121, South Africa.

(followed by mounting in anhydrous glycerin. Up to date this has never been reported for the genus *Histotylenchus* (M. Marais, pers. comm.[6]), but is known for other genera e.g. *Paratrichodorus* (De Waele *et al.*, 1990; Nasira & Maqbool, 1994; Zheng *et al.*, 2004).

[6]Dr. M. Marais. Nematology Unit Biosystematics Programme, ARC- Plant Protection Research Institute, Private Bag X134, Queenswood, Pretoria, 0121, South Africa.

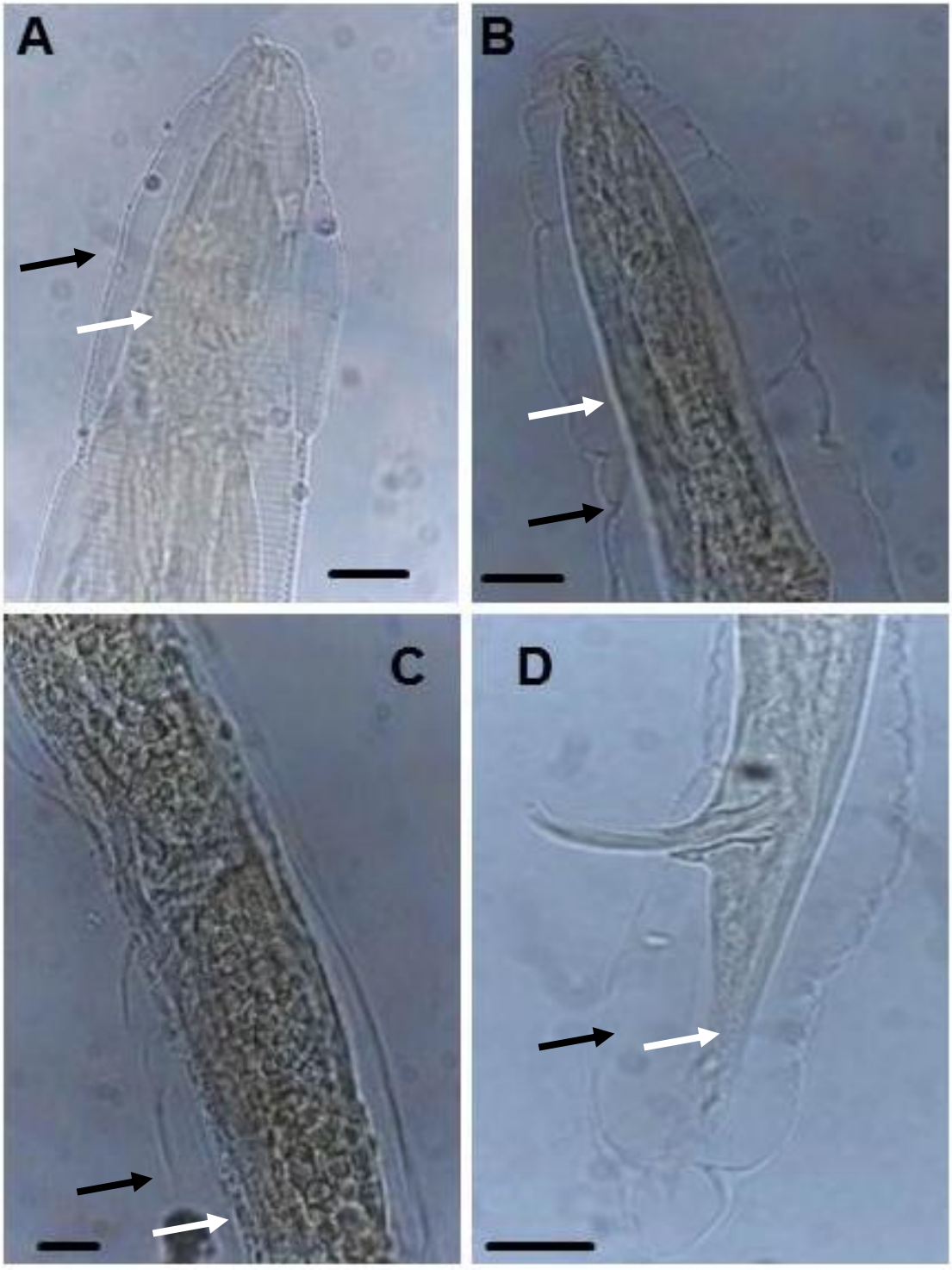


Figure 3.1: Light micrographs of some specimens from the genus *Histotylenchus* Siddiqi, 1971, illustrating the separation of the cuticle (black arrow) from the body (white arrow). **A**- separation of cuticle in lip region of female; **B**- separation of cuticle in lip region of male; **C**- separation of the cuticle from the body in midbody region of male; **D**- separation of the cuticle in tail region of male. **Scale bars** = 10 µm.

3.3 Abbreviations used in text

According to Baldwin & Perry (2004) nematode morphology is the starting point for correctly identifying pathogenicity, ecosystem roles and feeding biology of nematodes and therefore it is essential to understand the morphology of the genus. The classical method and system introduced by De Man (1880) is used in this dissertation. The different symbols and abbreviations used are as follows:

a = body length divided by greatest body width; **b** = body length divided by distance from anterior end to junction of oesophagus and intestine; **b'** = body length divided by distance from anterior end to posterior end of oesophageal gland; **c** = body length divided by tail length (anus or cloaca to tail terminus); **c'** = tail length divided by body width at anus or cloacal opening; **DGO** = dorsal gland opening posterior to stylet; **L** = total body length; **m** = length of anterior (conus) part of stylet expressed as a percentage of total stylet length; **OV_1** = length of anterior genital branch expressed as percentage of total body length; **OV_2** = length of posterior genital branch expressed as a percentage of total body length; **V** = distance of vulva from anterior end expressed as a percentage of total body length; **T** = distance from cloacal opening to tip of testis expressed as a percentage of total body length.

A number of morphological measurements are highlighted in Figure 3.2. Refer to Appendix II for a full description of all diagnostic characters used in this dissertation and why these characters were used specifically for *Histotylenchus.*

***Remark*:** This dissertation, specifically the descriptions, has been written according to guidelines set out for authors publishing in the journal '*NEMATOLOGY*'.

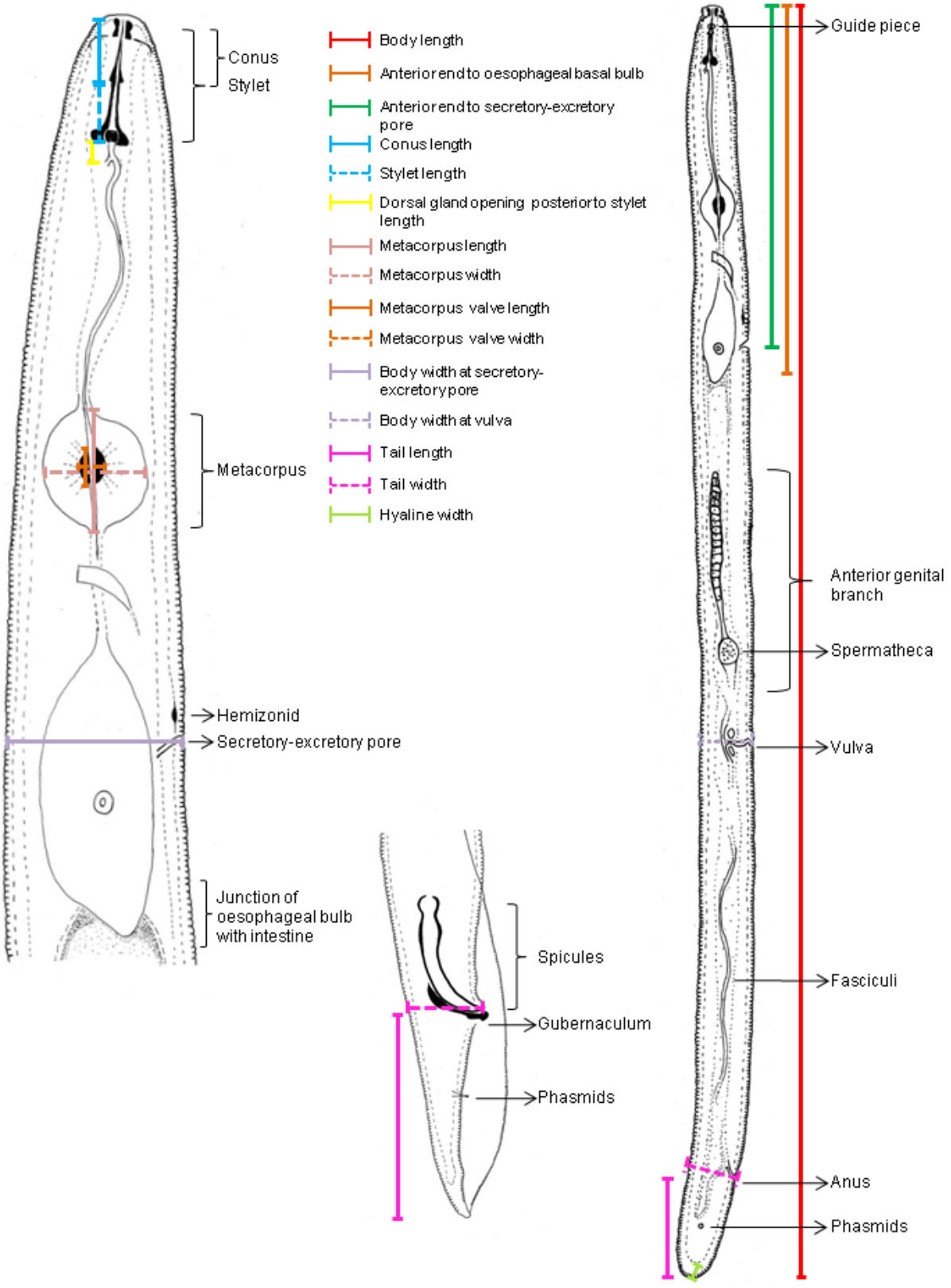
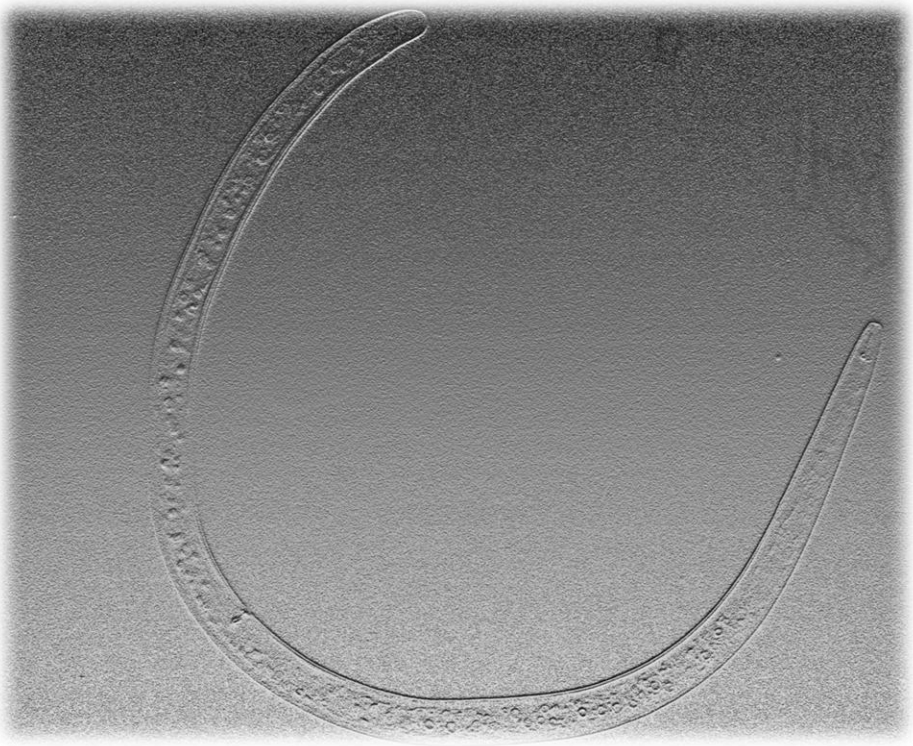


Figure 3.2: Diagram illustrating some of the morphological measurements used in the description of *Histotylenchus* Siddiqi, 1971.

CHAPTER 4



Histotylenchus species

4.1 Diagnosis of the genus *Histotylenchus* Siddiqi, 1971 (Fig. 4.1)

Habitus arcuate to strongly curved. Lateral field with four lines, usually areolated. Lip region continuous or slightly offset by a depression. Stylet heavy; conus characteristically asymmetrical; knobs large. Metacorpus large; metacorpus valve long, spindle-shaped. Isthmus short. Oesophageal glands extending over intestinum, deirids present. Hemizonid and secretory-excretory pore present. Phasmids postanal, small. *Female*: Vulva in a cavity, with single or double epiptygma. Genital tracts about equal, ovaries outstretched. Spermathecae axial. Tail short, hemispherical, subcylindrical to cylindrical. *Male*: Tail with moderately large bursa beginning just anterior to spicule and enclosing tail tip. Gubernaculum large, with modified proximal end directed backwards; protrusable or not. Hypoptygma absent.

Type species

Histotylenchus histoides Siddiqi, 1971

= *Paratrophurus histoides* (Siddiqi, 1971) Fortuner & Luc, 1987

Other valid species

Histotylenchus baoulensis (Netscher & Germani, 1969) Siddiqi, 1971

= *Telotylenchus baoulensis* Netscher & Germani, 1969

= *Trichotylenchus baoulensis* (Netscher & Germani, 1969) Jairajpuri, 1971

= *Paratrophurus baoulensis* (Netscher & Germani, 1969) Fortuner & Luc, 1987

Histotylenchus hedys Kleynhans, 1975

= *Paratrophurus hedys* (Kleynhans, 1975) Fortuner & Luc, 1987

Histotylenchus historicus (Jairajpuri & Baqri, 1968) Siddiqi, 1971

= *Telotylenchus historicus* Jairajpuri & Baqri, 1968

= *Trichotylenchus historicus* (Jairajpuri & Baqri, 1968) Jairajpuri, 1971

= *Paratrophurus historicus* (Jairajpuri & Baqri, 1968) Fortuner & Luc, 1987

Histotylenchus mohalei Kleynhans, 1992

Histotylenchus sudanensis Siddiqi, 1971

= *Paratrophurus sudanensis* (Siddiqi, 1971) Fortuner & Luc, 1987, *nec Paratrophurus sudanensis* Decker, Yassin & El Amin, 1975

= *Paratrophurus siddiqi* Fortuner & Luc, 1987

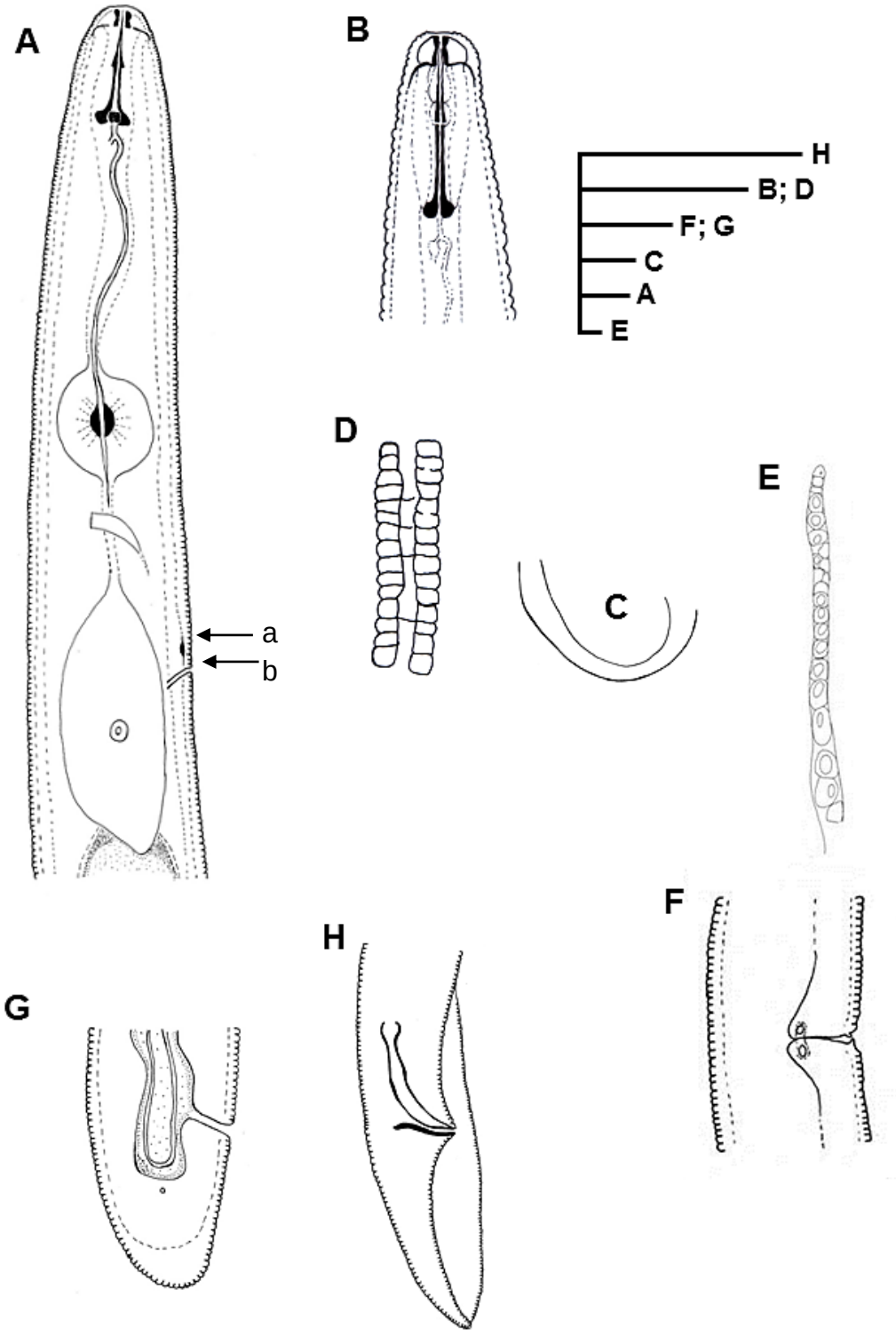


Figure 4.1: Line diagrams of *Histotylenchus* Sidddiqi, 1971. Female: **A-** anterior end with hemizonid (arrow a) and secretory-excretory pore (arrow b); **B**- lip region; **C**- habitus; **D**- lateral lines near vulva; **E**- section of anterior genital branch; **F**- vulva; **G**- tail. Male: **H**- tail. **Scale bars** = 10 µm.

4.2 Distribution of the genus *Histotylenchus* Siddiqi, 1971

Five of the six *Histotylenchus* species have been reported from Africa and one species from India (*Histotylenchus historicus*) (Fig. 4.2).

Species from Africa include (South African species highlighted):

1. *Histotylenchus baoulensis* : Côte d'Ivoire (Netscher & Germani, 1969; Siddiqi, 1971)
2. *Histotylenchus hedys*: **South Africa** (Kleynhans, 1975)
3. *Histotylenchus histoides*: Burkina Faso, Malawi, Namibia, and **South Africa** (Siddiqi, 1971; Kleynhans, 1975; Kleynhans & Heyns, 1984)
4. *Histotylenchus mohalei*: **South Africa** (Kleynhans, 1992)
5. *Histotylenchus sudanensis* (Fig. 4.7): Sudan (Siddiqi, 1977)

4.3 Compendium of the genus *Histotylenchus* Siddiqi, 1971

Currently there is no available compendium for the genus *Histotylenchus* and as previously mentioned only one dichotomous key by Siddiqi (1977) is available. In Appendix I a compendium for all six species in the genus *Histotylenchus* is given.

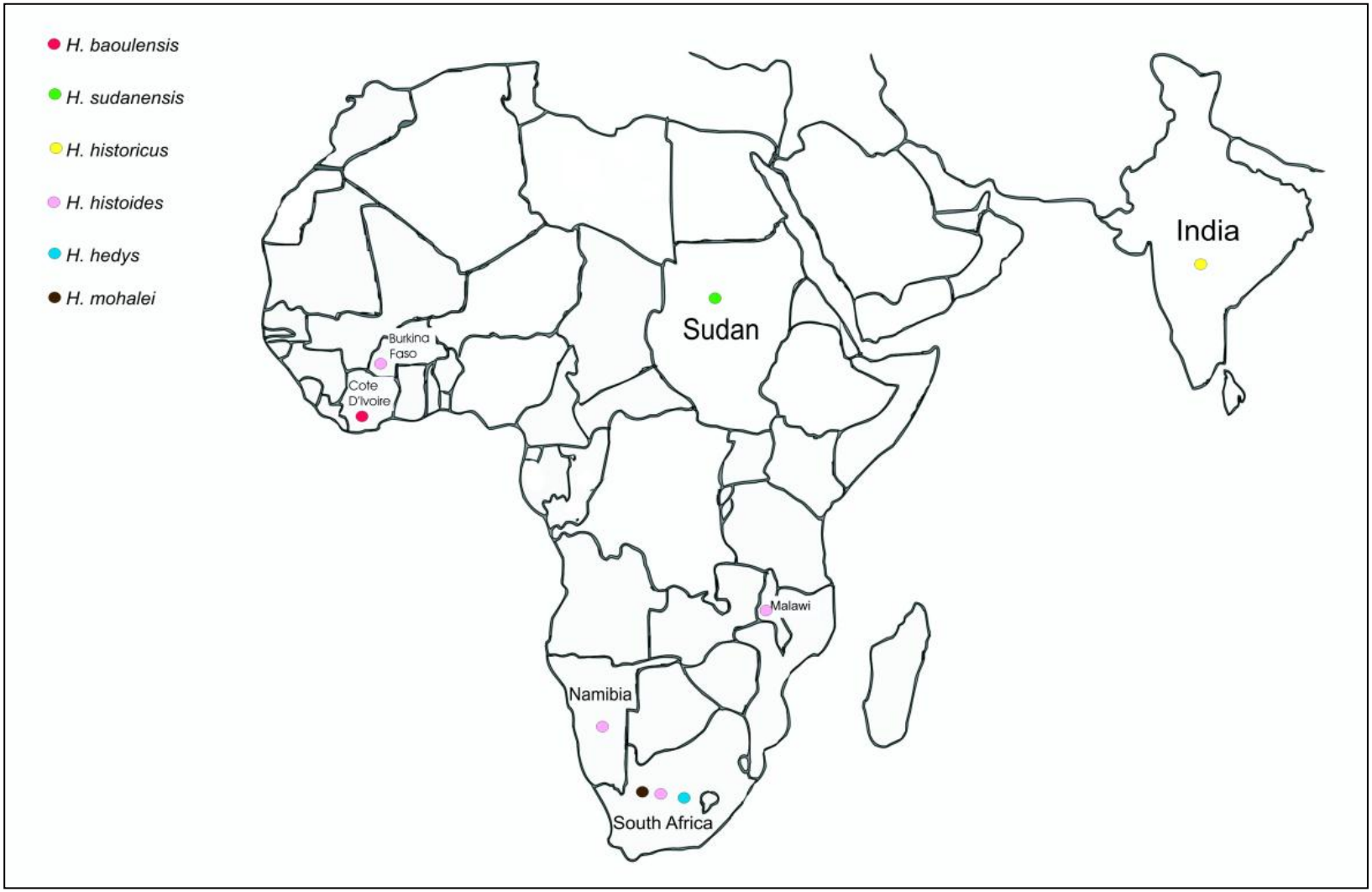


Figure 4.2: Distribution of the six valid species in the genus *Histotylenchus* Siddiqi, 1971 in Africa and India (compiled from: Jairajpuri & Baqri, 1968; Netscher & Germani, 1969; Siddiqi, 1971; Siddiqi, 1977; Kleynhans, 1975; Kleynhans & Heyns, 1984; Kleynhans, 1992 and SAPPNS database).

Histotylenchus hedys Kleynhans, 1975

(Figs 4.3 & 4.4)

The SAPPNS database (2010) recorded *Histotylenchus hedys* from roses and sugarcane in South Africa. It is interesting to note that up to date H. hedys has only been recorded from the Savanna biome, but the distribution of this species ranges from 100 to 1 200 meters above sea level.

During the present M. Sc study *Histotylenchus hedys* was described from material collected at one locality in the Gauteng Province, and several localities in the KwaZulu- Natal Province South Africa. Type material hosted at the National Collection of Nematodes, Biosystematics Programme, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa. No *H. hedys* specimens was present in fresh material collected during this study and thus there was none available for scanning electron micrographs.

Measurements: See Table 4.1

Description

Female (n = 6): Habitus straight (20 % of individuals), slightly curved (10 % of individuals) to curved ventrad (70 % of individuals), body cylindrical throughout except for anterior part of tail end. Lateral field with four incisures, originate as two lines just posterior to stylet knobs; third line appears after short distance dividing into two lines at level of metacorpus, outer two bands areolated throughout entire body, inner band incompletely areolated through entire body, but with areolations present more frequent posterior to vulva, outer and inner lines end in V-shaped pattern anterior to hyaline region on tail. Lip region continuous with body (67 % of individuals) or marked off by slight depression (33 % of individuals), 12 ± 1.4 (12-14) µm wide, more than twice lip region height of 5 ± 0.6 (5-6) µm; lip region with six to eight annuli; cephalic framework and basal ring usually not strongly sclerotised, vestibule wall strongly sclerotised. Stylet stout, stylet cone asymmetrical; stylet knobs mostly round, sloping backwards, flattened to slightly indented. Procorpus wider than isthmus. Metacorpus large, nearly rounded; metacorpus valve spindle-

shaped; hemizonid three annuli anterior to secretory-excretory pore, two to three annuli long; deirid not observed. Secretory-excretory pore situated opposite anterior part of postcorpus, i.e. at 15 ± 0.9 (15-17) % of body length. Postcorpus with ventral overlap; dorsal oesophageal gland distinct, posterior to secretory-excretory pore; oesophago-intestinal valve indistinct. Intestine with large, very prominent fasciculi extending well past anus. Vulva in deep boat-shaped depression situated at 53 ± 2.0 (53-57) % of body length with double, non-protruding or sunken epiptygma, vaginal musculature prominent; reproductive system amphidelphic with two outstretched ovaries, anterior genital branch slightly longer than posterior genital branch. Spermatheca not observed. Postanal diverticulum of intestine large, overlaps anus and reaches well past level of phasmids. Phasmids small, distinct, situated 10-18 annuli posterior to anus at about mid- tail. Tail short, symmetrically rounded, 42 ± 4.1 (42-48) µm long.

Male (n = 3): Similar to female, except in following: Habitus straight (67 % of individuals) to slightly curved (33 % of individuals). Dorsal lateral line extends past phasmids terminating near tail end, subdorsal and subventral lines extend to opposite cloacal opening or just beyond, ventral line terminating at beginning of bursa. Lip region with six to seven annuli; hemizonid not observed, secretory-excretory pore situated opposite to anterior part of postcorpus, i.e. at 16 % (n=1) of body length, deirid not observed. Fasciculi not observed. Spicule arcuate with prominent velum, gubernaculum non-protrusible, with pronounced swollen titillae, proximal end curved backwards. Phasmids small, located 12-15 µm posterior to cloacal opening. Tail slender with fingerlike ventral projection with rounded end.

Diagnosis

Histotylenchus hedys is characterised by the following combination of characters: Lip region continuous or marked off by depression, stylet stout (22-25 µm), stylet cone asymmetrical; stylet knobs mostly round, sloping backwards, flattened to slightly indented; lateral lines crenate, inner and outer lines irregular opposite vulva, all three bands areolated, inner band incomplete in some places, areolations of outer bands generally larger than body annuli; inner lines of lateral field at no place coalesce; vulva sunken anterior and posterior to epiptygma; postanal diverticulum of intestine large, overlaps anus and reaches well past phasmids; tail short, symmetrically

rounded with 29-42 ventral annuli in females; non-protruding gubernaculum with swollen titillae and proximal end curved backwards in males.

Discussion

Type material females, as described in Kleynhans (1975), differ from *Histotylenchus hedys* females from current study (NCN) populations in: Habitus (strongly curved ventrad *vs* straight to strongly curved ventrad) and body length (1 070-1 250 µm *vs* 828-982 µm). Males differ in: Stylet length (24-25 µm *vs* 17-22 µm), conus length (12-13 µm *vs* 7-10 µm) and ratio a (26-28 *vs* 38-53). *H. hedys* females from a South African population (Kleynhans & Heyns, 1984) differ from current study (NCN) females in: Habitus (strongly curved ventrad *vs* straight to strongly curved ventrad) and body length (1 017-1 222 µm *vs* 828-982 µm). The vulva position has the lowest coefficient of variation[7] (CV) value of 4 %, followed by stylet length (5 %). Males differ in: Stylet length (22-24 µm *vs* 17-22 µm) and ratio a (32-39 *vs* 38-53).

Locality and material examined

See Appendix III for full locality data and all material examined.

[7] Coefficient of variation (CV) represents the ratio of standard deviation to the mean and is used to compare the degree of variation in a diagnostic characteristic. The lower the value the more stable the diagnostic characteristic.

