A TAXONOMICAL AND ECOLOGICAL STUDY OF NEMATODES FROM THE SEEKOEIVLEI NATURE RESERVE, MEMEL

By

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Dissertation submitted in fulfilment of the requirements for the degree Magister Scientiae in the Faculty of Natural and Agricultural Sciences

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FEBRUARY 2014

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Chapter 1

INTRODUCTION

A famous quote by the man known as the Father of Nematology, Dr. Nathan Augustus Cobb (1914) stated that:

> "…if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes."

Nematodes are unsegmented roundworms found in virtually all environments that vary from pristine to extremely polluted (Abebe 2006). Nematology, which is the youngest of the zoological disciplines, is fractured along taxonomic lines into plant, insect, animal and human-parasitic and free-living nematode factions (Gaugler & Bilgrami 2004). In freshwater habitats, nematodes are the most diverse and numerically dominant metazoans and an array of functional roles has been attributed to them (Abebe 2006). Even so, freshwater nematology is currently the least studied of all the nematology disciplines (De Ley *et al.* 2006).

South Africa is a water-scarce country (Mathipa & Le Roux 2009) and there is an urgent need to endeavor appropriate management tools in an attempt to resolve this problem. Water quality is primarily assessed by chemical parameters which include: temperature, dissolved oxygen (DO), turbidity, pH, nitrates, suspended solids (SS), biological oxygen demand (BOD5), chemical oxygen demand (COD) and faecal coliforms (Wu *et al.* 2010). An advantage of this is that it provides accurate, standard and reliable information. However, according to Spellman & Drinan (2001), it only provides information about the water quality at the time of measurement. It cannot determine the impact of the previous events on ecology. Bio-indicators on the other hand, could offer information about past and episodic pollution (Wu *et al.* 2010).

The Seekoeivlei Nature Reserve (SKVNR) is one of the main wetlands of the Orange-Senqu River Basin and is situated in the north eastern corner of the Free State Province. It is the largest protected area of wetland on the Highveld and in South Africa. The area contains an assemblage of complex ecosystems and habitats and supports an appreciable assortment of rare, vulnerable and endangered species/subspecies of plants and animals. By recommendation of the World Conservation Strategy, this region is regarded as a priority Biogeographic Region in which major protected areas should be established (Du Preez & Marneweck 1996). The SKVNR covers an area of approximately 4 500 ha (45 km^2). It was designated as a Ramsar site on the 21 January 1997 (Ramsar 2013). The high altitude wetland plays a critical role in regulating water flow as well as maintaining the highest water quality standards (Du Preez & Marneweck 1996). This marshland is a very important sponge area for the Klip River which is a tributary to the Vaal River, providing water to the highly industrialised and densely populated Gauteng Province (Ramsar 2013). Human activities at the SKVNR include livestock grazing and tourism. A large percentage of the vegetation is made up of alien species such as Bluegums (*Eucalyptus* L'Her, 1789 spp.), Poplars (*Populus* L. spp.), Willows (*Salix* L. spp.) and Pines (*Pinus* L. spp.). According to McCarthy *et al.* (2010), investigations have indicated that whilst parts of the floodplain wetlands in the Klip valley have remained relatively pristine, there are wetlands within the reserve that have been seriously degraded. Sanitation and low-cost housing sections of Memel are poor and low quality water from this source discharges into tributaries of the Klip River at the upstream end of the wetland (McCarthy *et al.* 2010).

Questions that arise, are:

- Considering the unique ecosystem dynamics provided by the wetland, what species of nematodes would be present?
- What are these bio-indicators able to indicate concerning the ecological integrity of the system?
- How has human activities influenced the system?
- Are there key or new species of nematodes present at the reserve?

Bio-indicators can be a useful tool to assess real environmental impacts by pollution. The Maturity index (MI) is commonly used to assess environmental quality (Bongers 1990). Taxonomy and ecology have always been close allies and in therefore, in order to understand the position of any animal group in a biocoenosis, it is an absolute prerequisite that the identities of the taxa have been firmly established (Abebe 2006). Even though nematode taxonomy has at times had a tumultuous history (De Ley & Blaxter 2002), advances in molecular biology techniques has allowed for a more objective and empirical analysis of the evolutionary history of the phylum Nematoda (Meldal *et al.* 2007). According to Abebe *et al.* (2008), the current nematode conservative estimate seems to stabilise at approximately one million species. A staggering 97% of these are currently unknown. Of the 27000 species that are known, approximately seven percent have been documented from freshwater habitats.

Nematodes are major players in biodiversity worldwide. There is an urgency to improve our understanding of the species and how nematodes contribute to and regulate the operation of freshwater ecosystems if we are to meet the challenge of sustaining these ecosystems for the future. Greater attention is required for the discovery of needed information as to whether hot spots of biodiversity exist on global or local scales and whether there are key species for an ecosystem process (Abebe 2006).