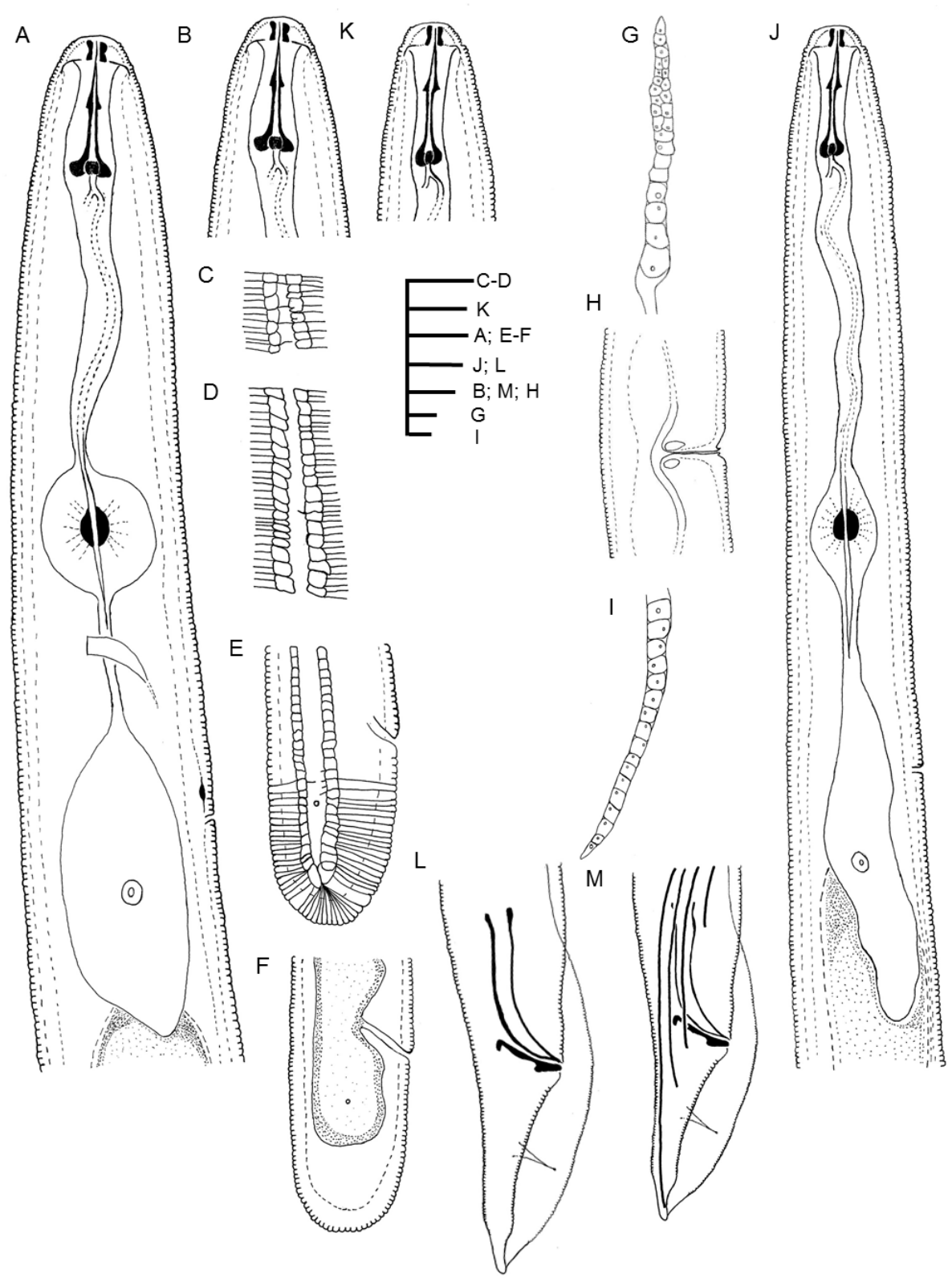


Figure 4.3: Line diagrams of *Histotylenchus hedys* Kleynhans, 1975. Female: **A**- anterior end; **B**- lip region; **C**- lateral lines in secretory-excretory pore; **D**- lateral lines near vulva; **E**- tail with lateral field; **F**- tail, showing postanal diverticulum; **G**- section of anterior ovarium; **H**- vulva; **I**- section of posterior ovarium. Male: **J**- anterior end; **K-** lip region; **L**- tail and **M**- tail, showing lateral lines. **Scale bars** = 10 µm.

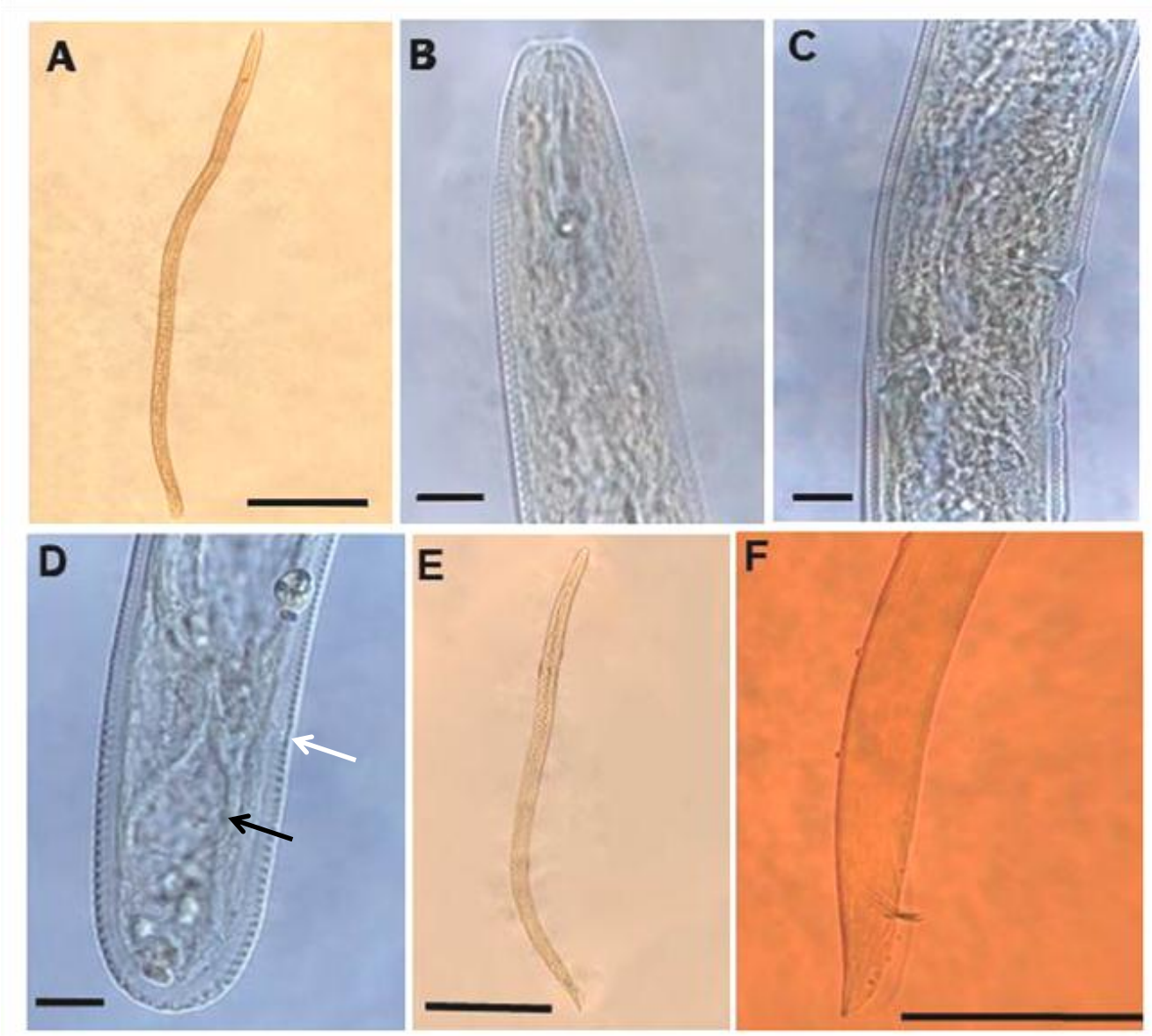


Figure 4.4: Light micrographs of *Histotylenchus hedys* Kleynhans, 1975. Female: **A**- habitus; **B**- lip region; **C**- vulva region; **D**- tail region showing anus (white arrow) and fascicule (black arrow). Male: **E**- habitus and **F**- tail region. **Scale bars** = 100 µm (A; E-F) and 10 µm (B-D).

Table 4.1: Morphometric data of *Histotylenchus hedys* Kleynhans, 1975 paratype material and current study populations (NCN). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	**Mposa (Kleynhans, 1975)**		**Roodeplaat (Kleynhans, 1975)**	**Gauteng & Kwa-Zulu Natal (Kleynhans & Heyns, 1984)**		**N291: Loteni Nature Reserve (NCN)**	**N414: Mposa (NCN)**	**N620: Hluhluwe (NCN)**	
n	8♀♀	2♂♂	1♀	11♀♀	9♂♂	1♀	1♀	4♀♀	1♂
L	1 150 (1 070-1 250)	1 070-1 110	1 250	1 143 (1 017-1 222)	1 068 (987-1 124)	982	922	879 ± 38.5 (828-922)	822
Number of lip annuli	6-7	6-7	6-7	-	-	7	7	7 ± 0.9 (6-8)	6
Lip region height	5 (4-5)	5-6	5	-	-	4	5	4 ± 0.4 (4-5)	4
Lip region width	12 (12-13)	13-13	12	13 ± 0.6 (12-13)	11 (10-15)	11	12	12 ± 2.1 (10-14)	10
Basal ring: annuli	-	-	-	-	-	4	-	4 ± 0.6 (3-4)	4
Outer margins of basal ring: length	-	-	-	-	-	3	-	4 ± 0.5 (3-4)	5
Stylet length	24 (23-26)	24-25	23	24± 0.9 (23-25)	23 (22-24)	-	23	23 ± 1.5 (22-25)	-
Conus length	12 (10-12)	12-13	11	-	-	10	11	12 ± 2.7 (10-15)	13
Stylet base length	-	-	-	-	-	-	12	12 ± 1.5 (10-13)	-
Stylet knobs height	-	-	-	-	-	-	3	4 ± 0.5 (3-4)	-
Stylet knobs width	7 (6-8)	8	6	-	-	-	7	6 ± 0.4 (6-6)	-
Stylet length/ lip region width	-	-	-	-	-	-	2	2 ± 0.4 (2-3)	-
Dorsal gland opening posterior to stylet (DGO)	-	-	-	-	-	-	3	3 (n = 1)	-
Anterior end to secretory-excretory pore	145 (137-157)	-	149	150 ± 7.8 (138-162)	181 (141-172)	-	152	137 ± 1.7 (135-139)	133

Table 4.1 continued: Morphometric data of *Histotylenchus hedys* Kleynhans, 1975 paratype material and current study populations (NCN). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	**Mposa (Kleynhans, 1975)**		**Roodeplaat (Kleynhans, 1975)**	**Gauteng & Kwa-Zulu Natal (Kleynhans & Heyns, 1984)**		**N291: Loteni Nature Reserve (NCN)**	**N414: Mposa (NCN)**	**N620: Hluhluwe (NCN)**	
n	8♀♀	2♂♂	1♀	11♀♀	9♂♂	1♀	1♀	4♀♀	1♂
Anterior end to mid metacorpus	94 (77-100)	97-100	98	-	-	-	91	90 ± 2.8 (87-93)	86
Metacorpus length	-	-	-	-	-	20	17	18 ± 2.5 (15-21)	12
Metacorpus width	16 (15-18)	19-20	13	-	-	16	20	13 ± 0.9 (12-13)	10
Metacorpus length/ metacorpus width	-	-	-	-	-	1	1	1-2	1
Metacorpus valve length	-	-	-	-	-	6	4	5 ± 0.9 (4-6)	6
Metacorpus valve width	-	-	-	-	-	5	3	5 ± 0.9 (4-6)	2
Position of deirid	Opposite secretory-excretory pore	-	Opposite secretory-excretory pore	-	-	-	-	-	-
Position of hemizonid	2-4 annuli anterior to secretory-excretory pore	-	2-4 annuli anterior to secretory-excretory pore	-	-	-	-	-	3 annuli anterior to secretory-excretory pore
Anterior genital branch length	-	-	-	-	-	-	279	-	-
Posterior genital branch length	-	-	-	-	-	-	229	-	-
Vagina length	-	-	-	-	-	-	21	13 ± 1.7 (11-15)	-

Table 4.1 continued: Morphometric data of *Histotylenchus hedys* Kleynhans, 1975 paratype material and current study populations (NCN). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	Mposa (Kleynhans, 1975)		Roodeplaat (Kleynhans, 1975)	Gauteng & Kwa-Zulu Natal (Kleynhans & Heyns, 1984)		N291: Loteni Nature Reserve (NCN)	N414: Mposa (NCN)	N620: Hluhluwe (NCN)	
n	8♀♀	2♂♂	1♀	11♀♀	9♂♂	1♀	1♀	4♀♀	1♂
Vagina length/corresponding body width	-	-	-	-	-	-	0.8	-	-
Spermatheca length	-	-	-	-	-	-	15-18	-	-
Spicule length	-	32-33	-	-	33 (32-35)	-	-	-	33
Gubernaculum length	-	13-13	-	-	13 (12-14)	-	-	-	15
Width of annuli at: secretory- excretory pore	-	-	-	-	-	-	1	2 ± 0.1 (2-2)	1
Width of annuli at: midbody or vulva	-	-	-	-	-	1	1	2 ± 0.2 (1-2)	1
Body width at secretory-excretory pore	-	-	-	-	-	-	32	28 ± 1.0 (27-29)	26
Body width at midbody or vulva	35 (32-39)	39-43	35	-	-	38	35	28 ± 3.7 (23-32)	26
Body width at anus/cloacal opening	-	-	-	-	-	32	25	25 ± 1.9 (22-26)	17
Lateral field width	-	-	-	-	-		8	6 ± 1.3 (5-8)	5
Position of phasmids posterior to anus	-	-	-	-	-	11-13	15-18	-	-
Phasmids to anus or cloacal opening length	22 (22-28)	25-26	22	-	-	14	-	16 ± 3.5 (13-21)	-
Phasmids diameter	-	-	-	-	-	1	1	1 ± 0.2 (1-1)	1
Tail length	43 (34-47)	44-51	39	42 ± 5.1 (31-47)	46 (37-52)	30	40	41 ± 5.4 (35-47)	44
Number of ventral annuli	27-44	-	27-44	27-44	-	29-31	35-42	-	-

Table 4.1 continued: Morphometric data of *Histotylenchus hedys* Kleynhans, 1975 paratype material and current study populations (NCN). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	Mposa (Kleynhans, 1975)		Roodeplaat (Kleynhans, 1975)	Gauteng & Kwa-Zulu Natal (Kleynhans & Heyns, 1984)		N291: Loteni Nature Reserve (NCN)	N414: Mposa (NCN)	N620: Hluhluwe (NCN)	
n	8♀♀	2♂♂	1♀	11♀♀	9♂♂	1♀	1♀	4♀♀	1♂
Tail projection length	-	-	-	-	-	-	-	-	6
Hyaline length	-	-	-	-	-	9	5	5 ± 0.6 (4-6)	-
Postanal diverticulum length	-	-	-	-	-	16 annuli posterior anus	20 annuli posterior anus	22 annuli posterior anus	-
a	33 (29-39)	26-28	36	32 (26-37)	36 (32-39)	26	26	32 ± 3.5 (28-36)	32
b	8 (8-9)	-	-	-	-	-	-	-	-
b'	6 (6-7)	-	-	6 (5-7)	6 (5-6)	-	-	-	-
c	27 (24-33)	22-24	32	28 (24-33)	23 (21-27)	33	23	22 ± 2.1 (20-24)	19
c'	-	-	-	-	-	0.9	1.6	2 ± 0.2 (1-2)	3
m (%)	-	-	-	-	-	-	48	49 ± 8.7 (44-59)	-
Anterior end to secretory-excretory pore/L (%)	-	-	-	-	-	-	17	15 ± 0.3 (15-16)	16
V (%)	52 (51-54)	-	53	53 (50-55)	-	53	52	55 ± 1.4 (54-57)	-
OV_1 (%)	-	-	-	-	-	-	30	-	-
OV_2 (%)	-	-	-	-	-	-	25	-	-
Posterior genital branch length/Anterior branch length (%)	-	-	-	-	-	-	82	-	-

Histotylenchus histoides Siddiqi, 1971

(Figs 4.5 & 4.6)

The most common species in South Africa is *Histotylenchus histoides*, often found associated with roots of Graminaceae (Kleynhans & Heyns, 1984). According to the South African Plant-Parasitic Nematode Survey, until 2010, *H. histoides* has been recorded from various biomes including Savanna, Thicket and Grasslands. This species has also been found associated with various crops, including wheat, pearl millet, certain pumpkin species, tomato, sugarcane, grass on bowling greens, pasture grass (Kleynhans & Heyns, 1984) and peach (Kleynhans 1975).

For this M.Sc study *Histotylenchus histoides* was described from the type material, from soil collected around Napier grass (*Pennisetum purpureum* Schumach) in the Limbe Province, Malawi. All *Histotylenchus histoides* material available in the National Collection of Nematodes, Biosystematics Programme, ARC-Plant Protection Research Institute, Pretoria, South Africa, was unsuitable for observation because of the separation of the cuticle from the body and no new *H. histoides* material was obtained during sampling for this genus. Paratype material was obtained from CABI and FERA for observation and based on this material *H. histoides* is re-described below. The fresh material collected during this study did not have any *H. histoides* specimens to be used in scanning electron micrographs.

Measurements: See Table 4.2

Description

Female (n = 4): Habitus strongly curved (100 % of individuals), body cylindrical throughout except for anterior end and tail. Lateral field with four incisures, originate as two lines in conus region; third line appears after short distance, dividing into two lines at level of metacorpus valve, outer two bands areolated throughout entire body, inner band doubles in width after secretory-excretory pore, incompletely areolated throughout body with areolations becoming more regular towards end of tail, outer and inner lines end in Y-shaped pattern (50 % of individuals) or in V-shaped pattern (50 % of individuals) three to six annuli anterior to hyaline region of tail. Lip region

continuous (25 % of individuals) or nearly continuous with body marked by slight depression (75 % of individuals), 12 ± 1.8 (11-15) µm wide, three times lip region height 4 ± 0.7 (3-5) µm; lip region with ten annuli; cephalic framework and basal ring usually not strongly sclerotised, vestibule wall strongly sclerotised. Stylet stout, stylet cone asymmetrical; stylet knobs large, round, sloping backwards, usually angular. Procorpus wider than isthmus. Metacorpus large, more round than oval in shape; metacorpus valve spindle-shaped. Postcorpus with ventral overlap; dorsal oesophageal gland distinct, situated posterior to secretory-excretory pore; oesophago-intestinal valve indistinct. Hemizonid three annuli long, situated three to four annuli anterior to secretory-excretory pore; deirid not observed. Secretory-excretory pore situated opposite anterior part of postcorpus, i.e. at 15 ± 3.2 (13-18) % body length. Intestine with fasciculi. Postanal diverticulum of intestine overlaps anus but does not reach phasmids. Vulva in deep depression situated at 53 ± 1.8 (51-55) % of body length with double, non-protruding or sunken epiptygma, vaginal musculature small and not prominent. Genital tracts not distinct in specimens. Phasmids small, distinct, situated 9-10 annuli posterior to anus at about mid- tail. Specimens with broad symmetrical rounded tail, 41 ± 2.1 (38-42) µm long.

Male (n = 5): Similar to female except in following: Dorsal lateral line extends past phasmids terminating near tail end, subdorsal and subventral lines extend to opposite cloacal opening or just beyond, ventral line terminating at beginning of bursa. Lip region with nine annuli. Spicule strongly arcuate to J-shaped with prominent velum, gubernaculum protrusible, with pronounced swollen titillae, proximal end sloping backwards. Phasmids small, located 13-18 µm posterior to cloacal opening. Tail slender with long 13 ± 3.6 (10-17) µm, fingerlike ventral projection with rounded end.

Diagnosis

Histotylenchus histoides is characterised by the following combination of characters: lip region nearly continuous with body marked by slight depression, outer two band of lateral field areolated throughout body, inner band incompletely areolated with areolations becoming more complete towards end of tail, stylet stout (17-23 µm), stylet cone asymmetrical, stylet knobs large, round, sloping backwards, outer edges usually angular, postanal diverticulum of intestine overlaps anus but does not reach

phasmids, tail with 24-45 ventral annuli and annulated tip in females. Tail with long, fingerlike ventral projection with rounded end; gubernaculum protrusible, with pronounced swollen titillae, proximal end sloping backwards in males.

Discussion

Current female specimens of *Histotylenchus histoides* (CABI & FERA material) differ from South African material in Kleynhans (1975) and Kleynhans & Heyns (1984) in: Habitus (straight to ventrally arcuate *vs* strongly curved) and body length (1 024-1 450 µm *vs* 861-964 µm). Lip region width and conus length have the lowest coefficient of variation (CV) values (2 %) followed by the position of the vulva (3 %). Males differ in body length (1 000-1 200 µm *vs* 873-946 µm); tail length (39-42 µm *vs* 43-55 µm) and ratio a (35-44 *vs* 21-33).

Locality and material examined

See Appendix III for full locality data and all material examined.

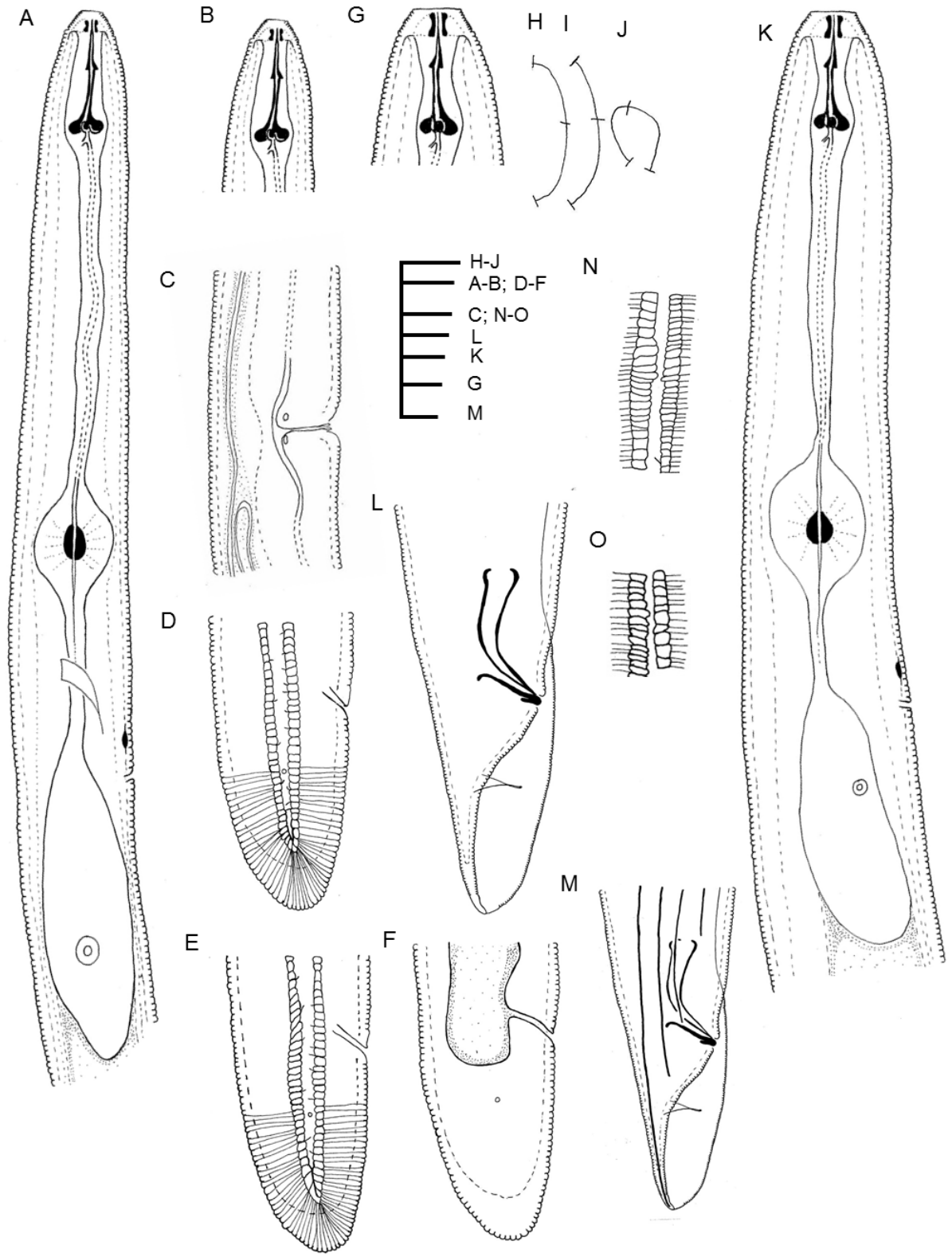


Figure 4.5: Line diagrams of *Histotylenchus histoides* Siddiqi, 1971. Female: **A**- anterior end; **B**- lip region; **C**- vulva region; **D**- tail with lateral field ending in V-shaped pattern; **E**- tail with lateral field ending in Y-shaped pattern; **F**- tail with postanal diverticulum; **H-J**- habitus; **N**- lateral field in vulva region and **O**- lateral field in secretory-excretory pore region. Male: **G**- lip region; **K**- anterior end; **L**- tail region and **M**- tail showing lateral lines. **Scale bars** = 10 µm.

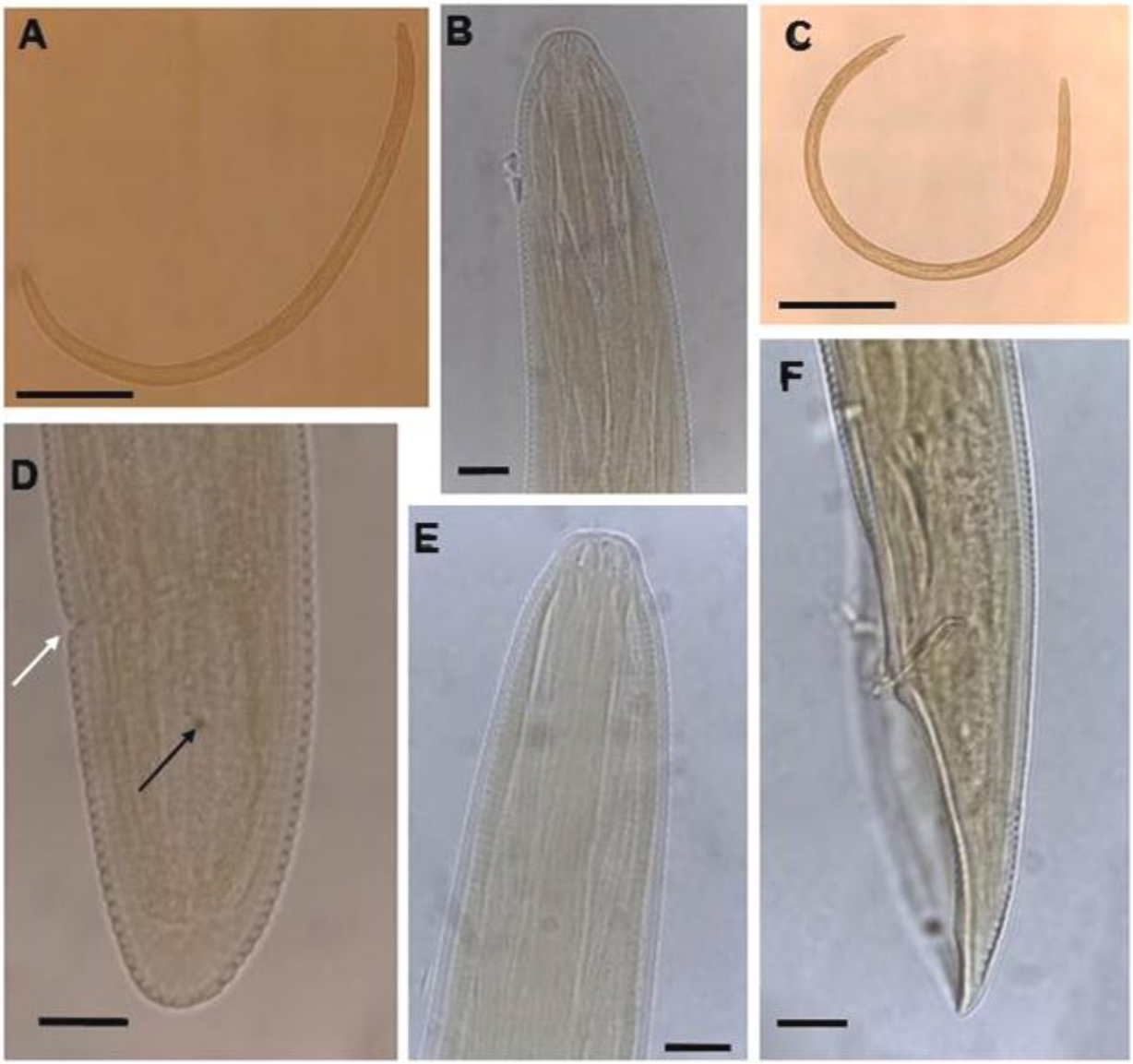


Figure 4.6: Light micrographs of *Histotylenchus histoides* Siddiqi, 1971. Female: **A**- habitus; **B**- lip region; **D**- tail region with anus (white arrow) and phasmids (black arrow). Male: **C**- habitus; **E**- lip region and **F**- tail region. **Scale bars** = 100 µm (A; C) and 10 µm (B; D-F).

Table 4.2: Morphometric data of *Histotylenchus histoides* Siddiqi, 1971 paratype material and current study populations (CABI & FERA). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	**Malawi (Siddiqi, 1971)**		**South Africa and Namibia (Kleynhans, 1975)**		**South Africa and Namibia (Kleynhans & Heyns, 1984)**		**Malawi Paratype CABI**		**Malawi Paratype FERA**	
n	7♀♀	14♂♂	13♀♀	5♂♂	13♀♀	7♂♂	1♀♀	2♂♂	3♀♀	3♂♂
L	1 080-1 180	1 030-1 200	1 200 (1 110-1 450)	1 070 (1 000-1 120)	1 136 ± 57.4 (1 024-1 204)	1 047 (1 010-1 107)	937	946-985	930 ± 59.1 (861-964)	898 ± 24.1 (873-922)
Number of lip annuli	8-11	-	-	-	-	-	10	10-10	10 ± 0.4 (10-10)	9 ± 0.4 (9-9)
Lip region height	-	-	-	-	-	-	15	12-13	11 ± 1.0 (11-13)	11 ± 1.2 (11-12)
Lip region width	11-12	-	11 (10-12)	10 (9-11)	11 ± 0.6 (10-12)	10 (10-11)	5	4-5	4 ± 0.8 (3-5)	5 ± 0.2 (4-5)
Basal ring: annuli	5-6	-	-	-	-	-	4	4	4 ± 1.0 (3-5)	4 ± 0.7 (3-4)
Outer margins of basal ring: length	-	-	-	-	-	-	3	3-4	3 ± 0.4 (3-3)	3 ± 0.7 (2-3)
Stylet length	22-24	22-23	23 (21-26)	22 (21-22)	24 ± 0.7 (22-24)	22 (22-23)	23	17-23	23 (n = 1)	23 (n = 1)
Stylet conus length	10-11	-	-	-	-	-	10	9-10	11 ± 0.6 (10-11)	9 (n = 1)
Stylet base length	-	-	-	-	-	-	14	7-14	12 (n = 1)	13 (n = 1)
Stylet knobs height	-	-	-	-	-	-	-	-	3 (n = 1)	7 (n = 1)
Stylet knobs width	6-7		-	-	-	-	-	-	8 (n = 1)	3 (n = 1)
Stylet length/ lip region width	-	-	-	-	-	-	-	-	2 (n = 1)	2 (n = 1)
Dorsal gland opening posterior to stylet (DGO)	2-3	-	-	-	-	-	-	-	-	2 (n = 1)
Anterior end to secretory-excretory pore	-	-	-	-	153 ± 9.9 (141-174)	143 (139-145)	-	-	147 ± 31.3 (124-169)	143 (n = 1)
Anterior end to nerve ring	-	-	-	-	-	-	-	-	-	-
Anterior end to mid metacorpus	138-170	-	-	-	-	-	-	-	96 ± 3.8 (93-100)	99 (n = 1)

Table 4.2 continued: Morphometric data of *Histotylenchus histoides* Siddiqi, 1971 paratype material and current study populations (CABI & FERA). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	**Malawi (Siddiqi, 1971)**		**South Africa and Namibia (Kleynhans, 1975)**		**South Africa and Namibia (Kleynhans & Heyns, 1984)**		**Malawi Paratype CABI**		**Malawi Paratype FERA**	
n	7♀♀	14♂♂	13♀♀	5♂♂	13♀♀	7♂♂	1♀♀	2♂♂	3♀♀	3♂♂
Metacorpus length	22	-	-	-	-	-	26	21-22	21 ± 2.6 (19-24)	20 ± 1.0 (19-21)
Metacorpus width	16	-	15 (13-19)	12 (11-13)	-	-	22	19	15 ± 1.5 (14-17)	18 ± 1.7 (17-19)
Metacorpus length/ metacorpus width	-	-	-	-	-	-	1	1	1-2	1
Metacorpus valve length	7	-	-	-	-	-	6	6-7	6 ± 1.0 (6-8)	7 (n = 1)
Metacorpus valve width	5	-	-	-	-	-	5	3-6	5 ± 0.5 (4-5)	5 (n = 1)
Position of hemizonid	Opposite to 2 annuli anterior to secretory-excretory pore	-	-	-	-	-	3 annuli anterior to secretory-excretory pore	3 annuli anterior to secretory-excretory pore	3 annuli anterior to secretory-excretory pore	3 annuli anterior to secretory-excretory pore
Vagina length	-	-	-	-	-	-	-	-	15 ± 2.8 (13-19)	-
Vagina length/corresponding body width	-	-	-	-	-	-	-	-	0.7	-
Testis length	-	-	-	-	-	-	-	-	-	368 (n = 1)
Spicule length	-	33-34	-	33 (31-34)	-	31 (29-34)	-	34-37	-	31 ± 0.7 (31-32)
Gubernaculum length	-	14-16	-	14 (12-15)	-	14 (13-15)	-	13-18	-	14 (n = 1)

Table 4.2 continued: Morphometric data of *Histotylenchus histoides* Siddiqi, 1971 paratype material and current study populations (CABI & FERA). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	Malawi (Siddiqi, 1971)		South Africa and Namibia (Kleynhans, 1975)		South Africa and Namibia (Kleynhans & Heyns, 1984)		Malawi Paratype CABI		Malawi Paratype FERA	
n	7♀♀	14♂♂	13♀♀	5♂♂	13♀♀	7♂♂	1♀♀	2♂♂	3♀♀	3♂♂
Width of annuli at: secretory-excretory pore	-	-	-	-	-	-	-	2 (n = 1)	2 (n = 1)	2 (n = 1)
Width of annuli at: midbody or vulva	1	-	-	-	-	-	1	1 (n = 1)	1	1 (n = 1)
Body width at secretory-excretory pore	-	-	-	-	-	-	-	36 (n = 1)	28 ± 5.3 (24-32)	24 (n = 1)
Body width at midbody/vulva	-	-	-	-	-	-	43	38-41	34 ± 6.6 (27-41)	35 ± 9.2 (28-41)
Body width at anus or cloacal opening	-	-	-	-	-	-	34	25-29	27 ± 4.9 (23-32)	22 ± 0.0 (22-22)
Lateral field width	-	-	-	-	-	-	-	9 (n = 1)	7 ± 0.7 (7-8)	8 (n = 1)
Phasmids to anus or cloacal length	10-20	-	-	-	-	-	-	23-25	14 ± 1.0 (13-14)	22 ± 2.2 (21-24)
Phasmids diameter	-	-	-	-	-	-	-	1	1 (n = 1)	1 (n = 1)
Tail length	37-43	-	-	-	35 ± 5.6 (27-43)	40 (39-42)	42	46-55	40 ± 2.3 (38-42)	47 ± 4.8 (43-50)
Number of ventral annuli	24-29	-	24-38	-	24-45	-	-	-	32-35	-
Tail projection length	-	-	-	-	-	-	-	15-17	-	10 ± 0.5 (10-10)
Hyaline length	-	-	-	-	-	-	5	-	9 ± 0.5 (8-9)	-
Postanal diverticulum length	-	-	-	-	-	-	-	-	10 annuli posterior anus	-
a	38-47	37-44	36 (29-45)	39 (35-41)	37 (29-42)	39 (35-41)	22	24-25	28 ± 7.0 (21-35)	27 ± 8.2 (21-33)
b	7-8	7-8	8 (7-9)	8 (7-8)	-	-	-	-	-	-
b'	5-6	5-6	6 (5-6)	6	6 (5-7)	6 (6-6)	-	-	-	-

Table 4.2 continued: Morphometric data of *Histotylenchus histoides* Siddiqi, 1971 paratype material and current study populations (CABI & FERA). All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

Characters	**Malawi (Siddiqi, 1971)**		**South Africa and Namibia (Kleynhans, 1975)**		**South Africa and Namibia (Kleynhans & Heyns, 1984)**		**Malawi Paratype CABI**		**Malawi Paratype FERA**	
n	7♀♀	14♂♂	13♀♀	5♂♂	13♀♀	7♂♂	1♀♀	2♂♂	3♀♀	3♂♂
c	25-31	23-28	28 (23-32)	22 (19-25)	33 (27-40)	26 (25-28)	22	17-21	23 ± 2.4 (21-26)	19 ± 1.3 (18-20)
c'	-	-	-	-	-	-	1	2	2 ± 0.2 (1-1)	2 ± 0.2 (2-2)
m (%)	-	-	-	-	-	-	-	-	49 (n = 1)	49 (n = 1)
Anterior end to secreoty-excretory pore/L (%)	-	-	-	-	-	-	-	-	15 ± 3.2 (13-18)	-
V (%)	52-58	-	53 (50-57)	-	54 (51-57)	-	55	-	53 ± 1.7 (51-54)	-
T (%)	-	40-51	-	-	-	-	-	-	-	40 (n = 1)

Histotylenchus mohalei Kleynhans, 1992

(Figs 4.7; 4.8; 4.9, 4.10 & 4.11)

The first records of *Histotylenchus mohalei* were from natural veld in the Golden Gate Highlands National Park, South Africa and in the Magaliesberg, South Africa (Kleyhans, 1992), but *H. mohalei* is now known from various other localities including Pretoria, South Africa (SAPPNS).

For this study *Histotylenchus mohalei* was described from material collected at various localities in the Free State, Gauteng, Limpopo and Mpumalanga Provinces, South Africa.

Measurements: Table 4.3 (paratype material) and table 4.4 (current study populations).

Description

Female (n = 63): Habitus straight (4 % of individuals), curved (61 % of individuals) to slightly curved (35 % of individuals), body cylindrical throughout except for anterior end and tail. Lateral field with four incisures, originate as two lines just posterior to stylet knobs; third line appears after short distance and dividing into two lines at level of metacorpus, outer two bands areolated throughout entire body, with some of areolations in region of phasmids being irregular; inner band incompletely areolated throughout entire body, with areolations irregular, but distinct, opposite secretory-excretory pore and more regular posterior to phasmids, outer and inner lines end in Y-shaped pattern (13 % of individuals) or in V-shaped pattern (87 % of individuals) anterior to hyaline region of tail. Guide piece situated three to four annuli anterior to junction between stylet cone and stylet base. Lip region continuous with body (76 % of individuals) or marked off by slight depression (24 % of individuals), 9 ± 1.1 (9-11) µm wide, more than twice lip region height of 4 ± 0.4 (4-5) µm; lip region with six to nine annuli; cephalic framework and basal ring usually strongly sclerotised, vestibule wall strongly sclerotised. Stylet stout, stylet cone asymmetrical; stylet knobs strong to moderately sloping backwards, anterior faces, slightly flattened. Procorpus wider than isthmus. Metacorpus large, nearly rounded; metacorpus valve spindle-shaped;

hemizonid four annuli anterior to secretory-excretory pore, two to three annuli long; deirid not observed. Secretory-excretory pore situated opposite to anterior part of postcorpus, i.e. at 13-17 % of body length. Postcorpus with ventral overlap in region of secretory-excretory pore; dorsal oesophageal gland distinct posterior to secretory-excretory pore; oesophago-intestinal valve indistinct. Intestine with large fasciculi starting from overlap of intestine. Postanal diverticulum of intestine present and overlaps anus, 4-16 annuli posterior anus but does not reach level of phasmids. Vulva in shallow to sunken boat-shaped depression at 49-65 % of body length with double epiptygma, vaginal musculature visible, but not prominent; reproductive system amphidelphic with two outstretched ovaries, anterior genital branch slightly shorter than posterior genital branch. Spermatheca not observed. Phasmids small, distinct, situate 8-26 annuli posterior to anus at about mid tail. Specimens with 46 ± 4.8 (32-56 µm) long, symmetrical rounded tail.

Male (n = 62): Similar to female except in following: Habitus straight (5 % of individuals), slightly curved (25 % of individuals) to curved (70 % of individuals). Dorsal lateral line extends past phasmids terminating near tail end, subdorsal and subventral lines extend to opposite cloacal opening or just beyond, ventral line terminating at beginning of bursa. Oesophageal gland situated posterior to secretory-excretory pore. Fasciculi not observed. Spicule arcuate with prominent velum, gubernaculum protrusible, with pronounced swollen titillae, proximal end curved backwards. Phasmids small, located 12-18 µm posterior to cloacal opening. Tail slender with fingerlike ventral projection with rounded end.

Diagnosis

Histotylenchus mohalei is characterised by the following combination of characters: Lip region continuous with body or marked off by slight depression, stylet stout (18-24 µm), stylet cone asymmetrical; stylet knobs strong to moderately sloping backwards, anterior faces, slightly flattened; lateral field outer two bands areolated throughout entire body, with some of areolations in region of phasmids being irregular; inner band incompletely areolated throughout entire body, with areolations irregular, but distinct, opposite secretory- excretory pore and more regular posterior to phasmids, outer and inner lines end in Y-shaped pattern or in V-shaped pattern anterior to hyaline region of tail; postanal diverticulum overlaps rectum but does not

reach the level of phasmids, tail with 25-53 ventral annuli in females. Stylet length has the lowest coefficient of variation (CV) value (4 %), followed by the position of the vulva (5 %). Gubernaculum protrusible with proximal end curved backwards in males.

Discussion

The current study material agrees with the type description and type material of Kleynhans (1992) but differs in that the upper range of the V-value is higher than that given by Kleynhans (1992). Compared with the type material in Kleynhans (1992) differ from *Histotylenchus mohalei* current study populations (NCN) of in position of vulva (42-60 % *vs* 49-58 %) and gubernaculum (protrusible with proximal end curved backwards *vs* proximal end unmodified or knobbed or directed towards rear), gubermaculum length (11-18 µm *vs* 9-15 µm).

Locality and material examined

See Appendix III for full locality data and all material examined.

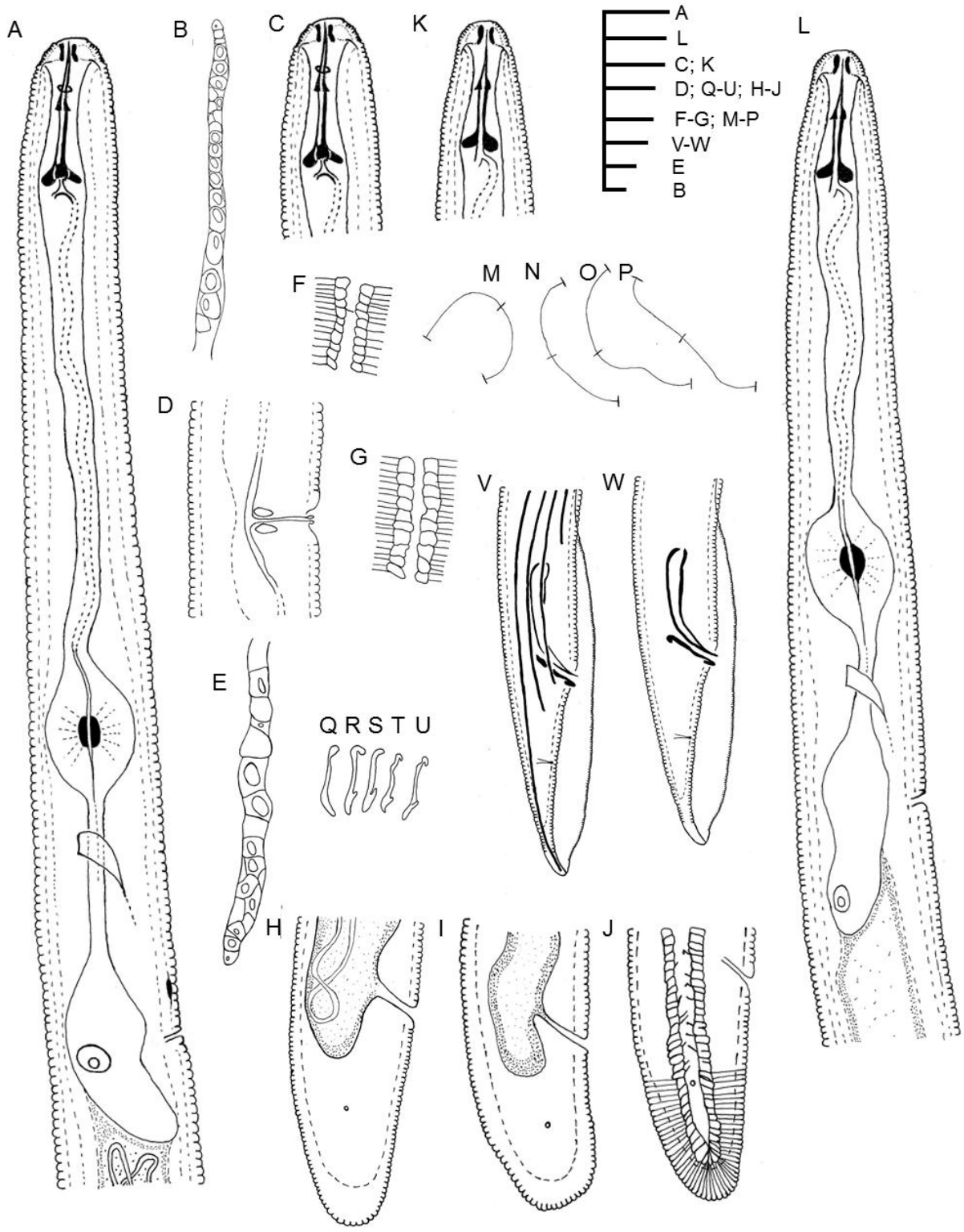


Figure 4.7: Line diagrams of *Histotylenchus mohalei* Kleynhans, 1992. Female: **A**- anterior part; **B**- part of anterior ovarium; **C**- lip region; **D**- vulva; **E**- part of posterior ovarium; **F**- lateral field in secretory-excretory pore region; **G**- lateral field in vulva region; **H-I**- tail with postanal diverticulum; **J**- tail indicating lateral field ending in V-shape; **M-P**- habitus. Male: **K**- lip region; **L**- anterior part; **Q-U**- gubernaculums; **V**- tail and **W**- tail indicating lateral field. **Scale bars** = 10 µm.

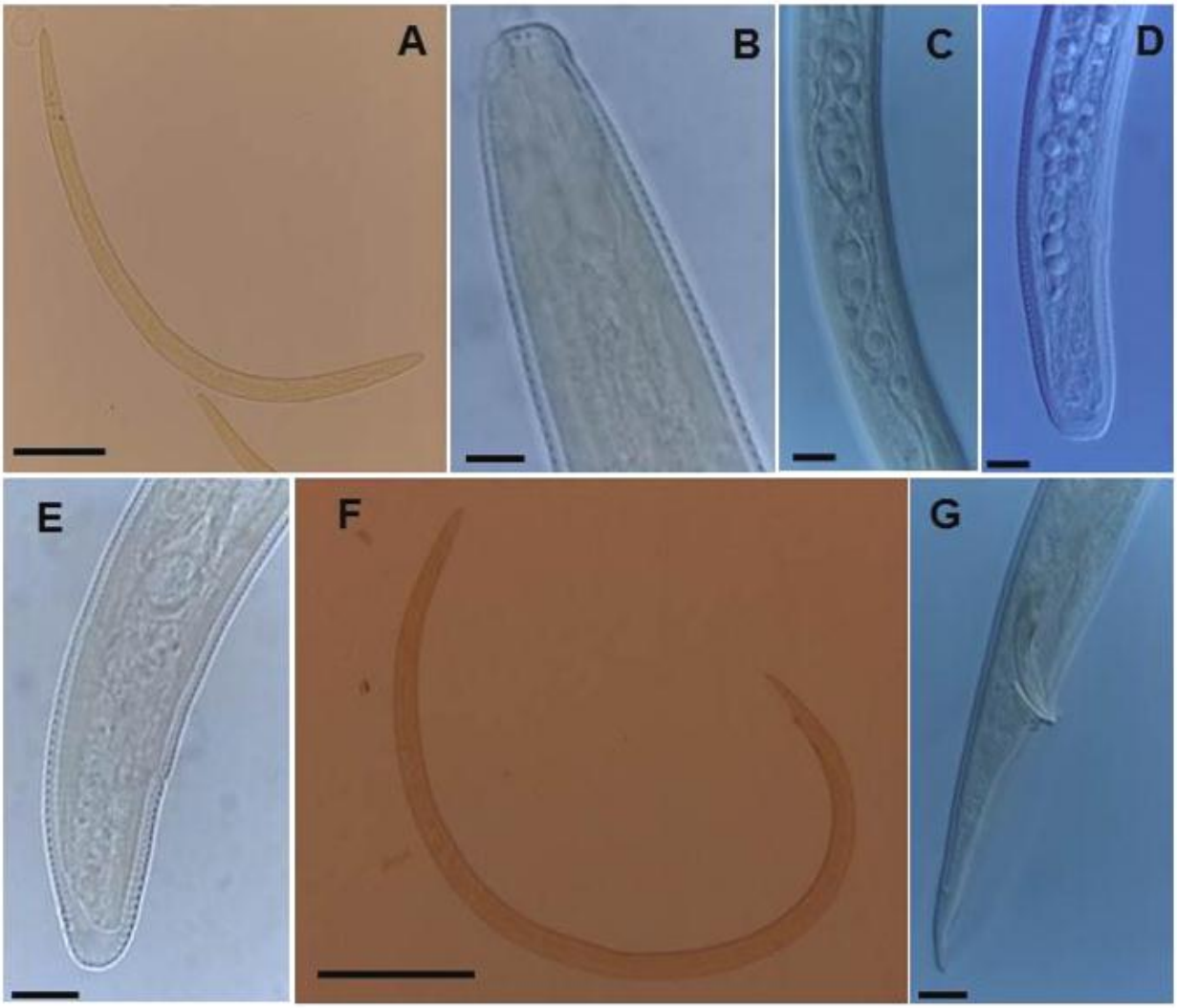


Figure 4.8: Light micrographs of *Histotylenchus mohalei* Kleynhans, 1992 paratype material. Female: **A**- habitus; **B**- lip region; **C**- fasciculi midbody; **D-E**- tail region. Male: **F**- habitus and **G**- tail region. **Scale bars** = 100 µm (A; F) and 10 µm (B-E; G).

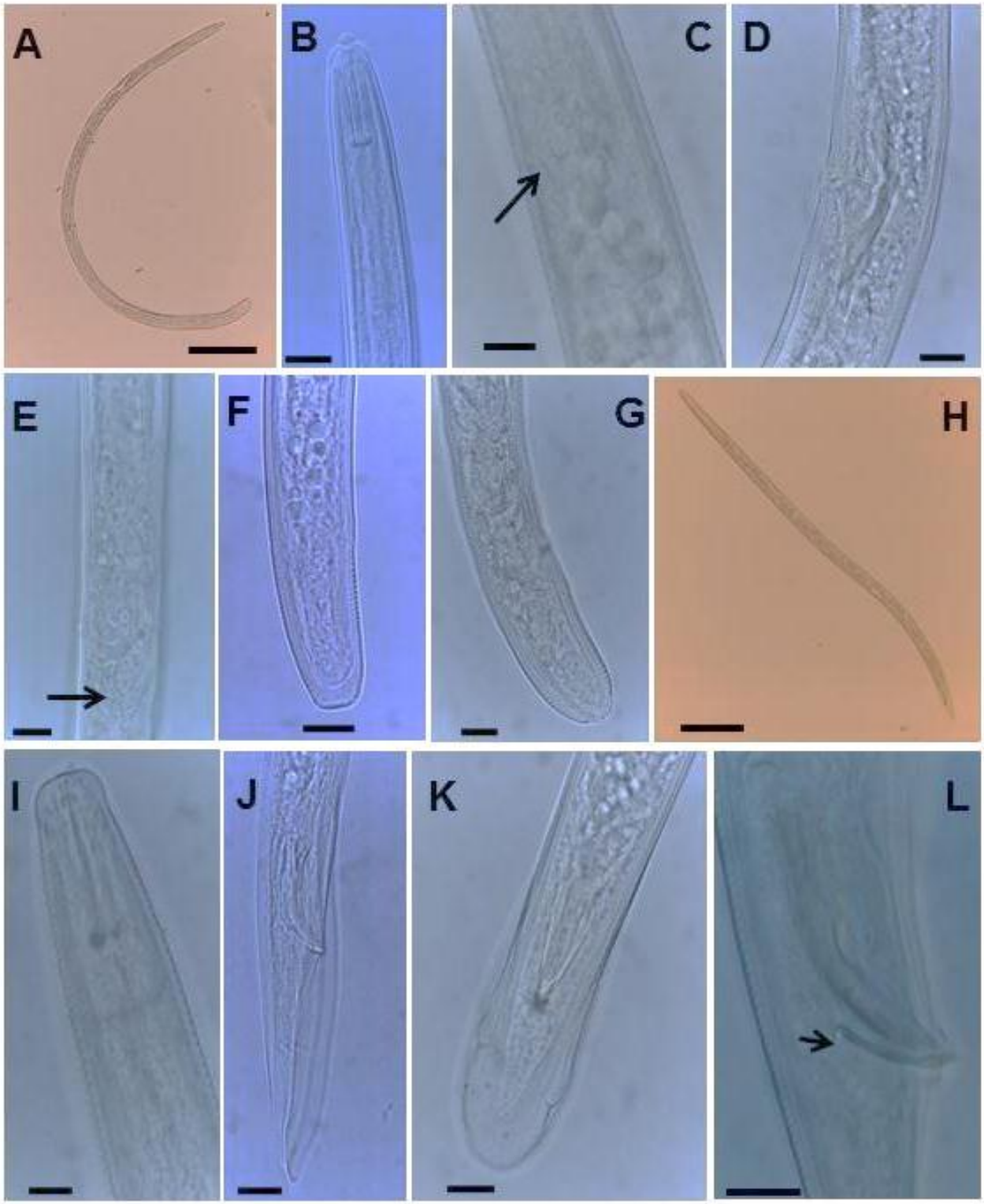


Figure 4.9: Light micrographs of current study populations (NCN) of *Histotylenchus mohalei* Kleynhans, 1992. Female: **A**- habitus; **B**- lip region; **C**- secretory-excretory pore (black arrow); **D**- vulva region; **E**- body anterior to vulva showing postanal diverticulum (black arrow); **F-G**- tail region; Male: **H**- habitus; **I**- lip region; **J**- tail region; **K**- dorsal view of spicule and **L**- spicule and gubernaculum with proximal end directed towards rear (black arrow). **Scale bars** = 100 µm (A; H) and 10 µm (B-G; I-L).

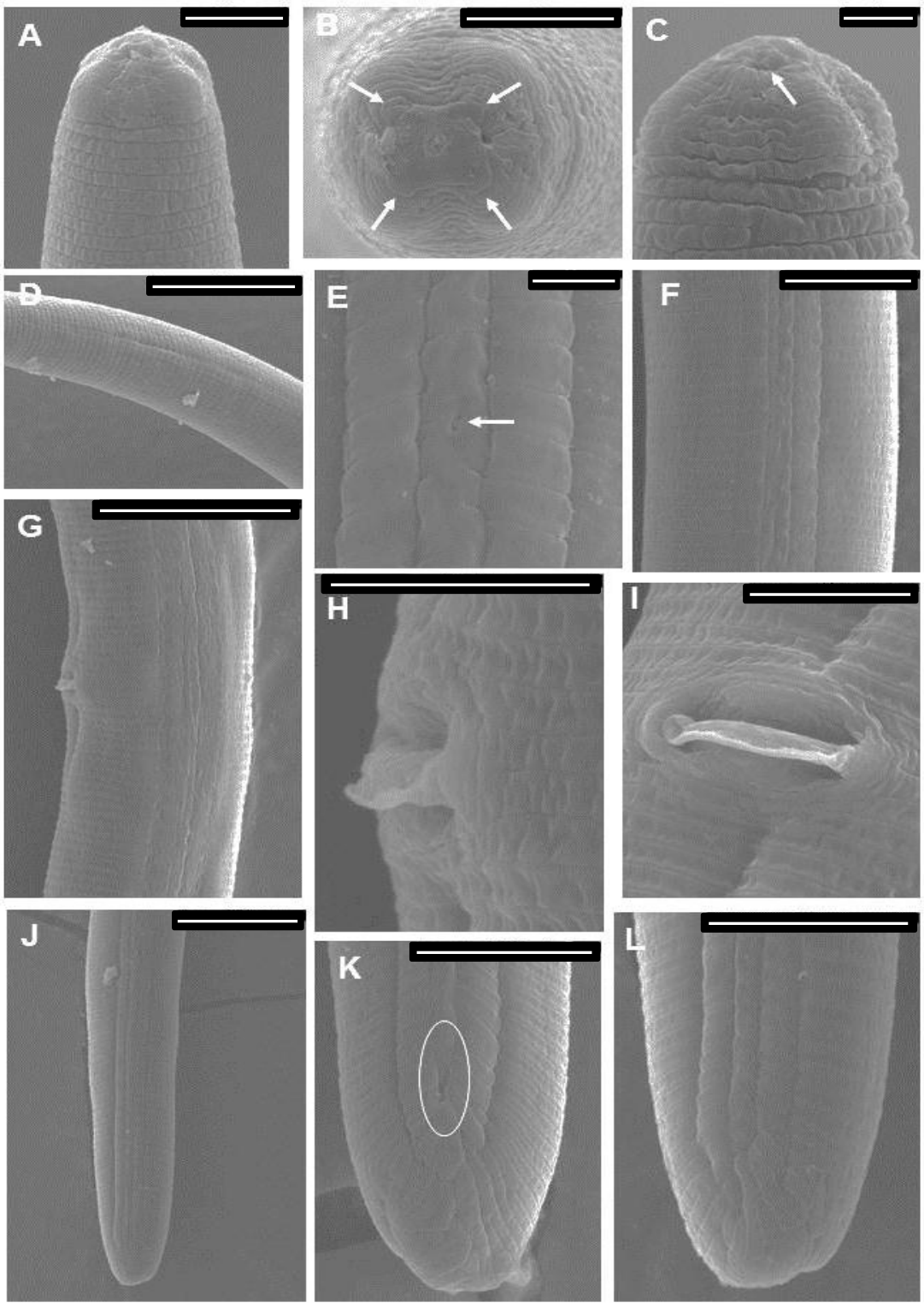


Figure 4.10: Scanning electron microscope (SEM) micrographs of *Histotylenchus mohalei* Kleynhans, 1992. Female: **A**- lip region; **B**- *en face* view with four lips (white arrows); **C**- lip region with amphidal apertures (white arrow); **D**- anterior part with start of lateral lines; **E**- lateral field with secretory-excretory pore opening (white arrow); **F**- four lateral lines with incomplete areolation of outer two bands and incomplete, irregular areolation on inner band; **G**- lateral view with irregularity in lateral lines in vulva region; **H**- double protruding epiptygma; **I**- ventral view of vulva; **J**- tail region; **K**- lateral field, inner lines ending in V-shaped pattern (white circle) and **L**- lateral field with lateral lines and areolation in tail region. **Scale bars** = 20 µm (D; F; G; J); 10 µm (K-L); 5 µm (A-B; H-I) and 2 µm (C; E).

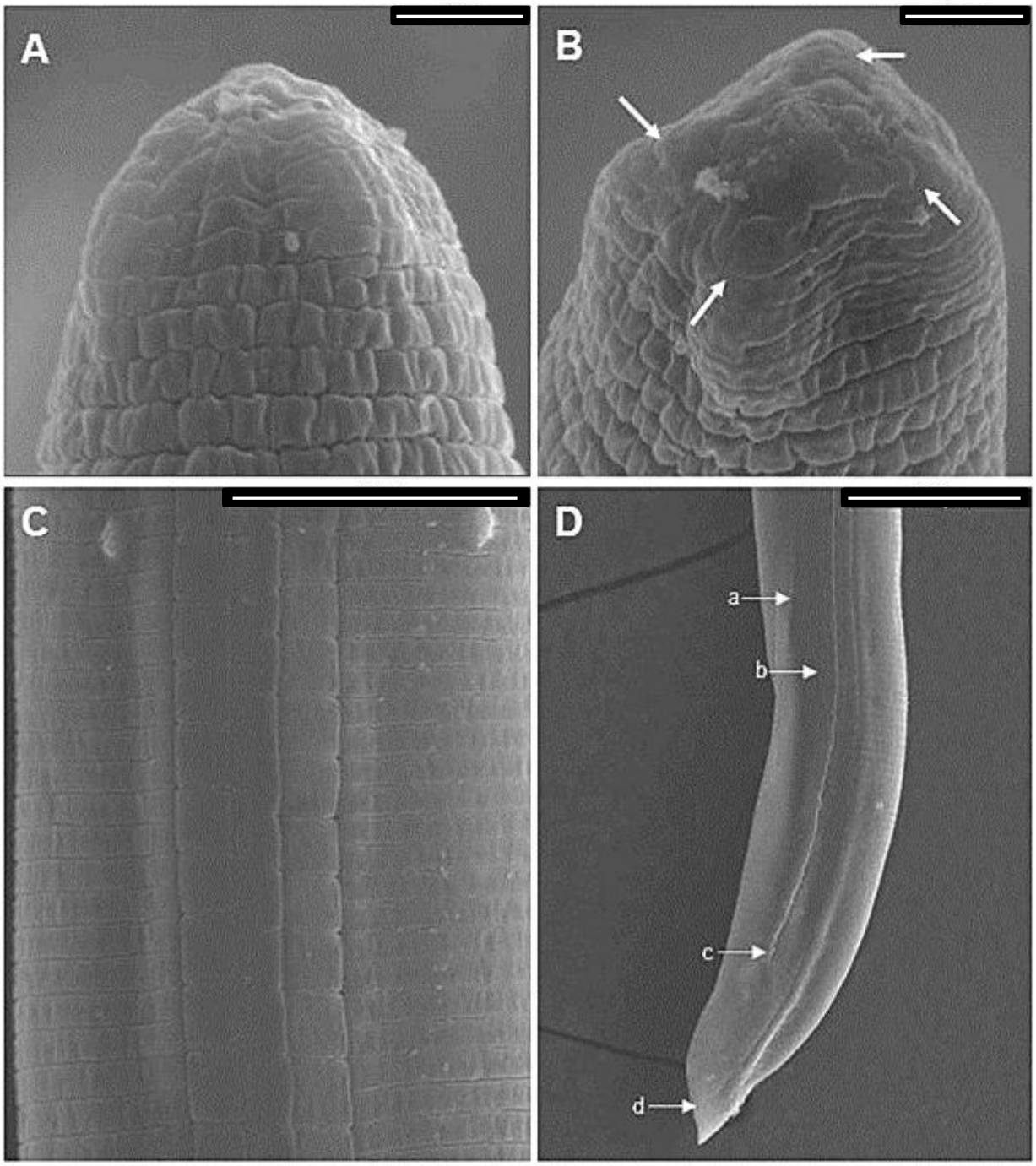


Figure 4.11: Scanning electron micrographs of male *Histotylenchus mohalei* Kleynhans, 1992: **A**- lip region; **B**- *en face* view showing four lips (white arrows); **C**- lateral field in midbody showing four lateral lines with complete areolation of outer two bands and incomplete, irregular areolations of inner band and **D**- tail region showing bursa and ending of different lateral lines. Ventral (white arrow a) and sub ventral (white arrow b) lateral lines end at bursa beginning, sub dorsal lateral line (white arrow c) ends in cloacal opening region and dorsal line (white arrow d) ends on tail tip. **Scale bars** = 20 µm (D); 10 µm (C) and 2 µm (A;B).