As a result, the aims of the study undertaken herein were to:

1) determine the genera of nematodes present
2) determine the ecological status of the wetland by using nematodes as bio-indicators
3) taxonomically describe new and key species

Overall, the above mentioned objectives contribute to the lacking knowledge of nematodes from the SKVNR, as well as to increase our current knowledge of free-living nematodes from South Africa. This project was carried out on a seasonal basis and may provide a baseline for further studies which may broaden our taxonomical and ecological perspective on the wetland.

Considering the:

- significance of nematodes,
- relatively low volume of literature and information on freshwater nematodes, not only in South Africa, but worldwide and
- high capacity of information nematode assemblages are able to facilitate;

there is now an increasing appreciation by scientists for the poorly known invertebrates such as nematodes.

Background information on the phylogeny, morphology and status of current freshwater nematology as well as the state of water resources in South Africa is discussed in **Chapter 2**. The study site which is a designated Ramsar site is discussed in detail in **Chapter 3**. The Material and Methods are discussed in **Chapter 4**. The process of collection, extraction and processing of soil samples as well as identification of nematodes is a time-consuming process and is discussed in this chapter. **Chapter 5** reveals the results obtained by the study. Two of the aforementioned aims are accomplished in this chapter. A list of genera of nematodes found at the SKVNR is summarized in **Table 5.1** and data describing the ecological status of the wetland is included here. **Chapter 6** described key species of nematodes found at the SKVNR, which conquers the third aim of this project. The results are discussed in **Chapter 7.** This dissertation ends with references in **Chapter 8**, followed by the **Appendix, Abstract & Opsomming** and **Acknowledgements**.

Chapter 2

LITERATURE REVIEW: WISE WORMS IN THE WATER

SYSTEMATICS OF FRESHWATER NEMATODES

Even though life cycles and relationships of nematodes have been studied for over three and a half decades (Meldal *et al.* 2007), nematode taxonomy has at times had a turbulent history (De Ley *et al.* 2006). This is not only a result of wider developments in animal systematics, but also since a comprehensive classification has only been produced by relatively few nematologists (De Ley *et al.* 2006). The majority of these classifications were based on comparatively few morphological characteristics which were derived primarily from light microscopy (Meldal *et al.* 2007). In addition to this, specialists remain in various taxonomic lines and rarely have marine and terrestrial or animal-and-plant parasitic species been studied by the same authors. In cases where the whole phylum was investigated, authors were limited to the nematode groups they specialised in. A lack of an informative fossil record, also led to the ontogeny and ultra-structure of nematodes being poorly understood (Meldal *et al.* 2007). Such difficulties have led to the formulation of multiple and in some cases conflicting classifications.

One of the earliest and most influential classifications within the phylum Nematoda was proposed by Chitwood (1937) and Chitwood & Chitwood (1950). The phylum Nematoda was divided into two subclasses: Aphasmidia and Phasmidia. This division was based on the fact that members of the subclass Phasmidia share several characteristics, including the presence of phasmids. Aphasmidia was later renamed Adenophorea which means gland bearers, by the same authors. This group included virtually all aquatic nematodes, belonging to the classes Enoplia and Chromadoria, as well as selected terrestrial omnivores and plant feeders belonging to the class Dorylaimia. Phasmidia was renamed Secernentea which means secretors. This group included almost all parasitic species (Tylenchina, Ascaridina and Spirurina) as well as the majority of terrestrial free living nematodes (Rhabditina) (Meldal *et al.* 2007).

This division was adhered to in many later classifications and had a strong influence on nematologists and zoology textbooks, especially in the western hemisphere. Thereafter, it was realised that Adenophorea was not a uniform group. As a result of an unweighted count of shared morphological characteristics, Andrássy (1976) proposed a tripartite system. The initial Adenophorea was divided into the Torquentia (roughly equivalent to the Chromadoria) and Penetranntia (roughly equivalent to Enoplia). The two groups had the same rank as the Secernentea (Holterman *et al.* 2006; Meldal *et al.* 2007).

In 1981, Lorenzen characterised the Adenophorea in more detail. He introduced the first taxonomic system based on cladistic principles. Important characteristics which are included in the analysis of Lorenzen (1981; 1994) were:

- The number, position, structure and postembryonic development of the anterior sensillae
- The structure, position and postembryonic development of amphids
- The structure of ovaries
- The position of copulatory organs
- The number of testes
- The position of gonads relative to intestine
- The position of caudal glands relative to tail
- The position of cervical gland relative to pharynx
- Metanemes
- The different terminal ducts of the epidermal glands in Enoploidea

Figure 2.1 shows the high level systematic inter-relationships within the free-living nematodes as depicted by Lorenzen (1981; 1994). His analysis made it clear that there was no support for the class Adenophorea as a monophyletic group. One differentiating characteristic was that the amphids in the order Enoplida are non-spiral in comparison to those of the order Chromadorida which are spiral. Based on this, members of the class Adenophorea could therefore be separated into two orders: Chromadorida and Enoplida. However, a lack of additional informative characteristics prevented the class Adenophorea to be separated into the two orders, at the time. (Holterman *et al.* 2006; Meldal *et al.* 2007). Today two classes can be distinguished namely the class Chromadorea and the class Enoplea (De Ley & Blaxter 2002) and this is the classification system that will be used further in this dissertation.