Table 4.3: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 paratype material. All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	**Pretoria (Kleynhans, 1992)**		**Golden Gate (Kleynhans, 1992)**		**OVS252: Golden Gate type material**		**TVL921: Pretoria type material**	
n	41♀♀	41♂♂	46♀♀	32♂♂	26♀♀	16♂♂	23♀♀	24♂♂
L	1 025 ± 67.2 (891-1 159)	978 ± 66.8 (844-1 112)	1 070 ± 57.0 (956-1 184)	985 ± 56.0 (873-1 097)	898 ± 46.3 (807-979)	820 ± 45.6 (765-892)	862 ± 79.4 (633-999)	808 ± 61.0 (723-946)
Number of lip annuli	8	8	8	8	8 ± 0.1 (8-9)	9	8 ± 0.3 (7-8)	8 ± 0.6 (7-9)
Lip region height	-	-	-	-	4 ± 0.4 (3-5)	4 ± 0.4 (3-4)	4 ± 0.4 (3-4)	4 ± 0.5 (3-5)
Lip region width	9 ± 0.43 (9-11)	9 ± 0.69 (8-11)	10 ± 0.36 (9-11)	9 ± 0.64 (8-11)	10 ± 0.9 (8-12)	9 ± 0.7 (8-10)	10 ± 0.9 (9-12)	9 ± 0.7 (7-10)
Basal ring: annuli	-	-	-	-	4 ± 0.7 (3-5)	4 ± 0.6 (3-5)	4 ± 0.4 (3-5)	4 ± 0.5 (3-4)
Outer margins of basal ring: length	-	-	-	-	3 ± 0.5 (2-4)	3 ± 0.4 (2-4)	3 ± 0.5 (2-4)	3 ± 0.6 (2-4)
Stylet length	20 ± 1.00 (18-22)	20 ± 1.2 (17-22)	21 ± 0.82 (19-22)	20 ± 0.80 (19-22)	20 ± 1.3 (17-22)	20 ± 0.9 (19-21)	20 ± 1.2 (18-23)	19 ± 2.2 (12-20)
Conus length	9 ± 0.69 (8-10)	9 ± 0.69 (7-10)	9 ± 0.47 (8-10)	9 ± 0.54 (8-10)	9 ± 0.7 (7-10)	9 ± 0.7 (8-10)	9 ± 0.6 (8-11)	9 ± 0.6 (8-10)
Stylet base length	-	-	-	-	11 ± 0.7 (10-12)	11 ± 0.4 (10-11)	11 ± 1.1 (9-13)	10 ± 1.9 (4-11)
Stylet knobs height	-	-	-	-	3 ± 0.3 (2-3)	2 ± 0.4 (2-3)	2 ± 0.4 (2-3)	3 ± 0.4 (2-3)
Stylet knobs width	-	-	-	-	5 ± 0.8 (4-6)	5 ± 0.6 (3-6)	5 ± 0.4 (4-6)	4 ± 0.5 (3-5)
Stylet length/ lip region width	-	-	-	-	2 ± 0.2 (2-2)	2 ± 0.2 (2-3)	2 ± 0.2 (2-2)	2 ± 0.3 (1-3)
Dorsal gland opening posterior to stylet (DGO)	2 ± 0.49 (1-3)	2 ± 0.36 (2-3)	3 ± 0.40 (2-3)	3 ± 0.25 (2-3)	2 ± 0.3 (2-3)	2 ± 0.4 (1-3)	2 ± 0.2 (2-2)	2 ± 0.3 (2-2)
Anterior end to secretory-excretory pore	-	-	-	-	133 ± 13.0 (112-159)	125 ± 6.3 (116-139)	131 ± 12.3 (98-149)	129 ± 9.1 (118-143)
Anterior end to mid metacorpus	-	-	-	-	88 ± 5.3 (80-98)	85 ± 4.4 (78-92)	89 ± 5.5 (76-99)	84 ± 4.3 (77-93)

Table 4.3 continued: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 paratype material. All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	**Pretoria (Kleynhans, 1992)**		**Golden Gate (Kleynhans, 1992)**		**OVS252: Golden Gate type material**		**Tvl921: Pretoria type material**	
n	41♀♀	41♂♂	46♀♀	32♂♂	26♀♀	16♂♂	23♀♀	24♂♂
Metacorpus length	-	-	-	-	19 ± 3.5 (7-23)	18 ± 1.1 (16-20)	20 ± 2.1 (16-25)	17 ± 1.8 (14-21)
Metacorpus width	-	-	-	-	17 ± 3.0 (11-20)	12 ± 1.7 (9-16)	16 ± 3.5 (9-22)	13 ± 3.2 (10-23)
Metacorpus length/ metacorpus width	-	-	-	-	1-2	2	1 -3	1-2
Metacorpus valve length		-	-	-	6 ± 0.6 (5-7)	5 ± 0.5 (4-6)	6 ± 0.8 (4-8)	5 ± 0.6 (3-6)
Metacorpus valve width	4 ± 0.39 (3-5)	4 ± 0.35 (3-5)	4 ± 0.34 (3-5)	4 ± 0.38 (3-4)	4 ± 0.5 (3-6)	4 ± 0.6 (3-5)	4 ± 0.7 (3-6)	4 ± 0.6 (3-5)
Anterior genital branch length	-	-	-	-	241 ± 94.8 (173-308)	-	233 ± 33.5 (178-272)	-
Posterior genital branch length	-	-	-	-	243 ± 59.3 (176-288)	-	240 ± 22.7 (220-271)	-
Vagina length	-	-	-	-	17 ± 1.9 (13-21)	-	14 ± 2.6 (11-20)	-
Vagina length/corresponding body width	-	-	-	-	0.7	-	0.7	-
Spermatheca length	-	-	-	-	-	-	16 ± 4.9 (9-21)	-
Spermatheca width	-	-	-	-	-	-	14 ± 3.5 (9-17)	-
Testis length	-	-	-	-	-	455 ± 42.7 (390-505)	-	435 ± 24.5 (405-474)
Spicule length	-	30 ± 1.7 (27-34)	-	-	-	30 ± 2.0 (26-33)	-	29 ± 2.2 (25-33)
Gubernaculum length	-	12 ± 1.1 (9-15)	-	13 ± 0.9 (11-15)	-	13 ± 0.9 (12-14)	-	13 ± 1.6 (11-18)
Width of annuli at: secretory-excretory pore	-	-	-	-	1 ± 0.2 (1-2)	1 ± 0.1 (1-1)	1 ± 0.2 (1-2)	1 ± 0.2 (1-2)
Width of annuli at: midbody/vulva	-	-	-	-	1 ± 0.2 (1-2)	1 ± 0.1 (1-1)	1 ± 0.2(1-2)	1 ± 0.2 (1-1)

Table 4.3 continued: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 paratype material. All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	Pretoria (Kleynhans, 1992)		Golden Gate (Kleynhans, 1992)		OVS252: Golden Gate type material		TVL921: Pretoria type material	
n	41♀♀	41♂♂	46♀♀	32♂♂	26♀♀	16♂♂	23♀♀	24♂♂
Body width at secretory-excretory pore	22 ± 1.2 (19-24)	20 ±1.2 (18-22)	22 ± 1.4 (19-24)	20 ± 1.1 (18-23)	29 ± 5.7 (20-37)	21 ± 2.1 (19-26)	27 ± 4.5 (22-37)	21 ± 2.5 (19-29)
Body width at midbody or vulva	24 ± 1.6 (21-27)	22 ± 1.4 (19-25)	26 ± 1.9 (23-30)	23 ± 1.40 (20-25)	34 ± 5.3 (25-41)	25 ± 4.7 (21-37)	31 ± 6.0 (22-41)	26 ± 5.0 (21-35)
Body width at anus or cloacal opening	20 ± 1.2 (18-23)	16 ± 1.3 (13-18)	20 ± 1.00 (18-22)	16 ± 0.68 (15-18)	25 ± 4.1 (19-31)	18 ± 1.4 (15-20)	23 ± 4.3 (18-31)	17 ± 2.8 (9-22)
Lateral field width	-	-	-	-	7 ± 0.6 (6-8)	6 ± 0.6 (6-7)	7 ± 0.6 (6-9)	7 ± 0.4 (6-7)
Position of phasmids posterior to anus	-	-	-	-	19-21 annuli	-	12-29 annuli	-
Phasmids to anus/ or cloacal opening length	21 ± 3.4 (14-28)	29 ± 3.4 (22-40)	21 ± 3.40 (14-28)	27 ± 3.80 (21-35)	22 ± 4.6 (16-31)	27 ± 4.2 (20-34)	22 ± 4.7 (17-31)	28 ± 2.9 (22-33)
Phasmids diameter	-	-	-	-	1 ± 0.3 (1-2)	1	1 ± 0.2 (1-1)	1 ± 0.3 (1-1)
Tail length	41 ± 4.3 (33-50)	51 ± 3.9 (43-57)	49 ± 4.10 (41-57)	55 ± 4.30 (46-64)	51 ± 3.7 (46-57)	55 ± 6.2 (41-62)	43 ± 7.4 (32-63)	50 ± 4.8 (43-57)
Number of ventral annuli	25-46	-	25-46	-	42-52	-	30-51	-
Tail projection length	-	-	-	-	-	10 ± 1.4 (7-13)	-	10 ± 2.0 (6-13)
Hyaline length	-	-	-	-	8 ± 1.2 (6-10)	-	7 ± 0.7 (6-8)	-
Postanal diverticulum length	-	-	-	-	4-8 annuli posterior anus	-	2-16 annuli posterior anus	-
a	43 ± 2.3 (38-47)	44 ± 3.00 (40-53)	41 ± 3.2 (34-47)	43 ± 3.00 (38-49)	27 ± 4.6 (23-37)	34 ± 4.0 (24-39)	29 ± 5.4 (21-40)	32 ± 5.0 (24-39)
b	8 ± 0.48 (7-9)	8 ± 0.51 (7-9)	8 ± 0.38 (7-9)	7 ± 0.49 (7-9)	-	-	-	-
b'	6 ± 0.54 (5-7)	6 ± 0.35 (5-6)	6 ± 0.34 (5-7)	6 ± 0.22 (5-6)	-	-	-	-
c	25 ± 2.7 (20-31)	19 ± 1.7 (16-23)	22 ± 2.00 (18-26)	18 ± 1.30 (15-21)	18 ± 1.0 (15-20)	15 ± 1.5 (13-19)	21 ± 3.0 (13-27)	16 ± 1.7 (14-19)
c'	2 ± 0.23 (2-3)	3 ± 0.41 (2-4)	3 ± 0.23 (2-3)	3 ± 0.28 (3-4)	2 ± 0.4 (2-3)	3 ± 0.4 (2-4)	2 ± 0.3 (1-3)	3 ± 0.7 (2-5)

Table 4.3 continued: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 paratype material. All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	Pretoria (Kleynhans, 1992)		Golden Gate (Kleynhans, 1992)		OVS252: Golden Gate type material		TVL921: Pretoria type material	
n	41♀♀	41♂♂	46♀♀	32♂♂	26♀♀	16♂♂	23♀♀	24♂♂
m (%)	45 ± 2.0 (41-49)	45 ± 2.30 (36-50)	44 ± 1.50 (41-47)	44 ± 2.20 (40-49)	46 ± 1.7 (44-49)	46 ± 2.0 (43-49)	44 ± 2.5 (41-49)	48 ± 6.6 (43-69)
Anterior end to secretory-excretory pore/L (%)	-	-	-	-	15 ± 1.2 (13-18)	15 ± 0.6 (14-16)	15 ± 1.1 (13-17)	16 ± 0.5 (15-17)
Anterior end to secretory-excretory pore/Oesophagus length (%)	-	-	-	-	73 ± 3.7 (68-78)	74 ± 5.5 (68-82)	82 ± 10.8 (62-96)	83 ± 5.7 (74-89)
V (%)	54 ± 1.8 (50-58)	-	52 ± 1.2 (49-54)	-	53 ± 2.3 (49-60)	-	53 ± 4.1 (42-57)	-
OV_1 (%)	-	-	-	-	-	-	26 ± 3.8 (20-31)	-
OV_2 (%)	-	-	-	-	27 ± 8.2 (21-33)	-	26 ± 3.8 (24-32)	-
Posterior genital branch length/Anterior branch length (%)	-	-	-	-	97 ± 5.5 (94-101)	-	93.5 ± 5.0 (90-97)	-
T (%)	-	-	-	-	-	55 ± 6.3 (45-63)	-	55 ± 2.5 (50-58)

Table 4.4: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 current study populations (NCN). All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	**TVL929: Pretoria**		**TVL941: Pretoria**		**TVL1960: Irene**	**TVL2005: Suikerbosrand Nature Reserve**		**TVL2026: Komatipoort**	
n	11♀♀	19♂♂	3♂♂	35♀♀	17♂♂	11♀♀	13♂♂	6♀♀	8♂♂
L	892 ± 52.3 (810-958)	838 ± 40.7 (765-922)	791 ± 30.9 (765-825)	926 ± 45.2 (822-1 027)	849 ± 49.5 (753-928)	966 ± 57.8 (874-1 078)	886 ± 36.1 (813-940)	939 ± 40.4 (861-970)	885 ± 80.9 (789-1012)
Number of lip annuli	7 ± 0.7 (6-8)	7 ± 0.6 (6-8)	8 ± 0.7 (7-8)	8 ± 0.6 (7-8)	7 ± 0.5 (7-8)	8 ± 0.5 (8-9)	8 ± 0.4 (8-9)	7 ± 0.4 (7-8)	8 ± 0.4 (7-8)
Lip region height	4 ± 0.4 (3-5)	4 ± 0.6 (2-4)	3 ± 0.1 (3-4)	4 ± 0.3 (3-5)	4 ± 0.3 (3-4)	4 ± 0.4 (4-5)	4 ± 0.3 (4-5)	5 ± 0.3 (4-5)	4 ± 0.2 (4-5)
Lip region width	9 ± 0.6 (8-10)	8 ± 0.4 (8-9)	8 ± 0.5 (8-9)	9 ± 1.1 (5-10)	8 ± 0.5 (8-10)	10 ± 0.5 (10-11)	9 ± 0.6 (8-10)	11 ± 0.3 (10-11)	11 ± 0.5 (10-11)
Basal ring: annuli	4 ± 0.4 (3-5)	4 ± 0.5 (3-4)	4	4 ± 0.6 (3-5)	4 ± 0.6 (3-5)	4 ± 0.2 (4-5)	4 ± 0.4 (4-5)	4 ± 0.6 (3-4)	3 ± 0.4 (3-4)
Outer margins of basal ring: length	3 ± 1.3 (2-6)	3 ± 0.4 (2-4)	4 ± 0.4 (4-4)	3 ± 0.4 (2-4)	3 ± 0.3 (2-3)	3 ± 0.4 (3-4)	3 ± 0.5 (3-5)	3 ± 0.6 (2-3)	3 ± 0.4 (2-3)
Stylet length	20 ± 0.7 (19-21)	20 ± 1.7 (18-24)	19 ± 0.8 (18-20)	21 ± 1.0 (18-24)	20 ± 1.4 (19-25)	23 ± 0.5 (22-23)	22 ± 0.5 (21-23)	23 ± 0.9 (22-24)	22 ± 0.7 (21-23)
Conus length	10 ± 1.7 (9-15)	10 ± 1.0 (8-12)	9 ± 1.5 (8-11)	10 ±0.9 (7-12)	9 ± 0.8 (8-11)	11 ± 0.5 (10-11)	10 ± 0.5 (9-11)	10 ± 0.5 (9-11)	10 ± 0.8 (9-11)
Stylet base length	10 ± 0.7 (9-11)	11 ± 1.7 (8-14)	10 ± 1.2 (10-11)	11 ± 0.8 (10-13)	11 ± 1.1 (10-14)	12 ± 0.6 (11-13)	12 ± 0.6 (11-13)	12 ± 0.8 (11-14)	12 ± 0.7 (11-13)
Stylet knobs height	3 ± 0.4 (2-3)	3 ± 0.5 (2-4)	2	3 ± 0.4 (2-3)	3 ± 0.3 (2-3)	3 ± 0.9 (3-6)	3 ± 0.4 (2-3)	3 ± 0.3 (3-3)	3 ± 0.3 (3-3)
Stylet knobs width	5 ± 0.5 (4-6)	5 ± 0.8 (4-7)	5 ± 0.5 (5-5)	5 ± 0.3 (5-6)	5 ± 0.3 (4-5)	5 ± 0.8 (3-6)	5 ± 0.5 (5-6)	6 ± 0.4 (6-7)	6 ± 0.3 (6-6)
Stylet length/ lip region width	2 ± 0.2 (2-2)	2 ± 0.2 (2-3)	2	2 ± 0.3 (2-4)	2 ± 0.2 (2-3)	2 ± 0.1 (2-2)	2 ± 0.2 (2-3)	2 ± 0.1 (2-2)	2 ± 0.1 (2-2)
Dorsal gland opening posterior to stylet (DGO)	1 (n=1)	2	2 (n=1)	2 ± 0.4 (1-3)	2 ± 0.4 (1-3)	2 ± 0.2 (2-2)	2 ± 0.3 (2-3)	2 ± 0.2 (2-3)	2 ± 0.3 (2-2)
Anterior end to secretory-excretory pore	129 ± 7.6 (118-139)	128 ± 8.8 (109-140)	139 ± 14.7 (130-156)	141 ± 5.9 (128-154)	133 ± 4.4 (124-139)	145 ± 10.0 (134-158)	139 ± 5.8 (129-150)	143 ± 5.3 (139-147)	147 ± 11.6 (132-161)
Anterior end to mid metacorpus	86 ± 5.7 (75-94)	85 ± 5.2 (75-94)	83 ± 3.8 (80-87)	91 ± 4.6 (75-99)	88 ± 3.1 (83-96)	92 ± 5.9 (79-99)	87 ± 3.6 (82-94)	92 ± 10.3 (86-104)	89 ± 3.8 (86-94)

Table 4.4 continued: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 current study populations (NCN). All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	TVL929: Pretoria		TVL941: Pretoria	TVL1960: Irene		TVL2005: Suikerbosrand Nature Reserve		TVL2026: Komatipoort	
n	11♀♀	19♂♂	3♂♂	35♀♀	17♂♂	11♀♀	13♂♂	6♀♀	8♂♂
Metacorpus length	21 ± 1.4 (18-22)	18 ± 2.1 (15-22)	19 ± 0.9 (18-20)	18 ± 1.6 (16-22)	18 ± 1.6 (15-23)	18 ± 1.3 (17-21)	18 ± 1.0 (16-19)	19 ± 1.9 (17-22)	18 ± 1.1 (17-20)
Metacorpus width	14 ± 1.6 (12-17)	12 ± 1.6 (10-17)	12 ± 1.4 (11-13)	14 ± 1.4 (12-20)	13 ± 2.2 (11-20)	13 ± 1.1 (11-15)	12 ± 0.9 (10-13)	14 ± 1.1 (13-16)	13 ± 1.3 (12-16)
Metacorpus length/ metacorpus width	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
Metacorpus valve length	6 ± 0.6 (5-7)	5 ± 0.8 (4-7)	5 ± 1.1 (4-6)	6 ± 0.4 (4-7)	5 ± 0.6 (4-6)	6 ± 0.5 (6-7)	6 ± 0.6 (4-7)	6 ± 0.9 (4-6)	5 ± 0.4 (5-6)
Metacorpus valve width	4 ± 0.5 (3-5)	4 ± 0.5 (3-5)	4 ± 1.1 (3-5)	4 ± 0.4 (3-5)	4 ± 0.4 (3-5)	4 ± 0.4 (4-6)	4 ± 0.6 (3-6)	5 ± 0.7 (4-6)	4 ± 0.4 (4-5)
Position of hemizonid	4 annuli anterior to secretory-excretory pore	-	4 annuli anterior to secretory-excretory pore	4 annuli anterior to secretory-excretory pore	-	4 annuli anterior to secretory-excretory pore	-	3 annuli anterior to secretory-excretory pore	3 annuli anterior to secretory-excretory pore
Anterior genital branch length	165 (n=1)	-	-	172 ± 46.5 (123-215)	-	212 ± 20.2 (189-230)	-	-	-
Posterior genital branch length	183 ± 5.3 (179-187)	-	-	204 ± 45.1 (157-305)	-	213 ± 30.5 (186-253)	-	-	-
Vagina length	12 ± 1.1 (10-14)	-	-	14 ± 1.0 (12-17)	-	15 ± 1.7 (12-17)	-	15 ± 1.8 (13-18)	-
Vagina length/corresponding body width	-	-	-	0.7	-	0.7	-	0.7	-
Testis length	-	428 ± 74.2 (297-519)	458 ± 30.9 (439-493)	-	363 ± 74.9 (230-465)	-	458 ± 1.4 (254-520)	-	488 ± 34.9 (464-513)
Spicule length	-	29 ± 2.3 (22-32)	29 ± 2.6 (26-32)	-	30 ± 1.5 (27-33)	-	30 ± 1.4 (28-33)	-	31 ± 1.6 (28-32)
Gubernaculum length	-	13 ± 1.3 (12-16)	14 ± 2.6 (12-17)	-	14 ± 1.0 (12-16)	-	14 ± 1.0 (12-16)	-	15 ± 1.6 (14-17)
Width of annuli at: secretory-excretory pore	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
Width of annuli at: midbody or vulva	1	1	1	1-2	1	1-2	1	1	1

Table 4.4 continued: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 current study populations (NCN). All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	**TVL929: Pretoria**		**TVL941: Pretoria**	**TVL1960: Irene**		**TVL2005: Suikerbosrand Nature Reserve**		**TVL2026: Komatipoort**	
n	11♀♀	19♂♂	3♂♂	35♀♀	17♂♂	11♀♀	13♂♂	6♀♀	8♂♂
Body width at secretory-excretory pore	25 ± 3.2 (21-30)	22 ± 2.4 (20-28)	21 ± 0.5 (21-21)	23 ± 1.2 (21-25)	21 ± 1.3 (19-24)	27 ± 3.1 (23-30)	23 ± 1.1 (22-25)	24 ± 1.1 (23-26)	25 ± 1.6 (24-28)
Body width at midbody or vulva	28 ± 3.4 (24-33)	25 ± 3.4 (22-34)	24 ± 0.6 (23-24)	27 ± 2.1 (24-32)	24 ± 1.6 (21-28)	32 ± 2.7 (27-35)	26 ± 0.7 (25-27)	30 ± 2.2 (27-32)	28 ± 2.6 (25-32)
Body width at anus or cloacal opening	21 ± 2.4 (19-27)	17 ± 1.1 (15-19)	17 ± 0.9 (16-17)	21 ± 1.6 (17-24)	17 ± 1.9 (11-19)	25 ± 2.9 (21-30)	19 ± 1.2 (18-22)	24 ± 1.7 (22-27)	20 ± 0.8 (19-21)
Lateral field width	6 ± 1.1 (5-9)	7 ± 2.0 (5-10)	7 ± 0.2 (7-7)	8 ± 1.2 (5-11)	7 ± 1.1 (5-9)	8 ± 1.5 (5-10)	8 ± 0.9 (6-9)	9 ± 1.4 (8-10)	7 ± 0.3 (7-8)
Position of phasmids posterior to anus	17-21 annuli	-	-	14-26 annuli	-	8-18 annuli	-	10-19 annuli	-
Phasmids to anus or cloacal opening length	22 ± 4.0 (17-28)	28 ± 3.2 (22-33)	27 ± 2.5 (25-30)	19 ± 3.0 (14-26)	22 ± 4.1 (14-29)	17 ± 3.0 (11-20)	22 ± 3.4 (15-27)	18 ± 2.4 (14-20)	23 ± 3.5 (17-28)
Phasmids diameter	1	1	1	1	1	1	1	1	1
Tail length	42 ± 4.4 (36-49)	50 ± 5.5 (35-60)	49 ± 3.5 (45-52)	47 ± 4.1 (38-56)	49 ± 4.7 (40-58)	37 ± 3.5 (32-44)	48 ± 3.8 (43-54)	39 ± 3.2 (36-43)	46 ± 5.3 (36-50)
Number of ventral annuli	36-48	-	-	36-53	-	28-39	-	28-40	-
Tail projection length	-	8 ± 3.0 (4-14)	12 ± 0.7 (11-12)	-	11 ± 2.6 (6-17)	-	10 ± 1.0 (8-12)	-	8 ± 2.5 (5-10)
Hyaline length	7 ± 1.3 (4-9)	-	-	6 ± 1.2 (4-9)	-	5 ± 0.7 (4-6)	-	6 ± 0.9 (6-8)	-
Postanal diverticulum length		-	-	4-13 annuli posterior anus	-	7-15 annuli posterior anus	-	6-16 annuli posterior anus	-
a	33 ± 4.3 (27-39)	34 ± 3.8 (25-39)	33 ± 0.5 (33-34)	34 ± 2.6 (29-39)	36 ± 2.0 (33-40)	31 ± 1.9 (28-34)	34 ± 1.2 (33-37)	31 ± 1.3 (29-32)	32 ± 5.0 (28-41)
c	22 ± 2.4 (18-25)	17 ±2.6 (14-26)	16 ± 1.0 (16-17)	20 ± 1.8 (17-24)	18 ± 1.3 (16-20)	26 ± 2.3 (23-29)	19 ± 1.5 (16-21)	24 ± 2.2 (21-26)	20 ± 1.8 (17-23)

Table 4.4 continued: Morphometric data of *Histotylenchus mohalei* Kleynhans, 1992 current study populations (NCN). All measurement given in µm. Multiple females (♀♀) and multiple males (♂♂).

Characters	TVL929: Pretoria		TVL941: Pretoria	TVL1960: Irene		TVL2005: Suikerbosrand Nature Reserve		TVL2026: Komatipoort	
n	11♀♀	19♂♂	3♂♂	35♀♀	17♂♂	11♀♀	13♂♂	6♀♀	8♂♂
c'	2 ± 0.2 (1-2)	3 ± 0.4 (2-4)	3. ± 0.4 (3-3)	2 ± 0.2 (2-3)	3 ± 0.5 (2-4)	2 ± 0.2 (1-2)	3 ± 0.3 (2-3)	2 ± 0.1 (2-2)	2 ± 0.3 (2-3)
m (%)	48 ± 3.3 (46-54)	48 ± 5.5 (41-62)	45 ± 3.8 (42-47)	47 ± 3.4 (40-56)	45 ± 3.3 (38-51)	47 ± 2.2 (44-52)	46 ± 2.3 (42-50)	45 ± 2.3 (43-48)	45 ± 3.2 (41-49)
Anterior end to secretory-excretory pore/L (%)	14 ± 1.1 (13-17)	15 ± 1.0 (13-17)	18 ± 1.2 (17-19)	15 ± 0.8 (14-17)	16 ± 0.8 (14-17)	15 ± 0.6 (14-16)	16 ± 0.6 (15-17)	15 ± 0.4 (15-15)	16 ± 1.0 (15-18)
Anterior end to secretory-excretory pore/Oesophagus length (%)	87 ± 7.0 (77-94)	82 ± 11.3 (68-113)	92 ± 25.3 (74-110)	88 ± 7.5 (73-97)	87 ± 5.1 (77-93)	-	-	-	-
V (%)	58 ± 5.0 (53-65)	-	-	52 ± 1.6 (46-56)	-	55 ± 3.2 (51-63)	-	53 ± 1.8 (51-55)	-
OV_1 (%)	20 (n=1)	-	-	19 ± 6.4 (14-26)	-	22 ± 0.7 (22-23)	-	31 (n = 1)	-
OV_2 (%)	21 ± 0.7 (21-22)	-	-	22 ± 5.2 (17-33)	-	22 ± 2.4 (19-25)	-	31 (n = 1)	-
Posterior genital branch length/Anterior branch length (%)	-	-	-	117 ± 25.6 (100-147)	-	102 ± 7.8 (92-110)	-	102 (n = 1)	-
T (%)	-	51 ± 8.0 (38-61)	58 ± 4.9 (53-63)	-	47 ± 6.8 (34-53)	-	52 ± 10.5 (28-64)	-	50 ± 5.2 (46-53)

Histotylenchus niveus sp. n.

(Figs 4.12 & 4.13)

Measurements: See Table 4.5

Description

Female (n = 1 holotype; **2 paratype)**: Habitus straight (33 % of individuals) to curved ventrad (67 % of individuals), body cylindrical tapering at anterior end and tail. Four lateral field incisures, originate as two lines posterior to stylet knobs; third line appears after short distance dividing into two lines at level of metacorpus; outer two bands areolated throughout entire body; inner band incompletely areolated anterior to cap cell with areolations becoming complete posterior to cap cell; areolations opposite vulva irregular, outer and inner lines end in V-shaped pattern opposite hyaline region of tail. Lip region with eight annuli, nearly continuous with body marked by slight depression, 13 ± 1.0 (12-14) µm wide, almost twice lip region height of 6 ± 0.2 (6-6) µm; cephalic framework and basal ring not strongly sclerotised, vestibule wall strongly sclerotised. Stylet stout, stylet cone asymmetrical; stylet knobs mostly rounded, sloping backwards, one specimen more angular in shape. Guiding piece situated three annuli anterior to junction between stylet cone and stylet base. Procorpus wider than isthmus. Metacorpus large, oval-shaped; metacorpus valve spindle-shaped; hemizonid six to seven annuli anterior to secretory-excretory pore, three annuli long; deirid not observed. Secretory-excretory pore situated opposite to anterior part of postcorpus, i.e. 13 ± 1.4 (12-14) % of body length. Postcorpus with dorsal overlap; dorsal oesophageal gland nucleus distinct, situated opposite secretory-excretory pore; oesophago-intestinal valve indistinct. Intestine with fasciculi. Postanal diverticulum of intestine overlaps anus but does not reach phasmids. Vulva in boat-shaped depression situated at 51 ± 2.6 (48-52) % of body length with double, non-protruding or sunken epiptygma, vaginal musculature very prominent; reproductive system amphidelphic with outstretched ovaries, anterior genital branch slightly longer than posterior genital branch. Anterior spermatheca offset, posterior spermatheca axial; filled with oval to round sperm. Phasmids small, distinct, situated 13-14 annuli posterior to anus mid tail in inner band of lateral field..

Two specimens with long, symmetrical rounded tails, 53- 56 µm; one specimen with markedly shorter tail of 28 µm.

Male (n = 2): Similar to female except in following characteristics: Dorsal lateral line extend past phasmids terminating near tail end, subdorsal and subventral lines extends to opposite cloacal opening or just beyond, ventral line terminating at beginning of bursa. Lip region with six to seven annuli. Hemizonid not observed, secretory-excretory pore situated opposite to anterior part of postcorpus, i.e. at 16 % (n=1) of body length, deirid not observed. Postcorpus overlap of intestine not observed. Fasciculi not observed. Spicule arcuate with prominent velum; gubernaculum protrusible with pronounced swollen titillae, proximal end unmodified. Phasmids small, located about 13 µm posterior to cloacal opening. Tail slender with fingerlike ventral projection with rounded end.

Diagnosis

Histotylenchus niveus sp. n. is characterised by combination of the following characters: lip region nearly continuous with body marked by slight depression; outer two bands of lateral field areolated throughout entire body, inner band incompletely areolated anterior to cap cell with areolations becoming complete posterior to cap cell; areolations opposite vulva irregular; stylet stout (28-30 µm); stylet cone asymmetrical; stylet knobs round to angular, sloping backwards; metacorpus large, oval-shaped; postanal diverticulum of intestine overlaps anus, does not reach phasmids; tail asymmetrical rounded with 18 ventral annuli, annulated tip in females. Spicule arcuate with prominent velum. Gubernaculum protrusible, with pronounced swollen titillae, proximal end unmodified.

Discussion

Histotylenchus niveus sp. n. morphologically resembles *H. hedys*, *H. histoides*, *H. mohalei* and *H. sudanensis*. Type material females of *H. hedys*, as described in Kleynhans (1975), differ from current population (NCN) females of *H. niveus* sp.n. in: Habitus (straight to slightly curved *vs* strongly curved ventrad), shape of stylet knobs (slightly indented *vs* round to angular, sloping backwards) and postanal diverticulum

(very large, extending to well past phasmids *vs* small not reaching level of phasmids). Males of new species differ from *H. hedys* males (Kleynhans & Heyns, 1984) in tail length (54-86 µm *vs* 37-52 µm), gubernaculum (protrusable, with pronounced swollen titillae, proximal end unmodified *vs* non-protrusable, proximal end directed towards rear) and ratio c (12-19 *vs* 21-27). Additionally H. *niveus* sp. n. males differ from current study populations (NCN) of *H. hedys* males in: Body length (1 012-1 024 µm *vs* 822 µm), outer margins of basal ring (3 µm *vs* 5 µm), metacorpus length (25-28 µm *vs* 12 µm), metacorpus width (17-19 µm *vs* 10 µm), metacorpus valve width (5 µm *vs* 2 µm), spicule length (40 µm *vs* 33 µm), gubernaculum length (21 µm *vs* 15 µm), body width at secretory-excretory pore (34 µm *vs* 26 µm), body width at midbody (35 µm *vs* 26 µm), body width at cloacal opening (11 µm *vs* 17µm) and ratio c (5 *vs* 3).

Histotylenchus niveus sp. n. differs from *H. histoides* type material (Siddiqi, 1971) in: Stylet length (28-30 µm *vs* 22-24 µm) and conus length (14-17 µm *vs* 10-11 µm). Females can further be distinguished from females as decribed in Kleynhans (1975) and Kleynhans & Heyns (1984) in: Stylet length (28-30 µm *vs* 21-26 µm). The males of the new species differ from males from the type locality (Siddiqi, 1971), in: Stylet length (22-28 µm *vs* 22-23 µm), spicule length (40 µm *vs* 33-34 µm), gubernaculum length (21 µm *vs* 14-16 µm), proximal end of gubernaculum (unmodified *vs* directed towards rear), ratio a (30 *vs* 37-44) and ratio c (12-19 *vs* 23-28). *Histotylenchus niveus* sp. n males further differ from South African population of *H. histoides* (Kleynhans, 1975; Kleynhans & Heyns, 1984) in: Spicule length (40 µm *vs* 29-34 µm), gubernaculum length (21 µm *vs* 12-16 µm), proximal end of gubernaculum (unmodified *vs* directed towards rear), tail length (54-86 µm *vs* 39-42 µm) and ratio a (30 *vs* 35-44).

The new species can be distinguished from *H. mohalei* type material (Kleynhans, 1992) females in: Body length (1 289-1 374 µm *vs* 891-1 184 µm), lip region width (12-14 µm *vs* 9-11 µm), stylet length (28-30 µm *vs* 18-22 µm) and stylet conus length (14-17 µm *vs* 8-10 µm).

Histotylenchus niveus sp. n can also be separated from *H. mohalei* current study populations (NCN) females in: Body length (1 289-1 374 µm *vs* 810-1 078 µm), lip region width (12-14 µm *vs* 9-11 µm), stylet length (28-30 µm *vs* 18-24 µm), stylet conus length (14-17 µm *vs* 9-15 µm) and position of secretory-excretory pore (159-186 µm *vs* 118-158 µm). *H. niveus* sp. n males differ from *H. mohalei* males described in Kleynhans (1992) in: Spicule length (40 µm *vs* 27-34 µm), gubernaculum length (21 µm *vs* 9-15 µm) and ratio a (30 *vs* 38-53).

Histotylenchus niveus sp. n. differs from *H. sudanensis* females described by Siddiqi (1977) in: Body length (1 289-1 374 µm *vs* 1 070-1 140 µm), lip region shape (continuous with body marked by a slight depression *vs* continuous with body), lip region width (12-14 µm *vs* 10 µm), stylet length (28-30 µm *vs* 22- 24 µm), position of secretory-excretory pore (159-186 µm *vs* 138-149 µm) and lateral field areolations (outer two bands areolated throughout entire body, inner band incompletely areolated anterior to cap cell with areolations becoming complete posterior to cap cell, areolations opposite vulva irregular *vs* without areolations, except in oesophageal region and a few irregular areolations in outer bands over rest of body). Males of new species differ from *H. sudanensis* males in: Body length (1 012-1 024 µm *vs* 1 030-1 140 µm), spicule length (40 µm *vs* 30-33 µm), gubernaculum length (21µm *vs* 13-14 µm), proximal end of gubernaculum (unmodified *vs* directed towards rear), ratio a (30 *vs* 41-50), ratio c (12-19 *vs* 22-27) and ratio c' (5 *vs* 2-3).

Etymology

The species name is Latin for white and refers to the unusual white dunes found among the red dunes at type locality.

Locality and material examined

See Appendix III for full locality data and all material examined.

REMARK: Permit (131/2000 of 29 September 2003) to collect soil samples in the reserve was authorized by the reserve manager of Witsand Nature Reserve and the Department of Tourism, Environment and Conservation of the Northern Cape Province.

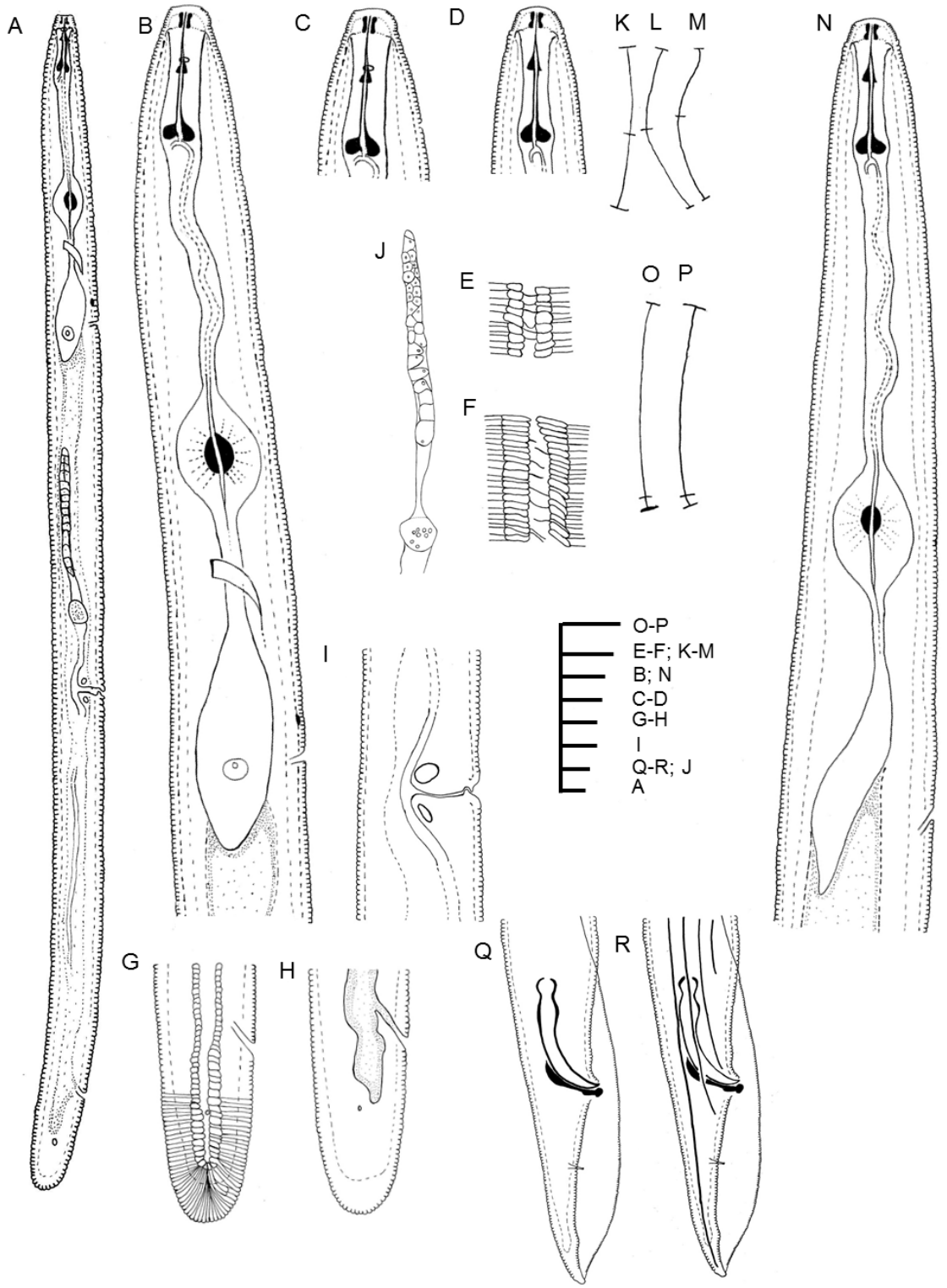


Figure 4.12: Line diagrams of *Histotylenchus niveus* sp. n. Female: **A**- whole specimen; **B**- anterior end; **C**- lip region; **E**- lateral field in secretory-excretory pore region; **F**- lateral field in vulva region; **G**- tail, indicating lateral field; **H**- tail indicating postanal diverticulum; **I**- vulva; **J**- part of anterior ovarium and **K-M**- habitus. Male: **D**- lip region; **N**- anterior of body; **O-P**- habitus; **Q**- tail and **R**- tail with lateral field. **Scale bars** = 20 µm (A) and 10 µm (B-R).

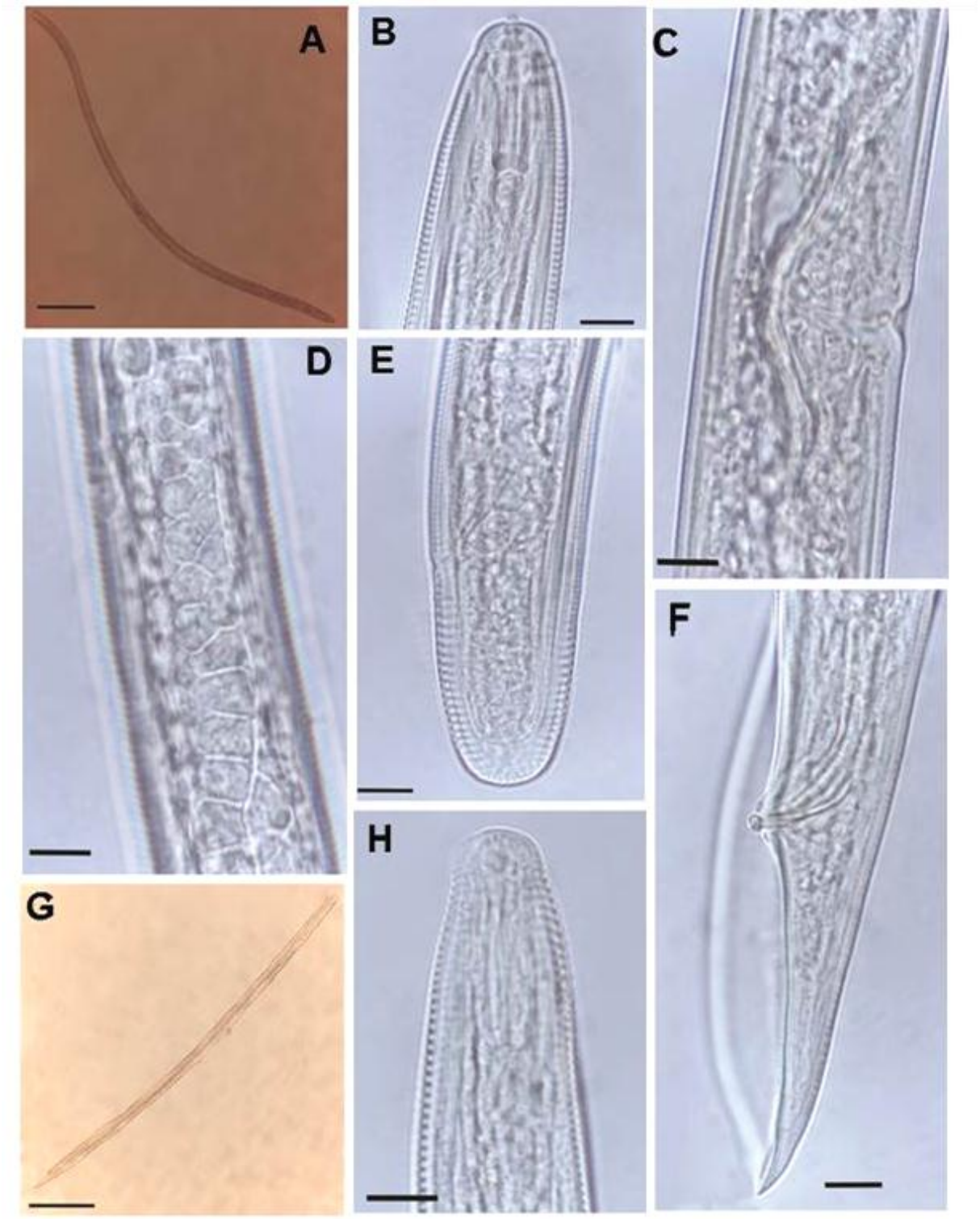


Figure 4.13: Light micrographs of *Histotylenchus niveus* sp. n. Female: **A**- habitus; **B**- lip region; **C**- vulva region; **D**- segment of anterior genital branch; **E**- tail region. Male: **F**- tail; **G**- habitus and **H**- lip region. **Scale bars** = 100 µm (A; G) and 10 µm (B-F; H)

Table 4.5: Morphometric data of current study populations (NCN) of *Histotylenchus niveus* sp. n, *Histotylenchus* sp. 1 and *Histotylenchus* sp. 2. All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

	***Histotylenchus niveus* sp. n.**			***Histotylenchus* sp. 1**		***Histotylenchus* sp. 2**
Characters	**KP2039: Witsand Nature Reserve Holotype**	**KP2039: Witsand Nature Reserve Paratype**	**KP2039: Witsand Nature Reserve Paratype**	**TVL754: Naboomspruit**	**TVL754: Naboomspruit**	**KP822: Port Elizabeth**
n	1♀	2♀♀	2♂♂	1♀	2♂♂	1♀
L	1 259	1 289-1 374	1 012-1 024	934	913-925	850
Number of lip annuli	8	8-9	6-7	8	8	8
Lip region height	6	6	5-6	4	4	4
Lip region width	12	12-14	11-13	9	9	10
Basal ring: annuli	4	6	3-4	-	4 (n=1)	4
Outer margins of basal ring: length	3	3-5	3	-	4 (n=1)	3
Stylet length	28	28-30	22-28	-	-	-
Conus length	16	14-17	6-15	10	10	10
Stylet base length	12	12-15	13-15	-	-	-
Stylet knobs height	6	7	5 (n=1)	-	-	-
Stylet knobs width	3	3-4	3 (n=1)	-	-	-
Stylet length/lip region width	2	2	2	-	-	-
Dorsal gland opening posterior to stylet (DGO)	-	2-3	2 (n=1)	-	-	-
Anterior end to secretory-excretory pore	167	159-186	166 (n=1)	81	78-85	-
Anterior end to mid metacorpus	101	108-115	113	-	-	77
Metacorpus length	23	23-24	25-28	21	19-21	17
Metacorpus width	19	18-20	17-19	20	15-19	19
Metacorpus length/ metacorpus width	1	1	2	1	1	1
Metacorpus valve length	7	6-7	6	7	6-7	5
Metacorpus valve width	5	5	5	5	5	4
Position of hemizonid	6- 7 annuli anterior to secretory-excretory pore	6- 7 annuli anterior to secretory-excretory pore	-	-	-	-
Anterior genital branch length	-	370 (n=1)	-	-	-	-
Posterior genital branch length	335	222-352	-	-	-	-
Vagina length	19	14 (n=1)	-	-	-	
Vagina length/corresponding body width	0.7	0.4	-	-	-	-
Spermatheca length	-	14-19 (n=1)	-	-	-	-
Spermatheca width	-	15-20 (n=1)	-	-	-	-
Spicule length	-	-	40 (n=1)	-	29 (n=1)	-
Gubernaculum length	-	-	21 (n=1)	-	13 (n=1)	-

Table 4.5 continued: Morphometric data of current study populations (NCN) of *Histotylenchus niveus* sp. n., *Histotylenchus* sp. 1 and *Histotylenchus* sp. 2. All measurements given in µm. Single female (♀), multiple females (♀♀), single male (♂) and multiple males (♂♂).

	***Histotylenchus niveus* sp. n.**			***Histotylenchus* sp. 1**		***Histotylenchus* sp. 2**
Characters	**KP2039: Witsand Nature Reserve Holotype**	**KP2039: Witsand Nature Reserve Paratype**	**KP2039: Witsand Nature Reserve Paratype**	**TVL754: Naboomspruit**	**TVL754: Naboomspruit**	**KP822:Port Elizabeth**
n	1♀	2♀♀	2♂♂	1♀	2♂♂	1♀
Width of annuli at: secretory-excretory pore	2	1(n=1)	1	-	2 (n=1)	-
Width of annuli at: midbody/vulva	2	1	1	1	1-2	1
Body width at secretory-excretory pore	31	33	34 (n=1)	-	-	-
Body width at midbody or vulva	33	34-37	35 (n=1)	43	37 (n=1)	36
Body width at anus or cloacal opening	33	26-32	11 (n=1)	-	35-41	29
Lateral field width	8	-	10 (n=1)	6-7	-	7
Position of phasmids posterior to anus	13-14 annuli	13-14 annuli	-	-	-	11 annuli
Phasmids to anus/cloacal length	13	19(n=1)	16-19	-	38 (n=1)	13
Phasmids diameter	1	1	1	1	-	1
Tail length	53	28-56	54-86	-	80 (n=1)	35
Number of ventral annuli	37	-	-	-	-	31
Tail projection length	-	-	9-11	-	8 (n=1)	-
Hyaline length	10	10-11	-	6	-	8
Postanal diverticulum length	5 annuli posterior anus	3- 5 annuli posterior anus	-	-	-	6 annuli posterior anus
a	38	37-38	30 (n=1)	22	23-26	24
c	24	23-56	12-19	-	12 (n=1)	25
c'	2	1-2	5 (n=1)	-	3 (n=1)	1
m (%)	57	49-59	29-55	-	-	-
Anterior end to secretory-excretory pore/L (%)	13	12-15	16 (n=1)	-	-	-
V (%)	48	52	-	52	-	55
OV_1 (%)	-	27 (n=1)	-	-	-	-
OV_2 (%)	27	17-26	-	-	-	-
Posterior genital branch length/Anterior branch length (%)	-	95 (n=1)	-	-	-	-

Histotylenchus sp. 1

(Fig 4.14)

Measurements: See Table 4.5

Description

Female (n = 2): Habitus curved, body cylindrical throughout, tapering towards anterior end and tail. Lateral field with four incisures, originate as two lines just posterior to stylet knobs; third line appears after short distance and dividing into two lines at level of metacorpus, outer two bands and inner band areolated throughout body, areolations irregular opposite vulva. Lip region with eight annuli, offset, 9 µm wide, more than twice lip region height of 4 µm; cephalic framework and basal ring usually not strongly sclerotised, vestibule wall strongly sclerotised. Stylet base not observed, probably as result of fixative used. Procorpus wider than isthmus. Metacorpus large, nearly rounded; metacorpus valve spindle-shaped; hemizonid not observed; deirid not observed. Secretory-excretory pore not observed. Postcorpus overlap of intestine ventral. Intestine with fascicule, ending one annuli width anterior to start of hyaline. Reproductive system not observed. Postanal diverticulum of intestine stretches well past phasmids. Phasmids small, distinct, in middle insicure. Tail rounded with annulated tip, tail length could not be determined, as result of effect of fixative used.

Male (n = 2): Similar to female except in following characteristics: Dorsal lateral line extends past phasmids terminating near tail end, subdorsal and subventral lines extend to opposite cloacal opening or just beyond, ventral line terminating at beginning of bursa. Secretory-excretory pore situated opposite anterior part of postcorpus, i.e. at 81 ± 5.3 (78-85) % of body length. Spicule arcuate with prominent velum, gubernaculum non- protrusible, with pronounced swollen titillae, proximal end curved backwards. Phasmids small, located 26-39 µm posterior to cloacal opening. Tail slender with fingerlike ventral projection with rounded end.

Diagnosis

Although these specimens probably represent a new species, a diagnosis is not made at this stage, due to the small number of specimens examined.

Discussion

Histotylenchus sp. 1 morphologically resembles *H. historicus, H. mohalei* and *H. niveus* sp. n. *Histotylenchus* sp. 1 females can be distinguished from *H. historicus* females as described by Jairajpuri & Baqri (1968) and Kleynhans (1975) in: Conus length (10 µm *vs* 7-9 µm), postanal diverticulum (large, extending well past phasmids *vs* none) and ratio c (12 *vs* 21-29). The males differ in lip region width (9 µm *vs* 11 µm), tail length (80 µm *vs* 41 µm), ratio a (23-26 *vs* 37-45) and ratio c (12 *vs* 21-29). *Histotylenchus* sp. 1 females differ from *H. mohalei* type material (Kleynhans, 1992) in: Position of secretory-excretory pore (81 µm *vs* 112-152 µm), postanal diverticulum (large, extending well past phasmids *vs* extending past anus, sometimes to midway between anus and phasmids) and ratio a (22 *vs* 34-47).

Additionally the females can be distinguished from current study (NCN) females of *H. mohalei* by the position of secretory-excretory pore (81 µm *vs* 118-158 µm), body width at vulva (43 µm *vs* 24-35 µm), ratio a (22 *vs* 27-39) and ratio c (12 *vs* 17-29). Males differ from *H. mohalei* type material (Kleynhans, 1992) in: Position of secretory-excretory pore (78-85 µm *vs* 112-148 µm) and tail length (80 µm *vs* 43-64 µm) and ratio a (23-26 *vs* 38-53). *Histotylenchus* sp. 1 differs from current study populations of *H. mohalei* (NCN) males in: Body width at midbody (37 µm *vs* 21-35 µm), body width at cloacal opening (35-41 µm *vs* 11-22 µm), tail length (80 µm *vs* 35-62 µm) and ratio c (12 *vs* 13-26). *Histotylenchus* sp. 1 females can be distinguished from *H*. *niveus* sp. n. females in: Body length (934 µm *vs* 1 289-1 374 µm), lip region height (4 µm *vs* 6 µm), lip region width (9 µm *vs* 12-14 µm), conus length (10 µm *vs* 14-17 µm), position of secretory-excretory pore (81 µm *vs* 159-186 µm), body width at vulva (43 µm *vs* 34-37 µm) and ratio a (22 *vs* 37-38). Males are distinguished from *H. niveus* sp. n. in: Body length (913-925 µm *vs* 1 012-1 024 µm), number of lip annuli (eigth *vs* six to seven), lip region width (4 µm *vs* 5-6 µm), lip region height (9 µm *vs* 11-13 µm), position of

secretory-excretory pore (78-85 µm *vs* 166 µm), spicule length (29 µm *vs* 40 µm), gubernaculum length (13 µm *vs* 21 µm), position of phasmids (38 µm *vs* 16-19 µm posterior to cloacal opening), ratio a (23-26 *vs* 30) and ratio c' (3 *vs* 5).

Locality and material examined

See Appendix III for full locality data and all material examined.

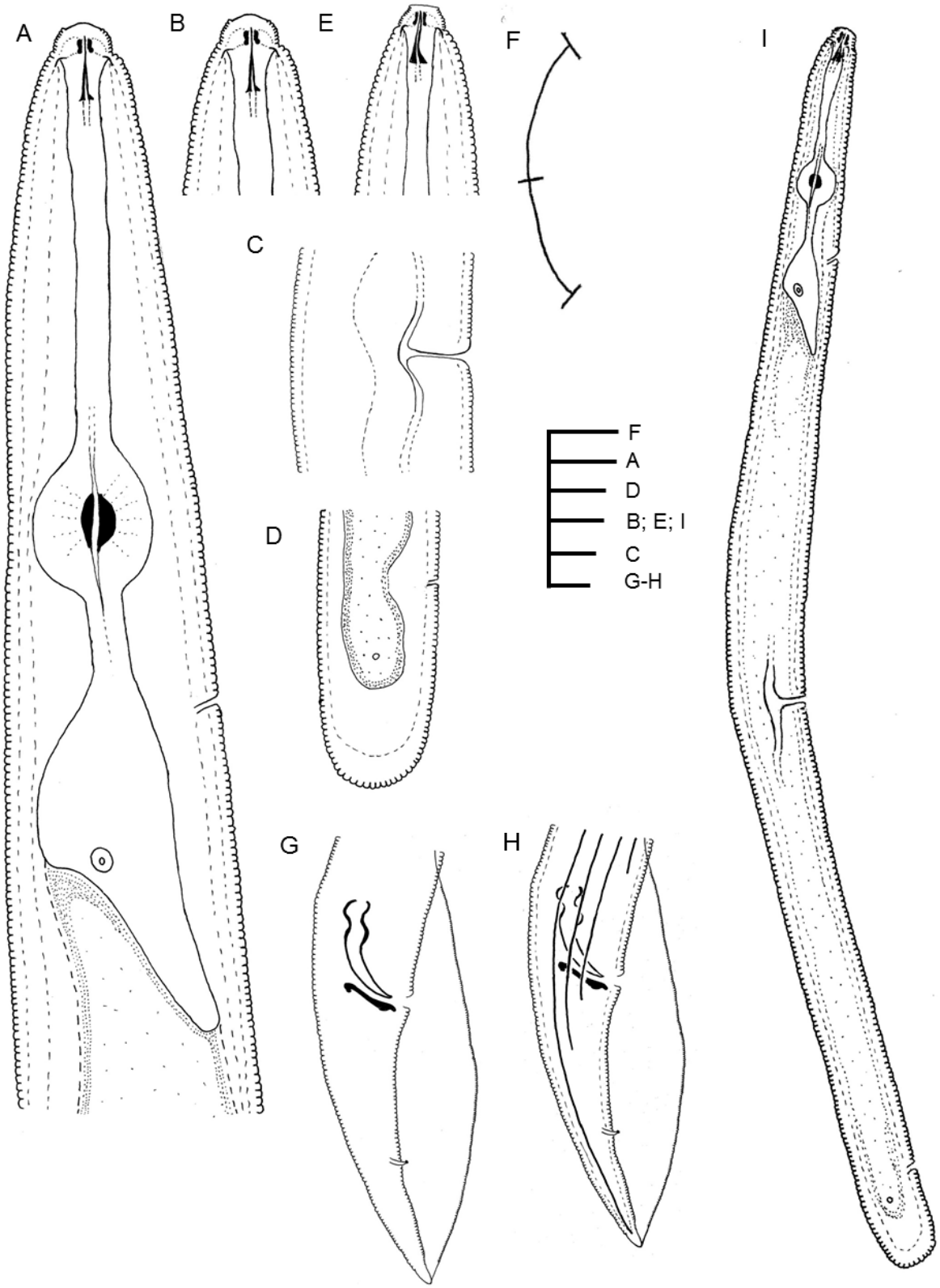


Figure 4.14: Line diagrams of *Histotylenchus* sp. 1. Female: **A**- anterior region; **B**- lip region; **C**- vulva**; D**- tail with postanal diverticulum and **F**- habitus. Male: **E**- lip region **G**- tail; **H**- tail with lateral field. Female: **I**- whole specimen. **Scale bars** = 20 µm (I) and 10 µm (A-H).

Histotylenchus sp. 2

(Figs 4.15 & 4.16)

Measurements: See Table 4.5

Description

Female (n = 1): Habitus curved, body cylindrical except for anterior end and tail. Lateral field with four incisures, originate as two lines just posterior to stylet knobs; third line appears after short distance, dividing into two lines at level of metacorpus, outer two bands areolated throughout entire body, with areolations posterior of anus incomplete; occasional areolations in inner band along whole length of body, areolations opposite vulva irregular, outer and inner lines end in Y-shaped pattern in hyaline region of tail. Lip region hemispherical continuous with body with eight annuli, 10 µm wide, more than twice lip region height of 4 µm; cephalic framework and basal ring usually not strongly sclerotised, vestibule wall strongly sclerotised. Stylet cone asymmetrical. Procorpus wider than isthmus. Metacorpus large, rounded; metacorpus valve spindle shaped; hemizonid, deirid and secretory-excretory pore not observed. Postcorpus overlap abuts intestine. Intestine with fasciculi. Postanal diverticulum of intestine overlaps anus but does not reach phasmids. Vulva in deep boat-shaped depression situated at 55 % of body length with double, non- protruding epiptygma; vaginal musculature prominent; genital tracts not distinct in specimen. Phasmids small, distinct, situated 11 annuli posterior to anus at about mid tail. Tail short, symmetrical rounded, 35 µm.

Males: Not found.

Diagnosis

Although these specimens probably represent a new species, as it does not conform to any of the known species, no diagnosis is made for three reasons: Firstly, due to the small number of specimens examined, secondly the effect of the fixative on specimens and thirdly the absence of male specimens to examine.

Discussion

Histotylenchus sp. 2 morphologically resembles *H. hedys*, *H. histoides*, *H. mohalei* and *H. niveus* sp. n. *Histotylenchus* sp. 2 females can be distinguished from type *H. hedys* as described by Kleynhans (1975) in: Body length (850 µm *vs* 1 070-1 250 µm), lip region width (10 µm *vs* 12-13 µm) postanal diverticulum (small, overlaps anus but does not reach phasmids *vs* very large, extending well past middle of tail) and ratio a (24 *vs* 29-39) and from females described by Kleynhans & Heyns (1984) in: Body length (850 µm *vs* 1 017-1 222 µm), lip region width (10 µm *vs* 12-13 µm), postanal diverticulum (small, overlaps anus but does not reach phasmids *vs* very large, extending well past middle of tail) and ratio a (24 *vs* 26-37). *Histotylenchus* sp. 2 females differ from current study (NCN) *H. hedys* populations in number of lip annuli (eight *vs* seven), body length up to mid metacorpus (77 µm *vs* 87-93 µm), postanal diverticulum (small, overlaps anus but does not reach phasmids *vs* very large, extending well past middle of tail) and ratio a (24 *vs* 26-36).

Histotylenchus sp.2 can be distinguished from *H. histoides* type females as described by Siddiqi (1971) in: Body length (850 µm *vs* 1 080-1 180 µm), and ratio a (24 *vs* 38-47) and further differs from a South African and a Namibian population as described in Kleynhans (1971) and Kleynhans & Heyns (1984) in: Body length (850 µm *vs* 1 024 -1 450 µm), and ratio a (24 *vs* 29-45). *Histotylenchus* sp. 2 also differs from *H. mohalei* type material (Kleynhans, 1992) by: Body length (850 µm *vs* 891-1 184 µm), ratio a (24 *vs* 34-47) and ratio c' (1.2 *vs* 1.7-3.0). *Histotylenchus* sp. 2 differs from current study populations (NCN) of *H. mohalei* females in: Ratio a (24 *vs* 27-39).

Histotylenchus sp. 2 differs from *Histotylenchus niveus* sp. n. in: Body length (850 µm *vs* 1 289-1 374 µm), lip region height (4 µm *vs* 6 µm), lip region width (10 µm *vs* 12-14 µm), body length at mid metacorpus (77 µm *vs* 108-115 µm), metacorpus length (17 µm *vs* 23-24 µm), metacorpus valve length (5 µm *vs* 6-7 µm), metacorpus valve width (4 µm *vs* 5 µm), hyaline length (8 µm *vs* 10-11 µm) and ratio a (24 *vs* 37-38). *Histotylenchus* sp. 2 females differs from *Histotylenchus* sp. 1 females in: Body length (850 µm *vs* 934 µm), lip region width (9 µm *vs* 10 µm), metacorpus length (17 µm *vs* 21 µm),

metacorpus valve length and width (5 µm *vs* 7 µm and 4 µm *vs* 5 µm), body width at midbody (36 µm *vs* 43 µm) and ratio a (24 *vs* 22).

Locality and material examined

See Appendix III for full locality data and all examined material.

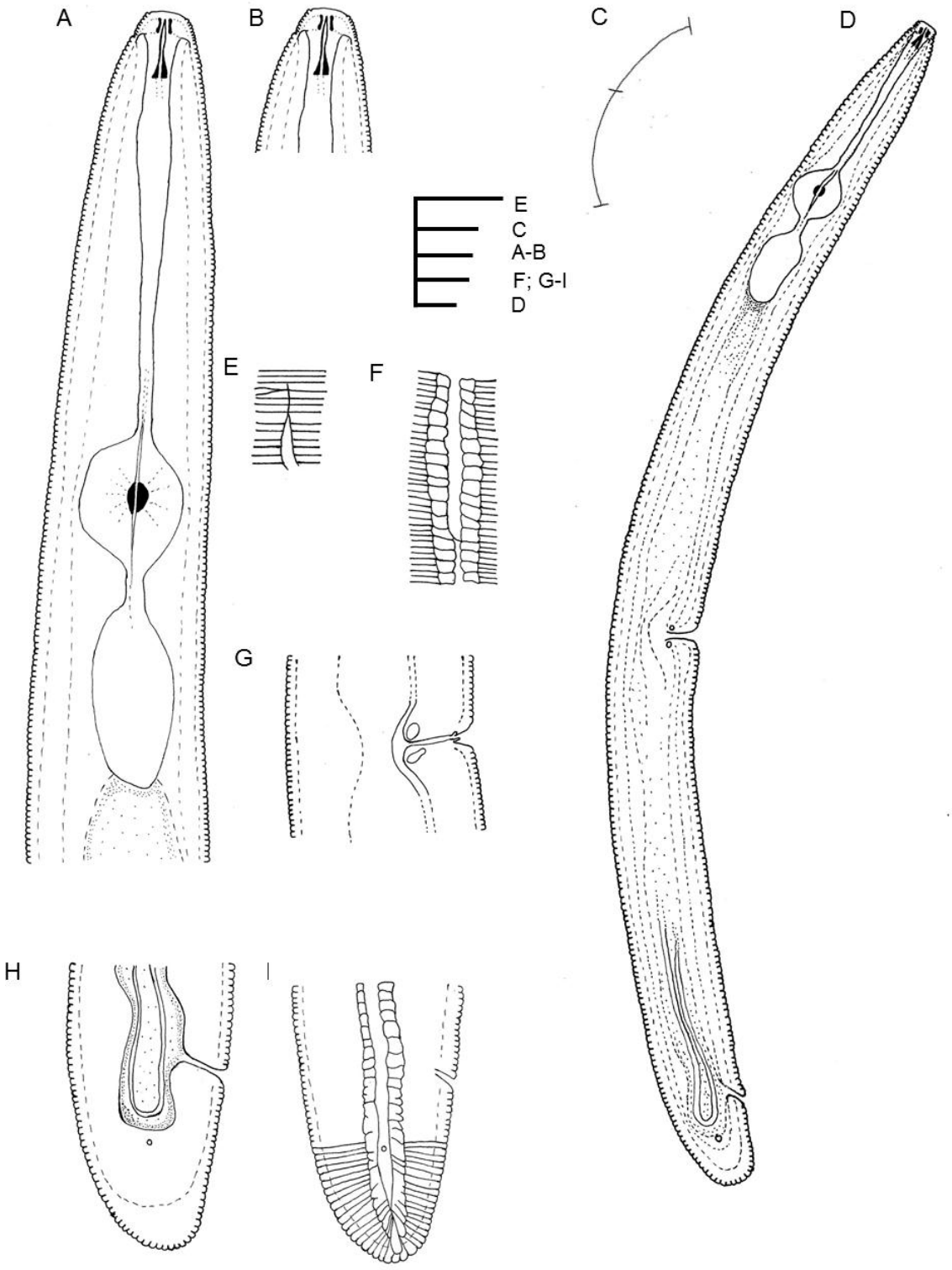


Figure 4.15: Line diagrams of *Histotylenchus* sp. 2. Female: **A**- anterior part of body; **B**- lip region; **C**- habitus; **D**- whole specimen; **E**- start of lateral lines; **F**- lateral field in vulva region; **G**- vulva with epiptygma; **H**- tail region showing postanal diverticulum and **I**- tail region with lateral field. **Scale bars** = 20 µm (A-D) and 10 µm (E-I).

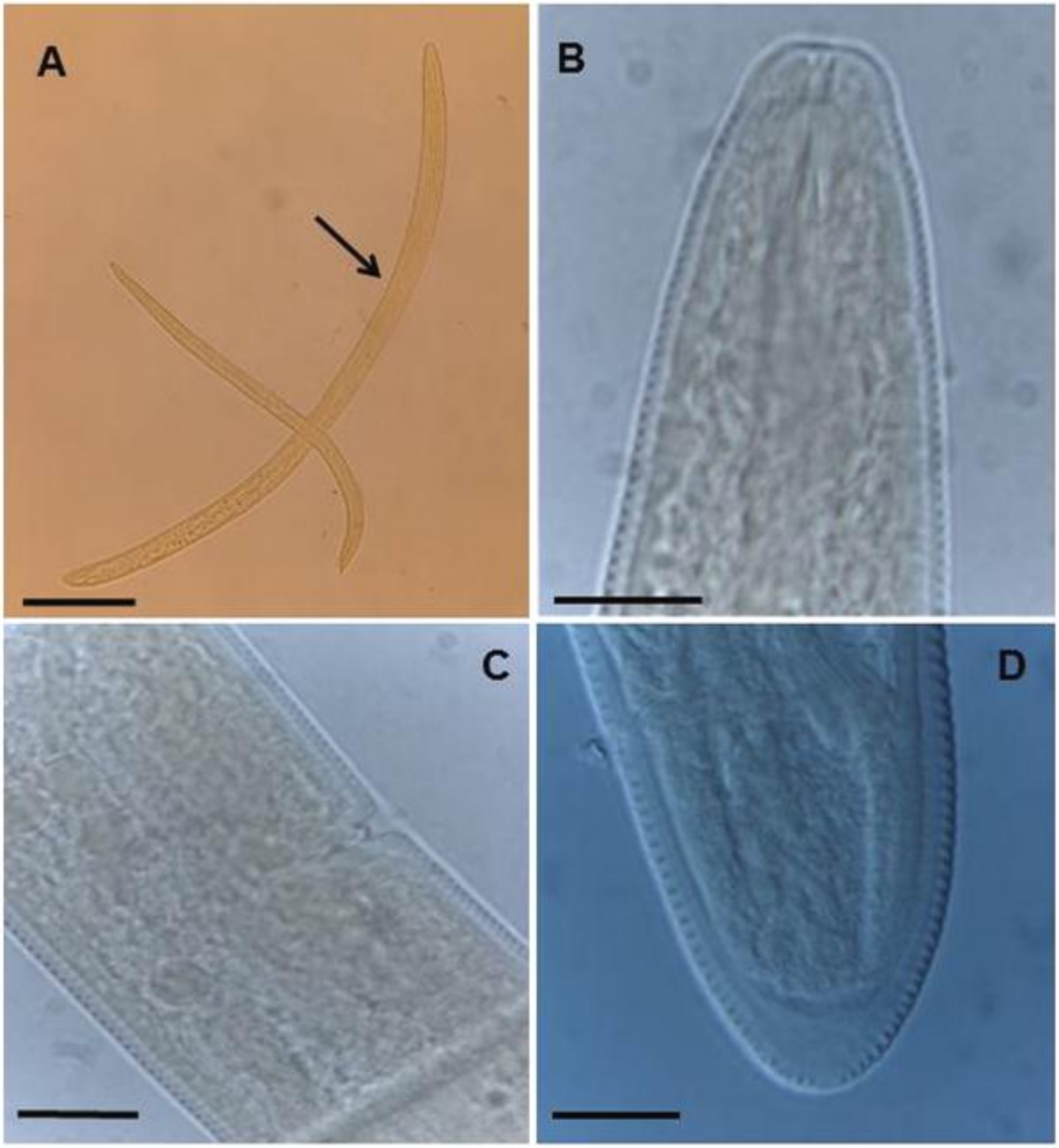
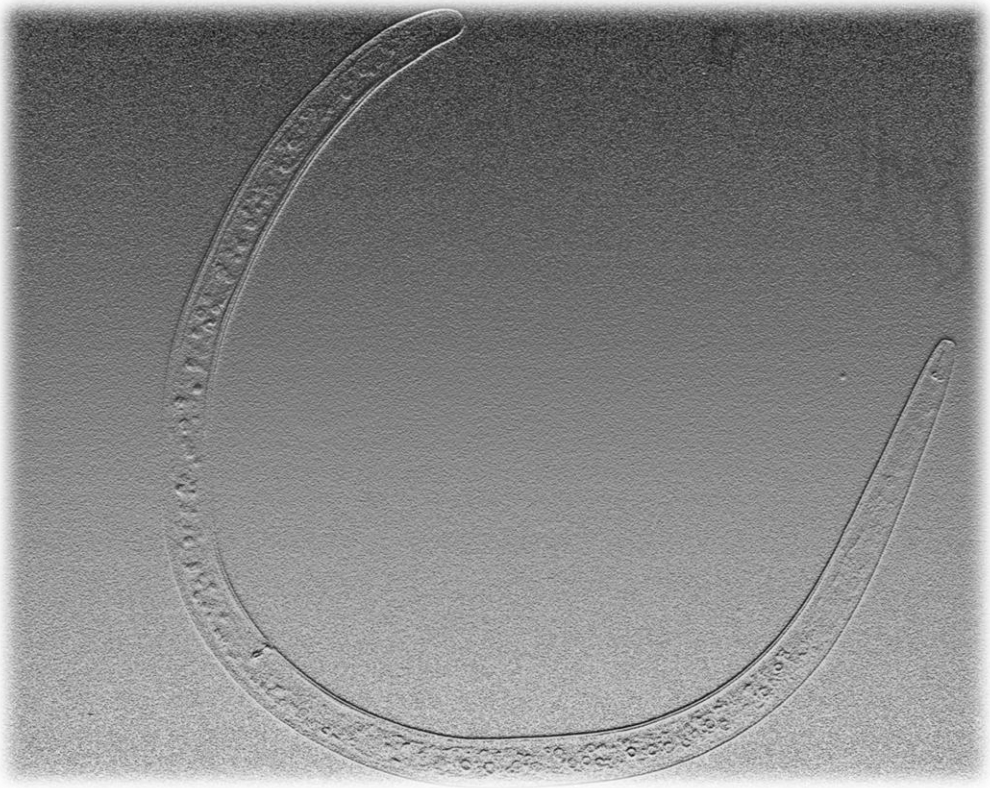


Figure 4.16: Light micrographs of *Histotylenchus* sp. 2. Female: **A**- habitus (black arrow); **B**- lip region, **C**- vulva region and **D**- tail. **Scale bars** = 100 µm (A) and 10 µm (B-D).

CHAPTER 5



Concluding remarks

In this study the morphological and morphometric data of the genus *Histotylenchus* in South Africa was studied. Representatives of this genus are not currently regarded as economically important nematodes and thus limited work has been done on these. As a result of this, there is a big gap in the understanding of these nematodes biology and little information is available on their distribution, host range and potential to cause economic damage.

As mentioned in Chapter 3, during this study it was noted that 44 % of the microscope slides studied were unsuitable for taxonomic study as a result of the anomaly of the cuticle separating from the body. In their guide, Coyne *et al*., (2007) doubted whether the killing process, which is a very important step in preparation for permanent microscope slides, can be responsible for anomalies such as the cuticle separating from the body. More likely the anomaly is due to the specific fixative used (Brown & Topham, 1984; Grewal *et al*., 1990). It is known that when using certain methods of fixing specimens in formalin (e.g. Coyne *et al*., 2007), the fixation method does not provide one with good quality specimens for long term storage. Hooper (1970), Ruzin (1999) and Hooper *et al.* (2005) further argued that due to the presence of ethanol in FAA fixative a certain amount of shrinkage of tissue nearly always occurs, rendering some of the specimens worthless. Hooper *et al.* (2005) noted that it is known that fixing specimens in TAF gives better results than when fixing in FAA or , but after a few hours the nematodes start to degenerate. However, specimens fixed in TAF and processed to glycerol by the slow method seem to remain in good condition. Timm & Hackney (1968) noted that in some cases FAA does give good results, e.g. when working with marine nematodes.

Since no *Histotylenchus histoides* material housed in the National Collection of Nematodes in South Africa was still in a condition to be studied, it is very important that research be continued on this genus and that new material be collected in order to maintain a complete collection and to fill in the gaps of information on this genus. Unfortunately the available information in the SAPPNS database on the distribution of

the genus *Histotylenchus* in South Africa gives an incomplete representation of the distribution of the genus in South Africa.

Another gap in the understanding of this nematode is the lack of molecular information for this genus. The availability of molecular information has the potential to influence the time spent on identification of certain nematodes (Ravichandra, 2010). Molecular information can help resolve contradictions in problematic diagnostic characters (Dorris *et al*., 1999). The availability of reliable molecular data will help to formulate more complete species descriptions.

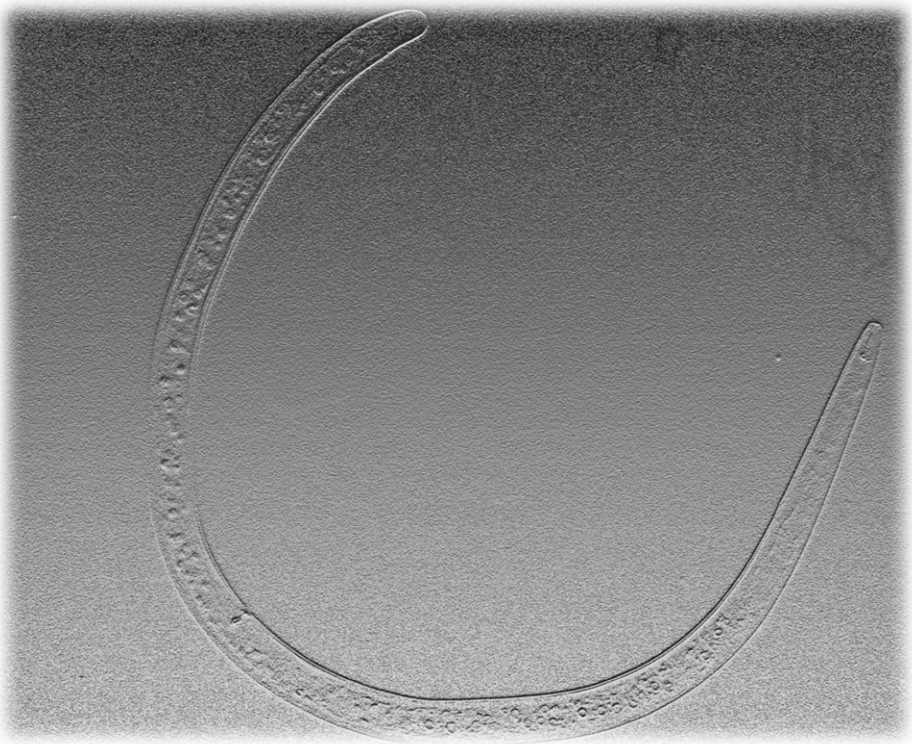
In short to summarise the various factors hampering the study of the genus *Histotylenchus* included:

1. The large amount of poor material available in nematode collections as a result of cut-backs in research funds, leading to the general decline in the curation of research collections, thus resulting in the subsequent deterioration of material making them of little use for study.
2. The small amount of paratype specimens available for some of the species. The lack of a standard set of morphometric characters used in the original species descriptions.
3. The lack of molecular data on the genus *Histotylenchus*.
4. The absence of scanning electron micrographs (SEM) in all species descriptions of the genus *Histotylenchus*.

FUTURE RESEARCH

Future work on representatives of the genus *Histotylenchus* will include expanding the available data in order to fully determine the distribution of the genus in South Africa. Further work has to be done in order to determine the best fixative to use when preparing *Histotylenchus* for long term (more than 50 years) storage (especially when storing type material). Determining a DNA sequence for the genus and expanding the molecular data will also be given attention.

CHAPTER 6



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Appendix I

Compendium of species characteristics and diagnostic species measurements for the genus *Histotylenchus* Siddiqi, 1971.

APPENDIX I

Compendium of species characteristics and diagnostic species measurements for the genus *Histotylenchus* Siddiqi, 1971. *Characteristic not provided in text of publication but derived from illustrations. Females (♀♀) and males (♂♂).

	Species											
	Histotylenchus baoulensis **Fig. I.I**		***Histotylenchus hedys*** **Fig. I.II**		***Histotylenchus histoides*** **Fig. I.III**		***Histotylenchus historicus*** **Fig. I.IV**		***Histotylenchus mohalei*** **Fig. V**		***Histotylenchus sudanensis*** **Fig. VI**	
	According to Netscher & Germani (1969)		According to Kleynhans (1975); Kleynhans & Heyns (1984)		According to Siddiqi (1971); Kleynhans (1975); Kleynhans & Heyns (1984)		According to Jairajpuri & Baqri (1968); Kleynhans (1975)		According to Kleynhans (1992)		According to Siddiqi (1977)	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Habitus	Variable	-	Strongly curved ventral	-	Straight to ventrally arcuate	-	Straight, irregularly curved or C shaped	-	Not indicated	-	Straight to arcuate	-
Lip annuli	6-7	-	6-7	-	8-11	-	8	-	8	-	7-9	-
Head shape	Continuous with body	-	Set off by a very shallow but distinct depression	-	Almost continues with body contour	-	Set off, flat at apex	-	Broad, low, continuous with body or marked off by depression or smaller or larger diameter	-	Conoid-rounded, continuous with body contour	-
Cephalic framework	Faintly sclerotised, vestibule wall thickened	-	Inconspicuous, but vestibule wall is thickened	-	Vestibule wall strongly sclerotised	-	Faintly sclerotised	-	Inconspicuous, but vestibule wall thickened	-	Sclerotized, cheilorhabdions prominent	-
Stylet knobs	*Round, sloping backwards	-	Laterally directed, slightly concave	-	Large, sloping to rear, outer edges angular	-	Round, sloping backwards	-	Rounded or transversely ovoid, flat or slightly convex anteriorly, slightly to strongly sloping backwards	-	Smoothly rounded	-
Deirids	Not indicated	-	Visible in lateral field opposite excretory pore	-	Probably opposite secretory-excretory pore	Probably opposite secretory-excretory pore	Present in lateral field at level of secretory-excretory pore	-	Not observed	-	Indistinct, on inner ventral incisure near excretory pore	-

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	Species											
	Histotylenchus baoulensis		***Histotylenchus hedys***		***Histotylenchus histoides***		***Histotylenchus historicus***		***Histotylenchus mohalei***		***Histotylenchus sudanensis***	
	According to Netscher & Germani (1969)		According to Kleynhans (1975); Kleynhans & Heyns (1984)		According to Siddiqi (1971); Kleynhans (1975); Kleynhans & Heyns (1984)		According to Jairajpuri & Baqri (1968); Kleynhans (1975)		According to Kleynhans (1992)		According to Siddiqi (1977)	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Reproductive tracts	Equally developed	Single testis	Not indicated	-	Ovaries symmetrical, spermatheca with sperm	Single testis, outstreched	Equally developed	-	Equally developed	-	Ovaries outstretched, with oocytes in one or two rows	Single testis, outstreched
Epiptygma	Single epiptygma present	-	Double, sunken anterior and posterior epiptygma	-	Double non-protruding epiptygma	-	*Not indicated	-	Reduced, double epiptygma	-	Indistinct, double epiptygma	-
Spicule	-	-	-	Cephalate	-	Ventral arcuate, slightly cephalated	-	Slightly arcuate	-	-	-	Indistinctly cephalated, arcuate near middle
Gubernaculum	-	Protrusible, proximal end directed towards rear	-	Non-protrusible, *proximal end directed towards rear	-	*Protrusible, proximal end directed towards rear	-	Proximal end directed towards rear, not known if protrusible	-	Protrusible, proximal end unmodified or knobbed or directed towards rear	-	Protrusible, proximal end posteriorly recurved, broad trough-shaped distal portion
Fasciculi	Present throughout entire body, including postanal diverticulum	-	Present	-	Present	-	*Not indicated	*Not indicated	Present	-	Present	-
Post-anal diverticulum	*Overlaps rectum, to level of phasmids	-	Very large, extending well past middle of tail and phasmids	-	Intestine with post-rectal blind diverticulum 1/6 to 1/3 into tail cavity	-	*None	-	Overlaps rectum to level of anus, sometimes to midway between anus and phasmids	-	*Overlaps rectum to level of anus, rarely reaching level of phasmids	-
Phasmids	*Small, middle of lateral field, posterior to anus	*Near middle of tail	*Small in middle incisure posterior to anus	-	*Small in middle incisure posterior to anus	Located 10-20 µm posterior to anus	Near middle of tail	Near middle of tail	Small in middle incisure posterior of anus	*Near middle of tail	Pore-like, usually anterior to middle of tail from anus, sometimes located in ventral incisure	Near middle of tail
Tail	Subcylindrical and broadly rounded	*Slender with fingerlike ventral projection with rounded end	Hemispherical or truncate at back	Not indicated	Subcylindrical and broadly rounded	Conoid	Cylindrical with irregularly indented terminus or very rarely with flat terminus	*Slender with fingerlike ventral projection with rounded end	Conoid or subcylindrical, straight or slightly curved ventral	*Slender with fingerlike ventral projection with rounded end	Subcylindrical, obtusely rounded annulated tip	*Slender with fingerlike ventral projection with rounded end
Tail annuli	20-35	-	27-44	-	24-43	-	-	-	24-46	-	29-42	-

APPENDIX I

Compendium of species characteristics and diagnostic species measurements for the genus *Histotylenchus* Siddiqi, 1971. All measurement given in µm. *Characteristic not given in text but derived from illustrations. Females (♀♀) and males (♂♂).

Species												
	Histotylenchus baoulensis		***Histotylenchus hedys***		***Histotylenchus histoides***		***Histotylenchus historicus***		***Histotylenchus mohalei***		***Histotylenchus sudanensis***	
	According to Netscher & Germani (1969)		According to Kleynhans (1975); Kleynhans & Heyns (1984)		According to Siddiqi (1971); Kleynhans (1975); Kleynhans & Heyns (1984)		According to Jairajpuri & Baqri (1968); Kleynhans (1975)		According to Kleynhans (1992)		According to Siddiqi (1977)	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
L	870-1090	840-1010	1017-1250	987-1124	1024-1450	1000-1200	910-1170	910-1110	891-1184	844-1112	1070-1250	1030-1140
Head width	-	-	12-13	10-15	10-12	9-11	-	11	8-11	8-11	10	-
Stylet length	20-22	18-23	23-26	22-25	21-26	21-23	19-21	23	18-22	17-22	22-24	21-23
Conus length	-	-	10-12	12-13	10-11	-	7-9	-	8-10	7-10	-	-
Anterior end to excretory pore length	116-145	113-144	137-162	141-172	141-174	139-145	-	-	112-152	112-148	138-149	-
Tail length	30-45	26-44	31-47	37-53	27-43	39-42	37	41	33-57	43-64	-	-
Spicule length	-	23-29	-	32-35	-	29-34	-	24-25	-	27-34	-	30-33
Gubernaculum length	-	-	-	12-14	-	12-16	-	10-14	-	9-15	-	13-14
a	28-42	28-39	26-39	26-39	29-45	35-44	40-49	37-45	34-47	38-53	38-46	41-50
b	4-6	4-5	8-9	-	7-8	7-8	-	5-7	7-9	7-9	7-9	7-8
b'	-	-	5-7	5-6	5-7	5-6	5-7	-	5-7	5-6	6	5-6
c	20-33	20-33	24-33	21-27	23-40	19-28	25-35	21-29	18-31	15-23	25-31	22-27
c'	-	-	-	-	2	-	2	-	2-3	2-4	2	2-3
m (%)	-	-	-	-	-	-	38-45	-	41-49	36-50	44-50	41-45
V (%)	52-61	-	50-55	-	50-58	-	50-63	-	49-58	-	50-56	-
T (%)	-	41-50	-	-	-	40-51	-	33-42	-	-	-	37-44

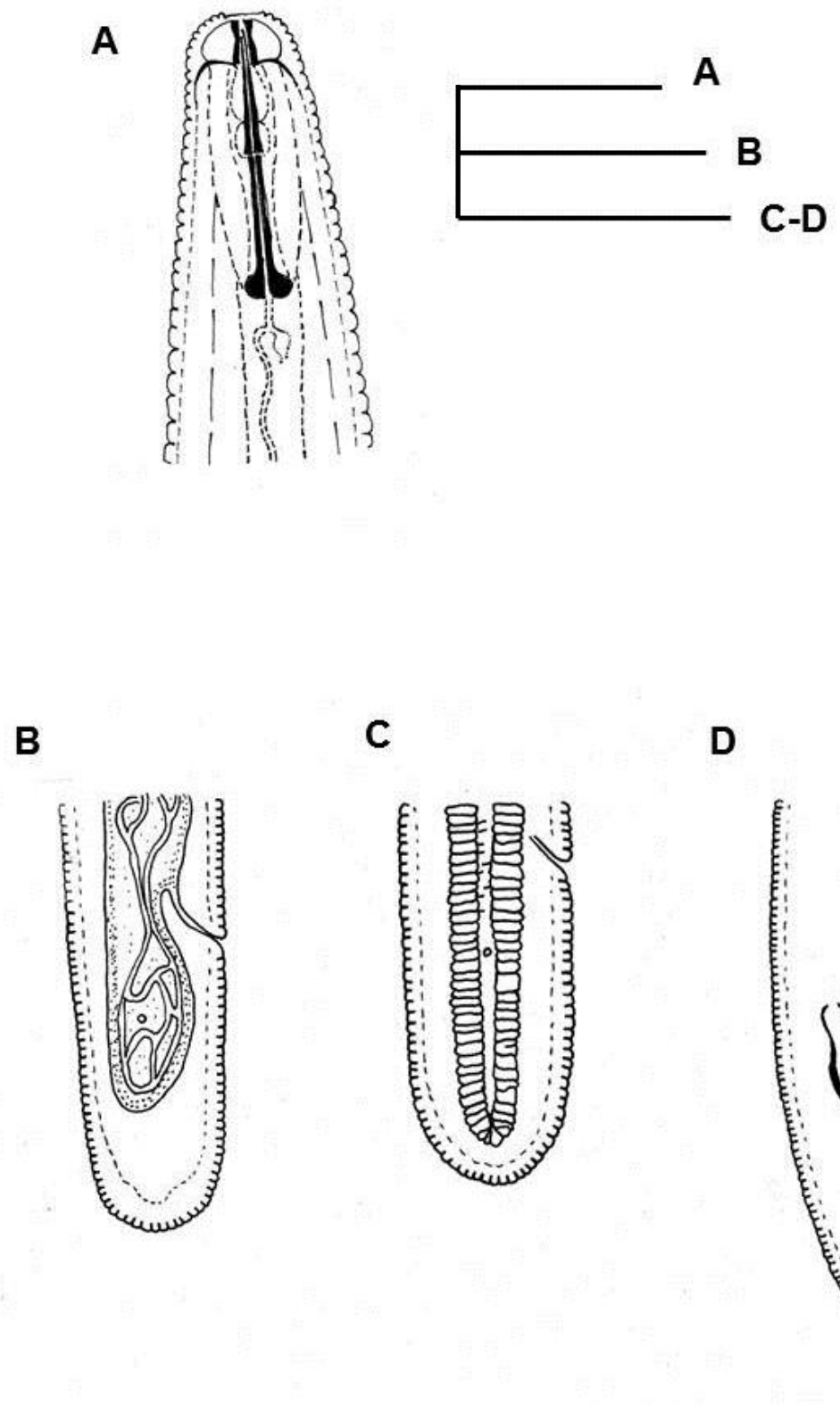

Figure I.I: *Histotylenchus baoulensis* Netscher & Germani, 1969. Female: **A**- lip region; **B**- tail with postanal diverticulum and fasciculi; **C**- tail with lateral field. Male: **D**- tail. **Scale bars** = 30 µm. Redrawn from Netscher & Germani (1969).

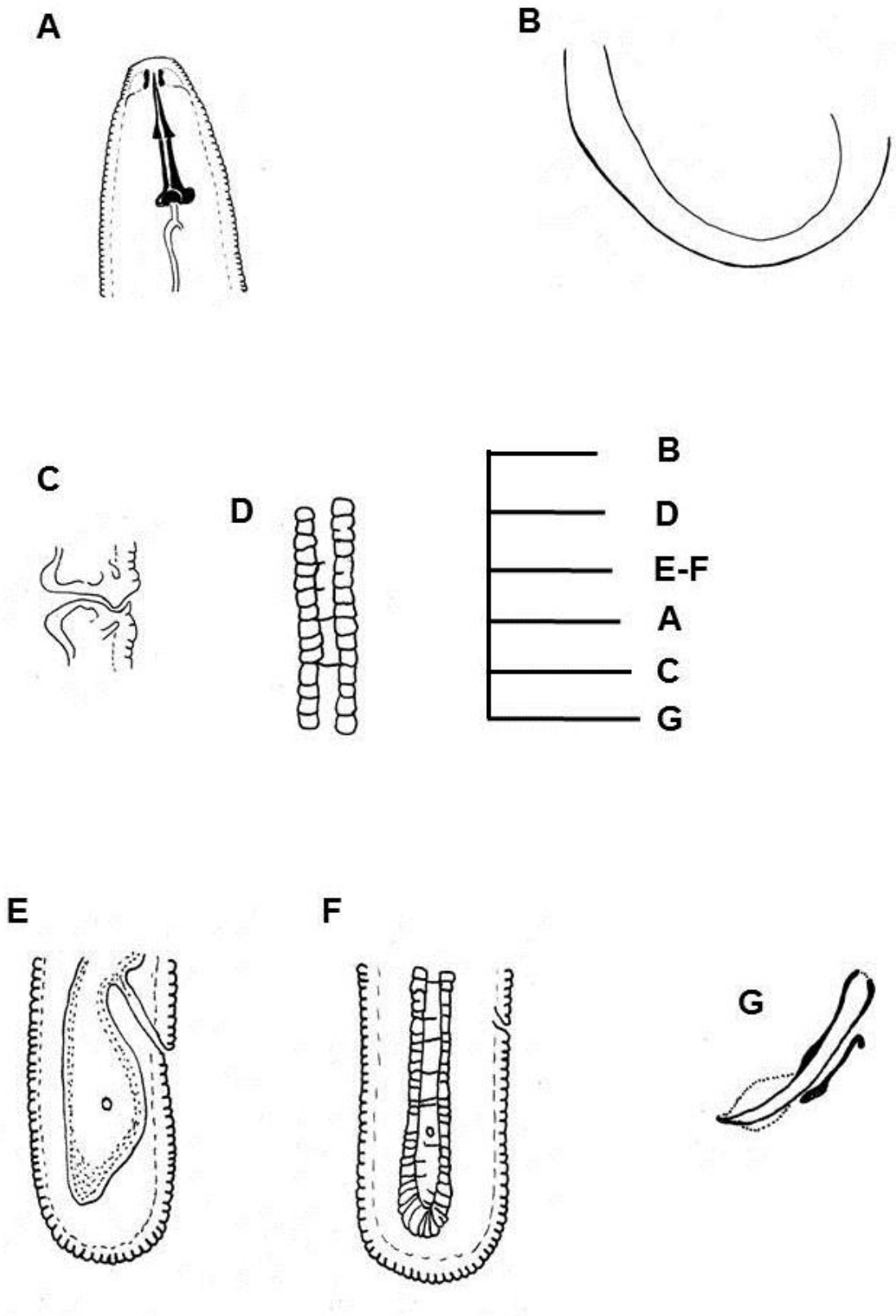

Figure I.II: *Histotylenchus hedys* Kleynhans, 1975. Female: **A**- lip region; **B**- habitus; **C**- vulva; **D**- lateral field opposite vulva; **E**- tail with postanal diverticulum; **F**- tail with lateral field. Male: **G**- spicules. **Scale bars** = 20 µm. Redrawn from Kleynhans (1975) and Kleynhans & Heyns (1984).

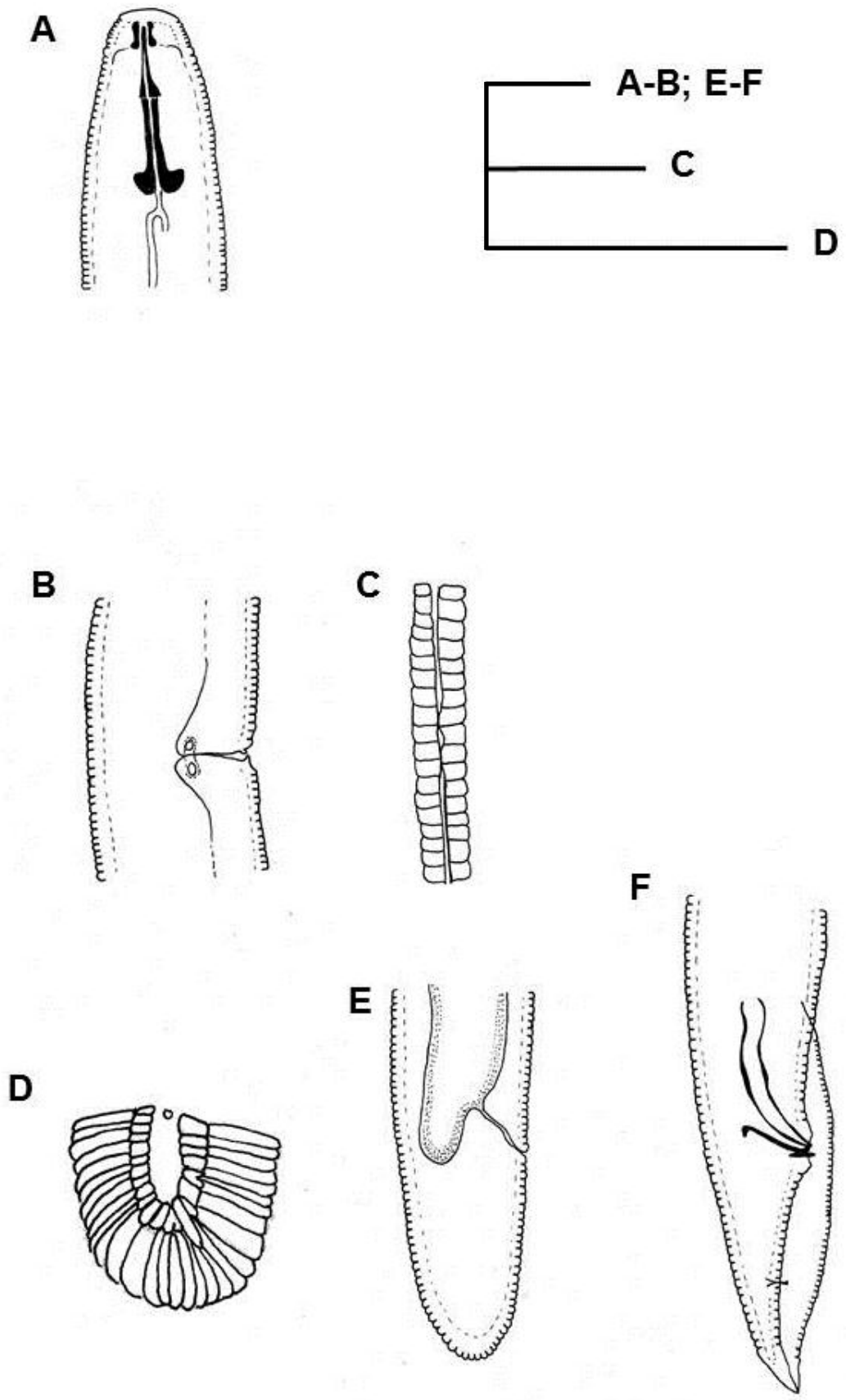

Figure I.III: *Histotylenchus histoides* Siddiqi, 1971. Female: **A**- lip region; **B**- vulva; **C**- vulva; **D**- tail with lateral fields; **E**- tail with postanal diverticulum. Male: **F**- tail**. Scale bars** = 15 µm (A-B; E-F) and 25 µm (C-D). Redrawn from Siddiqi (1971) and Kleynhans & Heyns (1984).

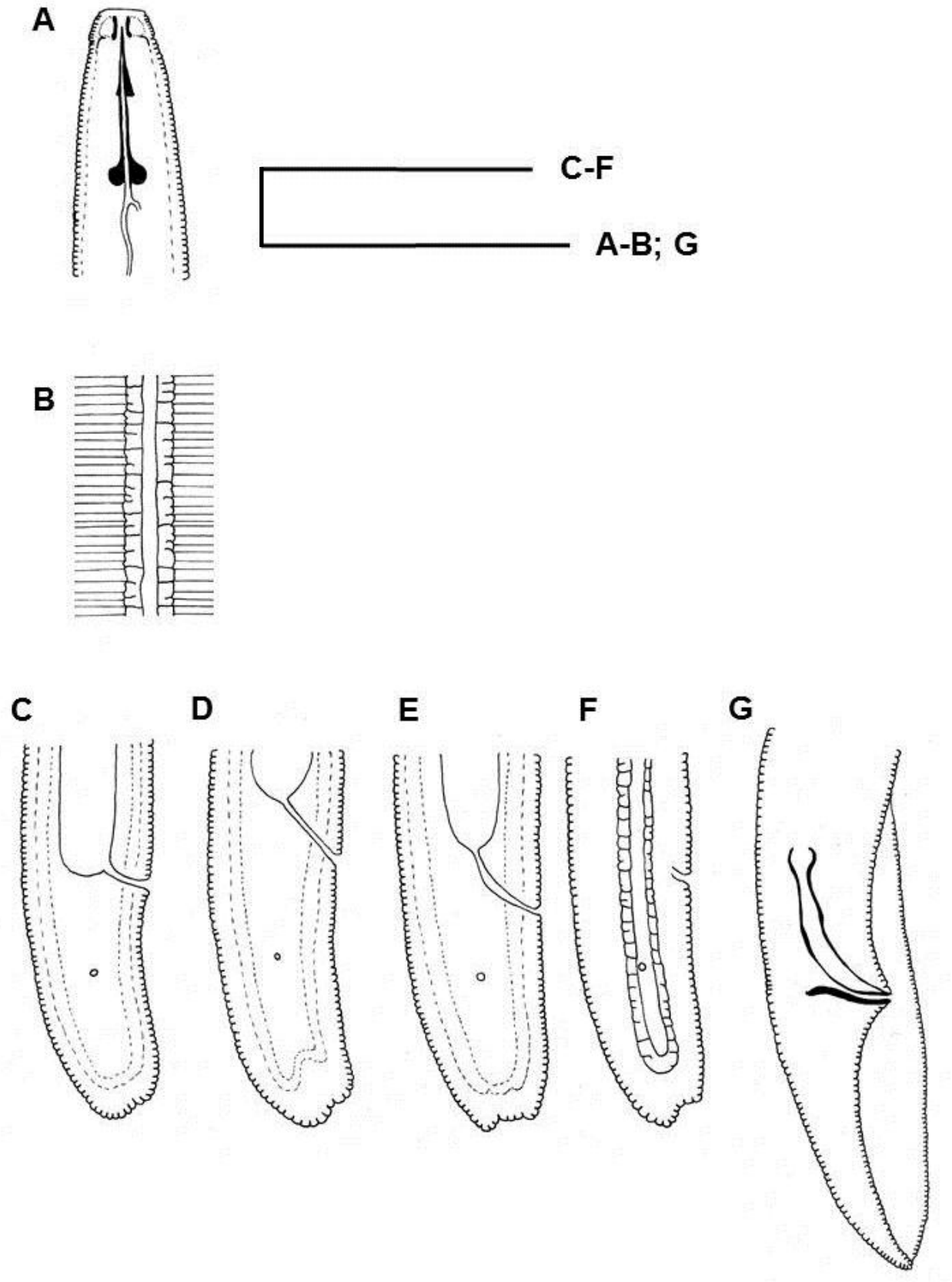

Figure I.IV: *Histotylenchus historicus* Jairajpuri & Baqri, 1968. Female: **A**- lip region; **B**- lateral field in opposite vulva; **C-E**- tail with postanal diverticulum; **F**- tail with lateral field. Male: **G**- tail. **Scale bars** = 40 µm. Redrawn from Jairajpuri & Baqri (1968).

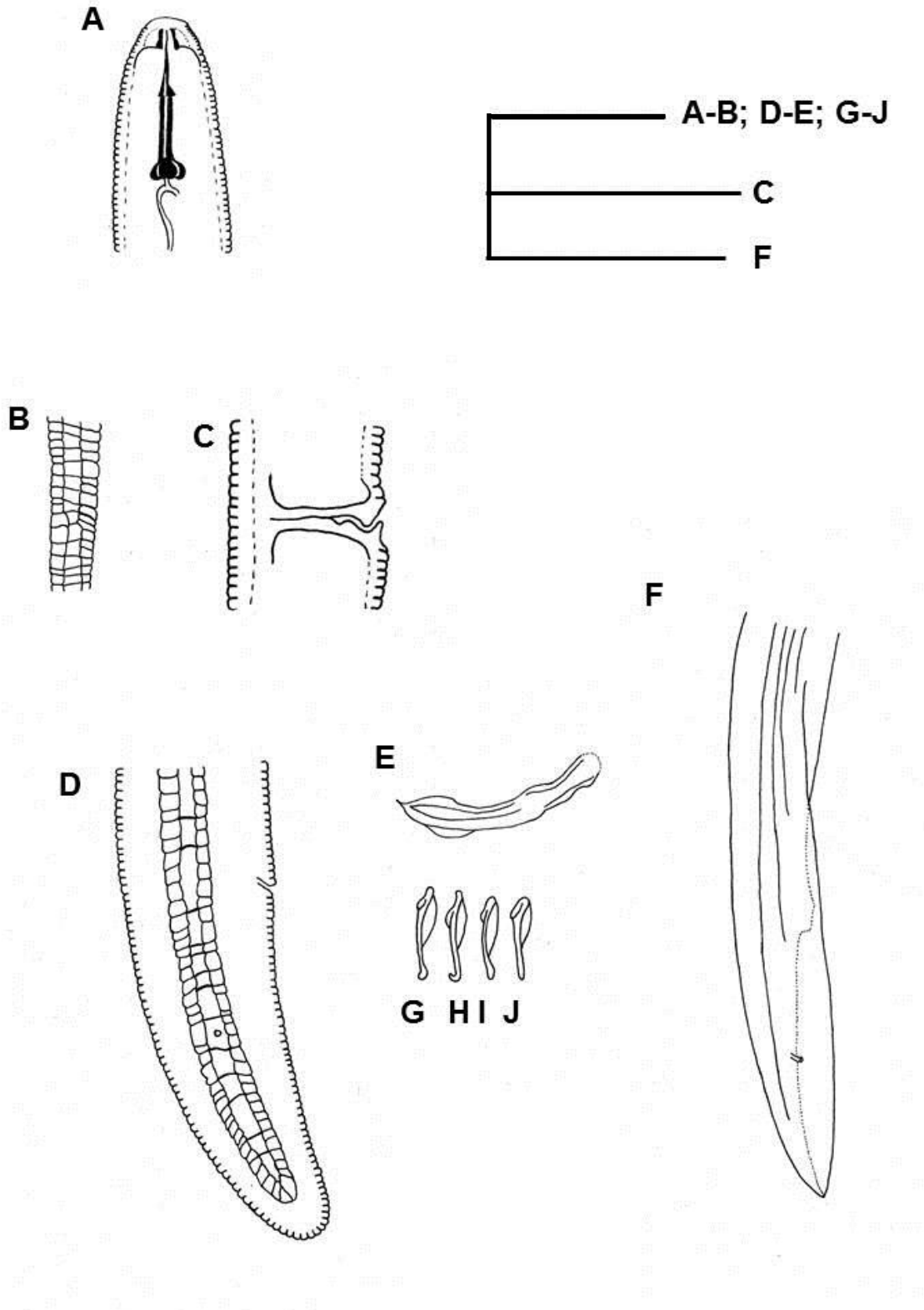

Figure I.V: *Histotylenchus mohalei* Kleynhans, 1992. Female: **A**- lip region; **B**- lateral field in vulva region; **C**- vulva; **D**- tail with lateral field. Male: **E**- spicule; **F**- tail with lateral lines; **G-J**- gubernaculums. **Scale bars** = 25 µm (A-E; G-J) and 50 µm (F). Redrawn from Kleynhans (1992).

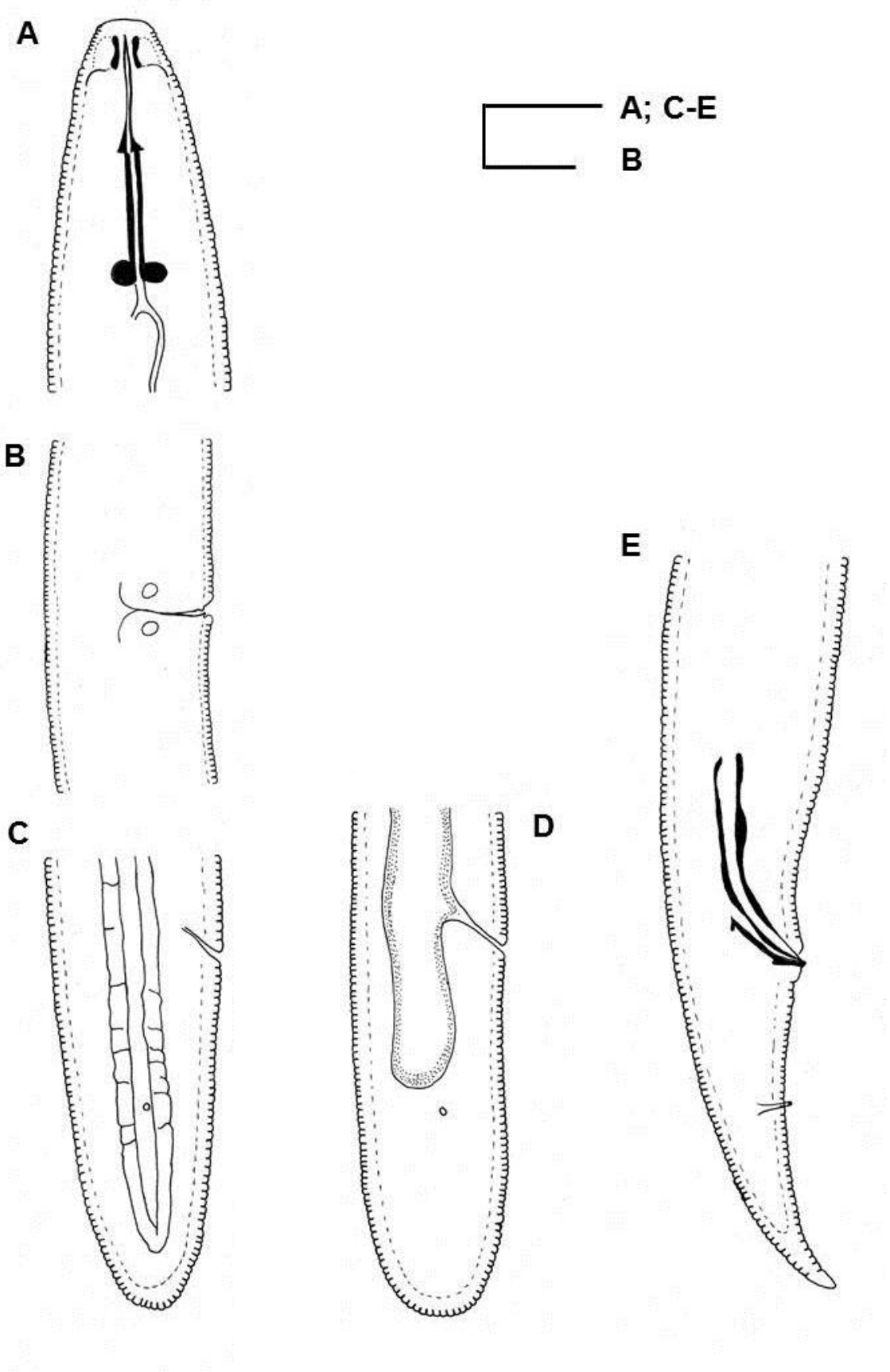

Figure I.VI: *Histotylenchus sudanensis* Siddiqi, 1977. Female: **A**- lip region; **B**- vulva; **C**- tail with lateral field; **D**- tail with postanal diverticulum. Male: **E**- tail. **Scale bars** = 10 µm. Redrawn from Siddiqi (1977).

Appendix II
Different diagnostic characters used in the description of *Histotylenchus* species.

BODY

Habitus

The characteristic body posture of heat-killed specimens can be used to distinguish between spiral-shaped species and species assuming a C-shape (ventrally arcuate), as seen in Fortuner (1984). In *Histotylenchus* the habitus is variable, ranging from straight to C-shaped.

Body length

Body length is indicated in all the *Histotylenchus* species descriptions, but because of the overlap that does occur between the different species, body length as a species differentiating character must be used with caution. The body length for *Histotylenchus* females ranges from 870 µm [*H. baoulensis* in Netscher & Germani (1969)] to 1450 µm [*H. histoides* in Siddiqi (1971)] and 840 µm [*H. baoulensis* in Netscher & Germani (1969)] to 1200 µm [*H. histoides* in Kleynhans (1975)] in males.

Lip region (labial region)

According to Kleynhans (1997), in members of Tylenchida the head is supported internally by a sclerotized cephalic skeleton. The cephalic skeleton consists of a basal plate, vestibule (cylindrical wall of the anterior stoma) and six blades connecting the basal plate with the stoma wall. The cuticular area from the basal ring forward, as defined by Caveness (1974), of the genus *Histotylenchus* is either hemispherical or marked off by a depression and continuous with the body or offset (Fig II.I).

Labial disc

Eisenback (1998) defines the labial disc as the more or less circular form of cuticle about the oral opening and that is delimited posteriorly by the first transverse striation. The form of the disc that can only be seen in an *en face* view, was not given in any of the *Histotylenchus* descriptions, but Gomez-Barcina *et al*. (1992) noted that *Histotylenchus* has a square disc. In the current study the *en face* view of *Histotylenchus mohalei* is shown as rectangular (Chapter 4).

Lip annuli

The number of lip annuli varies from six [*H. baoulensis* in Netscher & Germani (1969)] to eleven [*H. histoides* in Siddiqi (1971)]. Due to the fact that it is difficult to determine the number of lip annuli as seen under the light microscope and the inter-species overlap of number of annuli, the number of lip annuli can currently not be used as a diagnostic character.

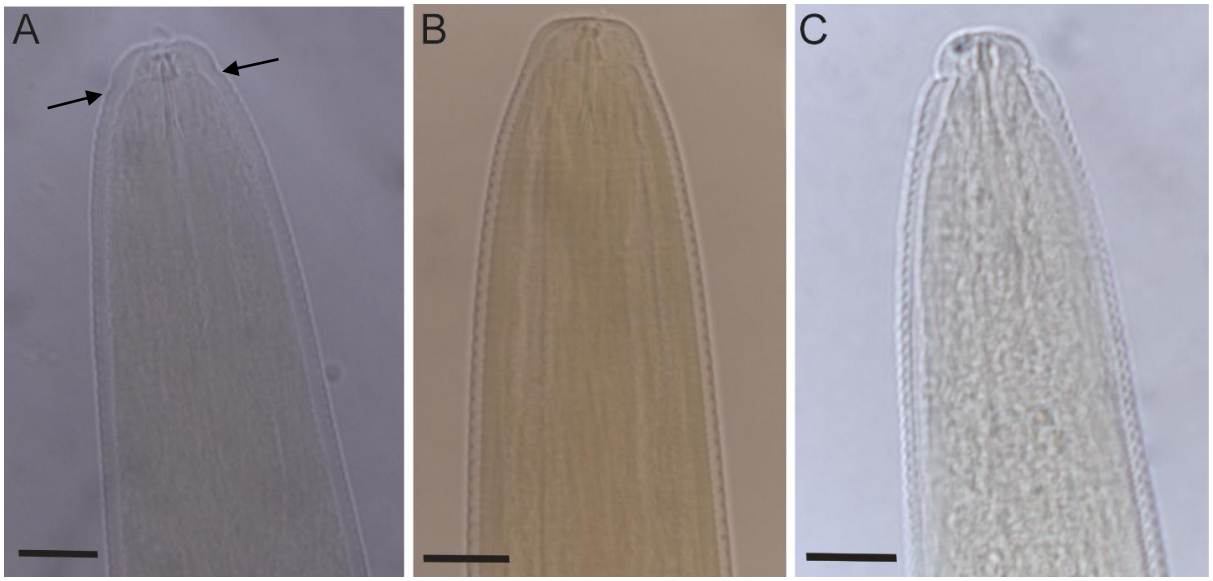

Figure II.I: Light micrographs of lip region of genus *Histotylenchus* Siddiqi, 1971. **A**- lip region hemispherical or anterior flattened marked off by a depression (depression indicated by arrows); **B**- lip region hemispherical and continuous with the body and **C**- lip region offset. **Scale bar** = 10 µm.

LATERAL FIELD

Caveness (1974) defined the lateral field as a form of cuticular configuration above the lateral cords. Eisenback (1998) broadened this definition to include longitudinal striae (=incisures) and transverse markings (areolations) that may divide the field. *Histotylenchus* species have four lateral lines or incisures in the lateral field. In all *Histotylenchus* species the two inner lines of the lateral field come together on tail in a specific pattern. For this dissertation the pattern of the inner lines is used as follow (Fig. II.II), described by Fortuner (1984):

1. Y-shaped: the two inner lines fuse posteriorly to the phasmids and continue as one line for some distance (Fig. II.II E).

2. V-shaped: the two inner lines fuse posteriorly to the phasmid but the shared leg after the connection is very short and is therefore described as V-shaped rather than Y-shaped (Fig. II.II D).

The presence of transverse striae in the lateral field (areolations) is mentioned in all original *Histotylenchus* species descriptions and the extent of the areolation can be used as a diagnostic character to some extent, e.g. *H. sudanensis* females lateral field is without areolations except opposite the oesophageal region and in the outer bands on the rest of the body (Siddiqi, 1977), whereas in the rest of the *Histotylenchus* species all three bands of the females are areolated to a greater or lesser degree. In both *H. baoulensis* and *H. histoides* the inner lines of the lateral field are markedly irregular opposite the vulva, and occasionally fuse in this region for a short distance (Kleynhans & Heyns, 1984).

TAIL

The tail can simply be defined as the portion of the body behind the anus to the tail terminus (Heyns, 1971; Eisenback, 1998). Tail shape is an important diagnostic characteristic mentioned in all *Histotylenchus* descriptions. Unfortunately there is no constant terminology used in the description of the tail shape. In this dissertation the tail shape is defined as follows: short, symmetrical rounded or as long symmetrical rounded (Fig. II.II A, B, C).

Tail length

Tail length is indicated in all species descriptions, either in µm or as the number of anal body widths. The length of the tail is given in four of the six original descriptions of *Histotylenchus* species and in females varies from 27 µm [*H. histoides* in Siddiqi (1971)] to 57 µm [*H. mohalei* in Kleynhans (1992)] and males from 37 µm [*H. hedys* in Kleynhans (1975)] to 64 µm [*H. mohalei* in Kleynhans (1992)] depending on the species.

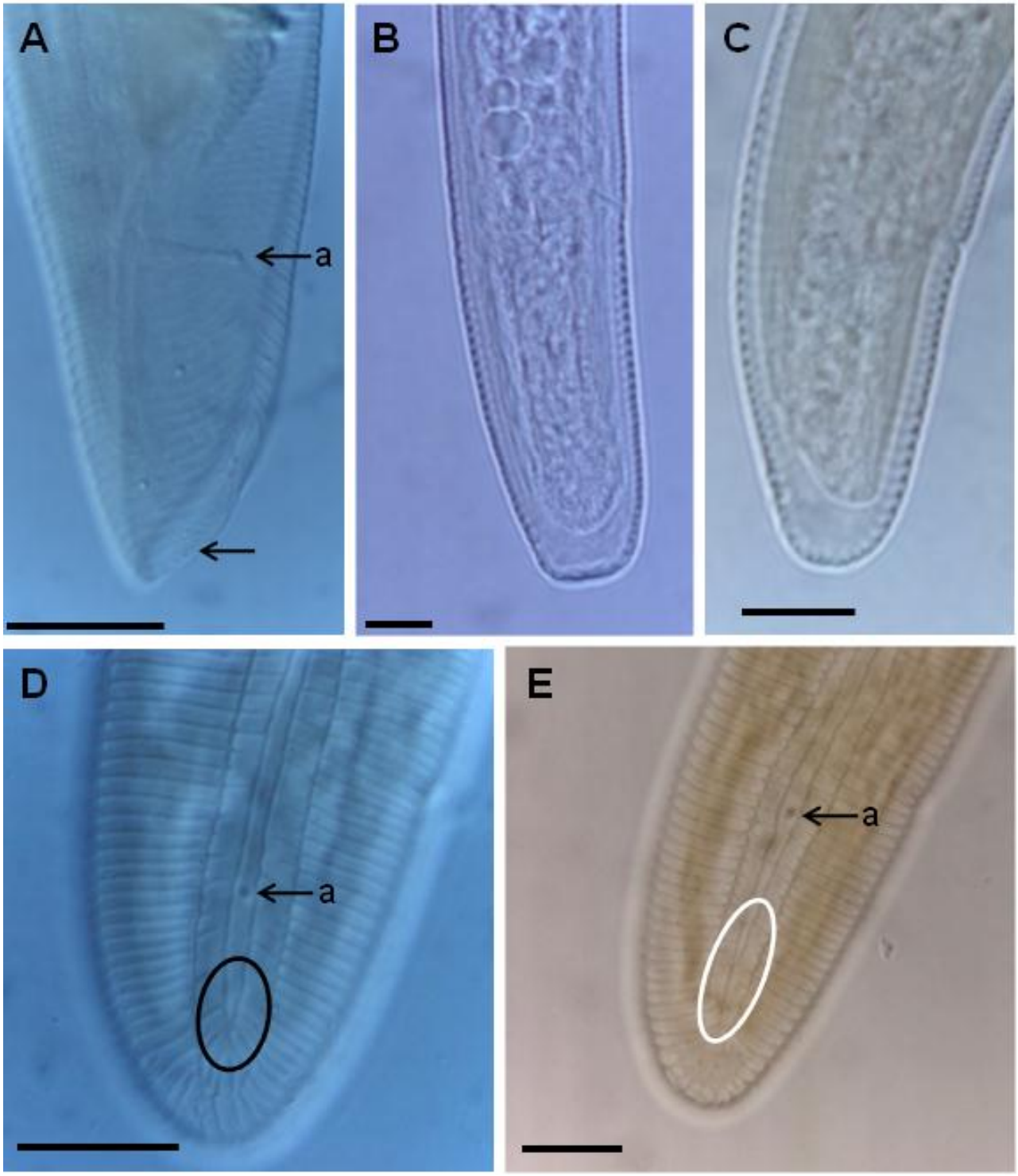

Figure II.II: Light micrographs of *Histotylenchus* Siddiqi, 1971. Male: **A**- tail region with phasmids (black arrow a) and bursa (black arrow). Female: **B**- short, symmetrical rounded tail; **C**- long, symmetrical rounded tail; **D**- tail with phasmids (black arrow a) and lateral field lines ending in V-shaped pattern (black circle) and **E**- tail with phasmids (black arrow a) and lateral field lines ending in Y-shaped pattern (white circle). **Scale bars** = 10 µm.

Ratio c

According to Fortuner (1990) a ratio is very objective and useful tool in taxonomy and is often used to discriminate between taxa (Roggen & Asselberg, 1971). Ratio c, is used to express the relative tail length when compared to the body length, and is given in all the original descriptions of *Histotylenchus* species. As is the case when comparing body lengths, ratio c can differentiate between some species. Ratio c in females of *H. mohalei* is 18-31 *vs* 23-40 for *H. histoides*, but inter species overlap does occur and the use of ratio c as a diagnostic character is therefore restricted as the variability of ratio c is generally higher than that of tail length e.g. in *H*. *hedys* where the coefficient of variability value for ratio c (11 %) is higher than tail length coefficient of variability value (7 %) and in *H. mohalei*, where the coefficient of variability value for ratio c (11 %) is higher than tail length coefficient of variability value (10 %).

Ratio c'

Ratio c' is only given in three of the six original descriptions of *Histotylenchus* species and only in the description of *H. mohalei* and *H. sudanensis* is this ratio given for both females and males (Jairajpuri & Baqri, 1968; Kleynhans, 1975; Siddiqi, 1977; Kleynhans, 1992). Ratio c' is used to indicated whether the tail is long and thin (high ratio c') or short and stubby (low ratio c') (Fortuner, 1984). The use of c' as a diagnostic characteristic in the genus *Histotylenchus* is debatable, because of the inter-species overlap. Reported c' values for *H. mohalei* females ranges from 2-3 and the large overlap in the range of values between species, e.g. when comparing *H. mohalei* (females: 2-3) with *H. sudanensis* (females: 2) (Siddiqi, 1977; Kleynhans, 1992). The value does, however, enable the taxonomist to make more accurate descriptions of tail length and form, based on measurements rather than just on subjective observations.

Number of ventral tail annuli

The number of ventral tail annuli is given in four of the six species descriptions for females and ranges from 24 [*H. histoides* in Siddiqi (1971) and *H. mohalei* in Kleynhans (1992)] to 46 [*H. mohalei* in Kleynhans (1992)]. This characteristic has no diagnostic value because of the inter- species overlap.

SENSORY SYSTEM

Components of the nervous and sensory system used as diagnostic characters include the phasmids, hemizonid and the secretory-excretory pore.

Phasmids

Phasmids, are pore-like sensory structures found in the tail region opening to the exterior of the lateral precaudal glands and probably have a sensory function (Eisenback, 1998; Decraemer & Hunt, 2006). In *Histotylenchus* the small but distinct phasmids are in the middle of the lateral field situated posterior to the anus and cloacal opening in the middle of the lateral field (Fig. II.II).

Hemizonid

The hemizonid is a nerve commissure of the longitudinal nerves extending round the ventral half of the body in the vicinity of the nerve ring, and is usually seen as a refractive subcuticular structure near the secretory- excretory pore (Decraemer & Hunt, 2006). In *Histotylenchus* the hemizonid is always anterior of the secretory-excretory pore.

Secretory-excretory pore

The secretory-excretory pore is the ventral opening of the secretory-excretory system as a transverse canal leading from one or two excretory cells, usually situated in the oesophagus region (Wallace, 1963; Eisenback, 1998; Gibbons, 2006). The position of the pore when measured from the anterior end, was given in four of the six species descriptions. The range for the genus is 116 µm (*H. baoulensis* in Netscher & Germani, 1969) to 174 µm [*H. histoides* in Kleynhans & Heyns (1984)] for females and 112 µm [*H. mohalei* in Kleynhans (1992)] to 172 µm [*H. hedys* in Kleynhans (1975)] for males. In this dissertation the position of the secretory-excretory pore is expressed in terms of its positon relative to the oesophageal lobe (basal lobe), either anterior to oesophageal lobe, middle of oesophageal lobe or posterior to oesophageal lobe and also the distance from the anterior end to the secretory-excretory pore and the position as a percentage of the body length and the oesophageal lobe.

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ALIMENTRY TRACT

Nematodes can be classified to some degree based on the form of the alimentary tract (Heyns, 1981; Von Lieven, 2003). According to Maggenti (1991) the alimentary tract subdivides into the stomodeum, mesenteron and proctodeum. The stomodeum consists of the stoma, oesophagus and the oesophago-intestinal valve. The mesenteron (derived from embryonic endoderm) is commonly referred to as the intestine and starts after the junction of oesophageal bulb with intestine. The proctodeum or rectum forms the most posterior part of the alimentary tract (Maggenti 1981; 1991). According to Maggenti (1981; 1991) and Baldwin & Perry (2004) in males the cloacal opening is formed by the junction of the rectum and the reproductive tract.

Stoma

The stoma is the cavity transition located between the oral opening and the oesophagus (Heyns, 1971; Baldwin & Perry, 2004) and according to Heyns (1971) often incorrectly referred to as the pharynx. From anterior to posterior the stoma consists of: the cheilostome and esophastome, with the esophastome dividing into the prostome (prostome, mesostome and metostome) and the telostome (Maggenti, 1981; 1991) but according to Eisenback (1998) and Baldwin & Perry (2004), the stoma consists of the cheilostom, gymnostom and stegostom with the stegostom subdivided into (from anterior to posterior) the prostom, mesostom, metastom and telostom. For this dissertation the structure of the stoma is based on Maggenti (1981).

In *Histotylenchus* the cheilostome usually includes the cephalic or labial framework, the stomatal cavity and the stylet conus. Stylet length in females range from 18 µm [*H. mohalei* in Kleynhans (1992)] to 26 µm [*H. histoides* in Kleynhans (1975)] and 30 µm in *Histotylenchus niveus* sp. n. and in males from 17 µm [*H. mohalei* in Kleynhans (1992)] to 26 µm [*H. histoides* in Kleynhans (1975)] and 28 µm in *H. niveus* sp. n. In *Histotylenchus* the asymmetrical stylet conus is considered the diagnostic characteristic for the genus (Siddiqi, 1971). The stylet cone is hollow with a subterminal ventral opening. In *Histotylenchus* the stylet conus ranges in length from 7 µm [*H. historicus* in Jairajpuri & Baqri (1968)] to 12 µm [*H. hedys* in Kleynhans (1975)] and 17 µm (*H. niveus* sp. n.) in females and 7 µm [*H. mohalei* in

Kleynhans (1992)] to 13 µm [*H. hedys* in Kleynhans (1975)] and 15 µm (*H. niveus* sp. n.) in males. The esophastome includes an elongate tubular shaft, ending in three stylet knobs. The stylet knobs mainly function as apodemes for stylet muscles (Maggenti, 1981; 1991). In *Histotylenchus* the stylet knobs are mostly round, sloping backwards but specimens with stylet knobs more angular in shape are observed. Andrássy (1962) redefined the stylet conus to the metenchium and the shaft (including the stylet knobs) to the telenchium. In *Histotylenchus* the metenchium is shorter to half the length of the telenchium with m-value of 38-50 % (Jairajpuri & Baqri, 1968; Siddiqi, 1977).

Oesophagus

The oesophagus, according to Maggenti (1991), is the most complex organ in the nematode body, and includes nerve, gland, muscle and hypodermal tissue. The oesophagus of *Histotylenchus* subdivides into the corpus, the isthmus and the posterior bulb also known as the postcorpus. The corpus subdivides anteriorly into the procorpus (a narrow nonmuscular region) and posteriorly the metacorpus (a larger muscular pump) (Baldwin & Perry, 2004). In *Histotylenchus* the postcorpus can overlap the intestine ventral (as in *H. hedys*; *H. histoides* and *H. mohalei*) or dorsal (as in *Histotylenchus niveus* sp. n.). The coefficient of variation (CV) value for oesophagus length (7 %) is much lower than CV value of ratio b (26 %).

Intestine

According to Baldwin & Perry (2004), the intestine (mesenteron) is the largest organ in the digestive system linking the oesophagus with the rectum (Heyns, 1971). The main functions of the intestine include absorption, storage and secretion of digestive enzymes (Baldwin & Perry, 2004). In the females of some species of *Histotylenchus* the intestine extends past the rectum (referred to as a post- anal diverticulum). The length of the postanal diverticulum is used as a species differentiating characteristic in *Histotylenchus*. The postanal diverticulum ranges from absent as *H. historicus* in Jairajpuri & Baqri (1968) to present. The presence of the postanal diverticulum can further be distinguished into either being present but the overlap does not reach the level of the phasmids as in *H. baoulensis*, *H. sudanensis*, *H. mohalei* and *H. histoides* (Netscher & Germani, 1969; Siddiqi, 1971; Siddiqi, 1977; Kleynhans, 1992)

or present and very large, extending to well past phasmids as in *H. hedys* (Kleynhans, 1975).

Fasciculi, also known as lateral- or sinuous canals (Fig II.III), are present (*H. baoulensis*, *H. hedys*, *H. mohalei*, *H. sudanensis* and *H. histoides*) or absent (*H. historicus*) in the intestine. According to Byers and Anderson (1973), and Eisenback (1998), fasciculi are composed of filaments and rod-like elements organised parallel to one another and parallel to the axis of the fasciculi, Byers and Anderson (1973) further propose three possible functions for fasciculi. Firstly that these structures are paracrystalline storage enclosures, secondly that fasciculi form as a result of aggregation of virus particles or the inclusion of virus particles as a result of a viral infection or thirdly that fasciculi act as contractile elements or supportive elements. The authors went on to dismiss the first and second theory and concluded that the function of fasciculi is either contractile or supportive.

REPRODUCTIVE SYSTEM

Female reproductive system

According to Maggenti (1981), the female reproductive system includes the tubular ovary (terminating in a cap cell), oviduct, uterus and the vagina (opening to the exterior through the vulva). The female reproductive system of *Histotylenchus* is didelphic and amphidelphic with outstretched ovaries, as defined by Maggenti (1981). The position of the vulva is a good taxonomic characteristic and ranges from 48 % (*H. niveus* sp. n) to 65 % [*H. mohalei* in Kleynhans (1992)] and has a low coefficient of variation (CV) value, 3 % (*H. hedys* and *H. histoides*) to 5 % (*H. mohalei*).

Vulva and epiptygma

The vulva, in adult females, can be described as a cuticle-covered opening in the hypodermis at the ventral exterior of the nematode. *Histotylenchus* vulva form is similar to vulva form of nematodes in general and the opening can be described as a transverse or a longitudinal slit or symmetrical pore on the ventral side of the body (Filip'ev & Schuurmans Stekhoven, 1959; Carta *et al*., 2009) and in *Histotylenchus* is situated at midbody. Eisenback (1998) defined the epiptygma as a vulval flap. In

Histotylenchus the epiptygma is single as (*H. baoulensis*) or double (*H. hedys*, *H. histoides*, *H. mohalei* and *H. sudanensis*).

Male reproductive system

The monorchic male reproductive system consists of a testis anteriorly followed by a vas eferens, seminal vesicle (main function is for storing sperm), and a vas deferens and ductus ejaculatorius (Baldwin & Perry, 2004).

The spicule (specialised dorsal wall cells of the cloaca) of *Histotylenchus* includes a manubrium (head), shaft, lamina (blade) and vellum (membraneous extension) (Maggenti 1981). The spicule in *Histotylenchus* is clearly visible and according to Filip'ev & Schuurmans Stekhoven (1959) of great value for all nematode species diagnosis. The gubernaculum is a cuticular thickening of the dorsal wall of the spicular pouch acting as a guide during spicule protrusion (Maggenti, 1981; Decreamer & Hunt, 2006). In *Histotylenchus* the gubernaculum is protrusible as found in *H. histoides*, *H. mohalei*, and *H. sudanensis* (Siddiqi, 1971; Siddiqi, 1977; Kleyhans, 1992) or non protrusible as in *H. baoulensis* (Netscher & Germani, 1969) and *H. hedys* (Kleynhans, 1975). The entire sub-ventral cuticular wings are referred to as the bursa copulatrix or simply the bursa (Filip'ev & Schuurmans Stekhoven, 1959) that clasps the female during copulation (Eisenback, 1998) and in all *Histotylenchus* species terminate in the tail tip, except in *H*. *historicus*.

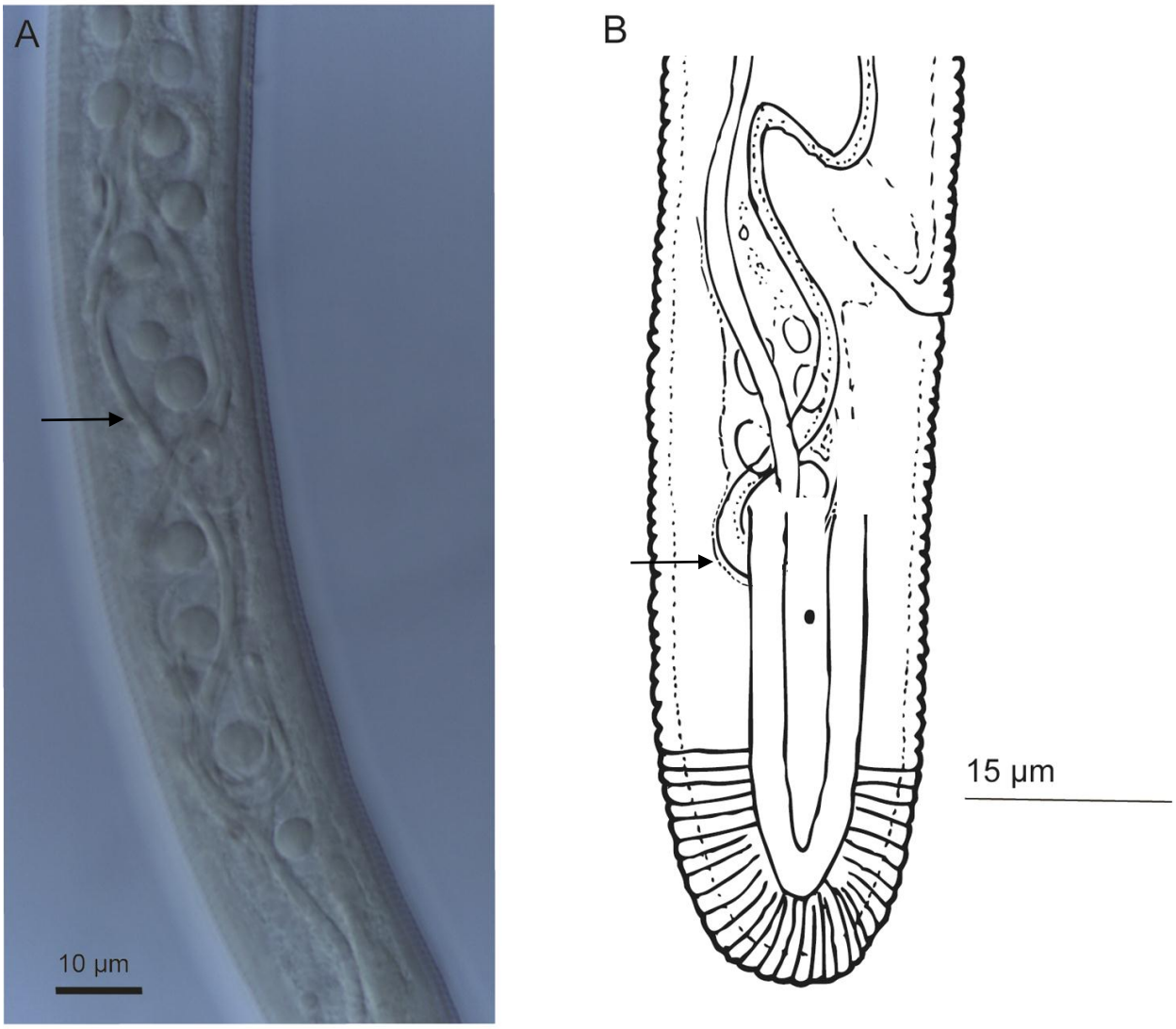

Figure II.III: Fasciculi indicated by black arrows in **A**- Differential Interference Contrast (DIC) light micrograph of intestine of *Histotylenchus mohalei* Kleynhans, 1992 and **B**- tail of *Histotylenchus sudanensis* Siddiqi, 1977 (redrawn from Siddiqi, 1977). **Scale bars as indicated.**

Appendix III

Locality data of all material examined.

APPENDIX III

Table III: Locality data of all examined material. Biome and vegetation type identification according to Low & Rebelo (1996).

Locality number	Slide number	Date sampled	Collector	Country	Province	Locality	Georeference	Biome	Vegetation type	Associated plant
***Histotylenchus* species 1**										
TVL754	13324; 13325	31.03.1977	E. van Wyk & L. van Wyk	South Africa	Limpopo	Mookgopong	-24.333; 28.833	Savanna	-	*Vitis* sp. L.
***Histotylenchus* species 2**										
KP822	14365	06.09.1978	L. Radloff	South Africa	Eastern Cape	Port Elizabeth	-31.033; 25.516	Thicket	Dune Thicket	*Solanum lycopersicum* L.
Histotylenchus hedys										
N5 (Paratype)	706; 708	21.03.1962	J. Heyns	South Africa	KwaZulu-Natal	Empangeni	-28.633; 32.066	Savanna	Coastal Bushveld/ Grassland	*Saccharum* sp. L.
N291	15559	27.12.1978	E. van den Berg	South Africa	KwaZulu-Natal	uKhahlamba-Drakensberg Park, Loteni, Nature Reserve	-29.550; 29.566	Grassland	Moist Upland Grassland	*Cephalaria oblongifolia* (Kuntze) Szabó
N414	20724	12.11.1981	V.W. Spaull	South Africa	KwaZulu-Natal	Mposa	-28.616; 32.066	Savanna	Coastal Bushveld/ Grassland	*Saccharum* sp. L.
N620	26789; 26790	03.07.1990	-	South Africa	KwaZulu-Natal	Hluhluwe,farm, Nyalazi	-27.966; 32.316	Savanna	Coastal Bushveld/ Grassland	Grass (unidentified)

APPENDIX III

Table III continued: Locality data of examined material. Biome and vegetation type identification according to Low & Rebelo (1996).

Locality number	Slide number	Date sampled	Collector	Country	Province	Locality	Georeference	Biome	Vegetation type	Associated plant
Histotylenchus histoides (Paratype)	C.B.H. no 24/69	-	M. R. Siddiqi	Malawi	Limbe	Bvumbwe Agricultural Research Station	-15.808; 35.057	-	-	*Pennisetum purpureum* Schumach
(Paratype)	RNC 48F/1/2	-	W.H.T Peregrine	Malawi	Limbe	Bvumbwe Agricultural Research Station	-15.808; 35.057	-	-	*Pennisetum purpureum* Schumach
(Paratype)	RNC 48F/1/3	-	W.H.T Peregrine	Malawi	Limbe	Bvumbwe Agricultural Research Station	-15.808; 35.057	-	-	*Pennisetum purpureum* Schumach
(Paratype)	RNC 48F/1/4	-	-	Malawi	Limbe	Bvumbwe Agricultural Research Station	-15.808; 35.057	-	-	*Pennisetum purpureum* Schumach
Histotylenchus mohalei OVS227 (Paratype)	19022; 19023	06.02.1982	E. van den Berg	South Africa	Free State	Golden Gate Highlands National Park	-28.516; 28.516	Grassland	Wet, Cold Highveld Grassland	*Pelargonium luridum* (Andrews) Sweet
OVS252 (Paratype)	19222; 19225; 19229; 19231 - 19238; 19240	06.02.1982	E. van den Berg	South Africa	Free State	Golden Gate Highlands National Park	-28.516; 28.516	Grassland	Wet, Cold Highveld Grassland	Grass (unidentified)
OVS266 (Paratype)	19365	01.02.1982	E. van den Berg	South Africa	Free State	Golden Gate Highlands National Park	-28.516; 28.516	Grassland	Wet, Cold Highveld Grassland	*Rhus discolour* L.
TVL844 (Paratype)	17812	01.02.1981	E. van den Berg	South Africa	Limpopo	Bela-Bela	-24.766; 27.777	Savanna	Mixed Bushveld	Unknown

APPENDIX III

Table III continued: Locality data of examined material. Biome and vegetation type identification according to Low & Rebelo (1996).

Locality number	Slide number	Date sampled	Collector	Country	Province	Locality	Georeference	Biome	Vegetation type	Associated plant
Histotylenchus mohalei										
TVL921 (Paratype)	17549	01.02.1981	A.P. Venter	South Africa	Gauteng	Hartbeespoort, Meerhof Nursery	-25.750; 27.833	Savanna	Clay Thorn Bushveld	*Aralia* sp. L
TVL925 (Paratype)	17531	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	*Nidorella hottentotica* DC
TVL926 (Paratype)	17543	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-29.366; 29.966	Savanna	Mixed Bushveld	*Pellaea calomelanos* Link
TVL927 (Paratype)	17550	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	*Commelia* sp. L
TVL929 (Paratype)	17568; 17571; 17575; 17581; 17583; 17587; 17588	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	*Thesium transvaalense* Schltr.
TVL932 (Paratype)	17620; 17621;17624; 17625	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	Unknown
TVL936 (Paratype)	17664	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	*Trachyandra* sp. Oberm

APPENDIX III

Table III continued: Locality data of examined material. Biome and vegetation type identification according to Low & Rebelo (1996).

Locality number	Slide number	Date sampled	Collector	Country	Province	Locality	Georeference	Biome	Vegetation type	Associated plant
Histotylenchus mohalei TVL941 (Paratype)	17748; 17755; 17756; 17761	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	Unknown
TVL944 (Paratype)	17817	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	*Zornia* sp. Gmel
TVL929	19764 - 19768	01.02.1981	A. Madeira	South Africa	Gauteng	Mountain View in the Magaliesberg	-25.700; 28.166	Savanna	Mixed Bushveld	*Thesium transvaalense* Schltr.
TVL1960	40517-40528	10.05.2008	A. Swart	South Africa	Gauteng	Irene at the Bakwena Cave	-25.897; 28.222	Grassland	Rocky Highveld Grassland	Grass (unidentified)
TVL 2005	40529-40536	12.03.2009	F. van Oudtshoorn	South Africa	Gauteng	Suikerbosrand Nature Reserve	-26.513; 28.240	Grassland	Rocky Highveld Grassland	Grass (unidentified)
TVL 2026	39925-39929	01.07.2009	S. Berry	South Africa	Mpumalanga	Komatipoort	-25.436; 31.942	Savanna	-	*Saccharum* sp. L.
***Histotylenchus niveus* sp. n.** KP2039	37905; 38724	05.11.2003	M. Marais	South Africa	Northern Cape	Witsand Nature Reserve	-25.583; 22.466	Savanna	Karroid Kalahari Bushveld	*Acacia haematoxylon* Willd.

ABSTRACT

A diverse and abundant nematode fauna is known to exist in South Africa with plant parasitic nematodes being the most representative. Seven genera belonging to the family Belonolaimidae Whitehead, 1960 are known to occur in South Africa, including *Histotylenchus* Siddiqi, 1971, a small genus only found in Africa and India. Nematodes belonging to the family Belonolaimidae are obligatory, primarily migratory ectoparasites of roots. These nematodes are found in cultivated soils and natural veld and are usually seen as mild plant parasites. *Histotylenchus* is represented by three species from South Africa, namely *Histotylenchus hedys* Kleynhans, 1975, *Histotylenchus histoides* Siddiqi, 1971 and *Histotylenchus mohalei* Kleynhans, 1992. Diagnostic characters of *Histotylenchus* include a lip region confluent with body contour or offset, asymmetrical stylet conus with an angular lumen, a large metacorpus valve, genital tracts about equal, lateral fields with four lines usually areolated, spicules tapering distally with distinct vela and gubernaculum proximally recurved with titillae. The aim of this study was to review all existing literature concerning the genus *Histotylenchus* in Africa, and to examine and describe the species from this genus collected in South Africa, which are deposited in the National Collection of Nematodes, Biosystematics Programme, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa. Results showed that a total of 44% of the fixed material examined was not suitable for this morphological study as a result of the separation of the cuticle from the body. This phenomenon is reported from this genus for the first time. *Histotylenchus hedys* and *Histotylenchus mohalei* were re-described from material of the National Collection of Nematodes, while *Histotylenchus histoides* was re-described from type material of the Rothamsted Nematode Collection housed at the Food and Environment Research Agency (Fera), York, United Kingdom; and CABI Bioscience, Egham, United Kingdom. A new species, *Histotylenchus niveus* sp. n., was described from material collected in the Witsand Nature Reserve, Northern Cape, South Africa. Additionally, two species *Histotylenchus* sp. 1 and *Histotylenchus* sp. 2 were described during this study and could possibly be new to science. However, additional material needs to be collected to verify these results. The present study revealed that further research into the genus is necessary, especially since the current information is limited and incomplete.

KEYWORDS: asymmetrical stylet conus, Belonolaimidae, ectoparasites, *Histotylenchus*, *Histotylenchus niveus* sp. n., India, South Africa

OPSOMMING

Die Suid-Afrikaanse nematood-fauna is uiteenlopend van aard en kom algemeen voor, met plantparasitiese nematode die mees verteenwoordigende groep. Sewe genera, wat aan die familie Belonolaimidae Whitehead, 1960 behoort, kom in Suid-Afrika voor. Een van hierdie genera is die klein genus *Histotylenchus* Siddiqi, 1971 wat slegs in Afrika en Indië aangetref word. Aalwurms wat tot hierdie genus behoort is verpligte, hoofsaaklik migratoriese ektoparasiete van plantwortels, maar word tans nie as gevaarlike plantparasiete beskou nie. *Histotylenchus* kan in beide bewerkte grond en onversteurde veld voorkom. Die drie spesies van die genus *Histotylenchus* wat tans in Suid Afrika aangemeld word, is *Histotylenchus hedys* Kleynhans, 1975, *Histotylenchus histoides* Siddiqi, 1971 en *Histotylenchus mohalei* Kleynhans, 1992. Diagnostiese kenmerke van *Histotylenchus* sluit ondermeer lipvorm wat aaneenlopend met die ligaam of afgesnoer kan wees, ‘n asimmetriese stekelkonus met ‘n hoekige lumen, ‘n groot metakorpusklep, reproduktiewe buise van naastenby dieselfde lengte, laterale velde met vier laterale met aërolasies, spikulas distaal gebuig met velum en gubernakulum proksimaal omgebuig met titilla. Die doel van hierdie studie was om alle bestaande literatuur met betrekking tot die genus te analiseer en om die materiaal van die genus in die Nasionale Versameling van Nematodes, Biosystematiek Program, Navorsingsinstituut vir Plantbeskerming, Landbounavorsingsraad, Pretoria, Suid-Afrika, te ondersoek. Vier-en-veertig persent van hierdie materiaal was ongeskik vir taksonomiese studies, as gevolg van die skeiding van die kutikula vanaf die liggaam. Hierdie verskynsel word vir die eerste keer in hierdie genus aangemeld. *Histotylenchus hedys* en *Histotylenchus mohalei* word vanaf materiaal van die Nasionale Versameling van Nematode herbeskryf, terwyl *Histotylenchus histoides* vanaf van die Rothamsted Nematode Collection gehuisves ter Food and Environment Research Agency (Fera), York, Vereenigde Koninkryk en ook by CABI Bioscience, Egham, Vereenigde Koninkryk herbeskryf word. ‘n Nuwe spesie, *Histotylenchus niveus* sp. n., versamel vanaf die Witsand Natuurreservaat, Noord-Kaap, Suid-Afrika, word beskryf. Bykomend is twee waarskynlike nuwe spesies, *Histotylenchus* sp. 1 en *Histotylenchus* sp. 2 beskryf in hierdie studie, maar addisionele materiaal moet ondersoek word om die resultate te bevestig. Die huidige studie toon dat verdere navorsing op die genus noodsaaklik is, aangesien die inligting tans min en ook onvolledig is.

SLEUTELWOORDE: asimmetriese stekelkonus, Belonolaimidae, ektoparasiete, *Histotylenchus*, *Histotylenchus niveus* sp. n., Indië, Suid- Afrika

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SUBMISSION OF MSc DISSERTATION

DECLARATION

I, the undersigned, declare that the dissertation hereby for the qualification Magister Scientiae submitted by me to the University of the Free State, is my own original work and that I have not previously, in its entirety or in part, submitted it to any University for a degree.

I furthermore cede copyright of this dissertation in favour of the University of the Free State.

Henda Landman

Signature .....

Date .....