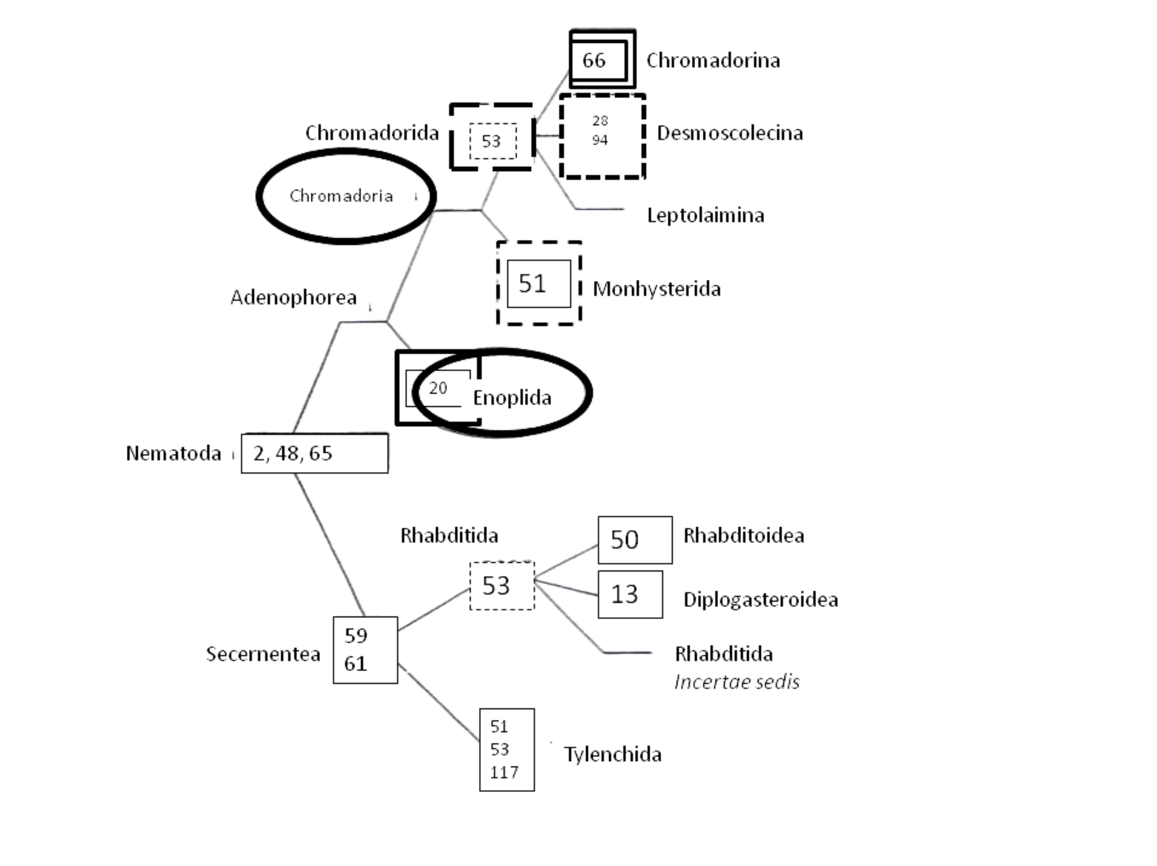

Figure 2.1: Systematic inter-relationships within free-living nematodes (redrawn from Lorenzen 1981, 1994)

An objective and empirical analysis of the evolutionary history of the phylum Nematoda was facilitated through advances in molecular biology techniques (Meldal *et al.* 2007). Whilst the classification by Chitwood (1937; 1958) has had a strong influence on nematology literature, small subunit (SSU) rDNA studies supports some substantially different hypotheses of taxonomic relationships. Interestingly, many of these were previously proposed but were not as successfully publicised as Chitwood's work (De Ley *et al.* 2006).

Using molecular analysis, De Ley & Blaxter (2002) showed the phylogenetic relationships derived from analysis of SSU rDNA. The SSU sequences differentiated between three nematode lineages (subclasses): Chromadoria, Enoplia and Dorylaimia (Fig. 2.2). The exact order of these lineages is currently unresolved. Previously, it was accepted that nematodes originated from marine habitats. SSU studies allows for the possibility that Dorylaimia diverged first. Considering the general absence of Dorylaimia from marine habitats, this could imply that the ancestor of all nematodes may have been a freshwater one. If this were the case, one has to then consider that even though Enoplia are particularly prevalent in marine habitats, their current osmotic requirements suggest that early enoplians were characterised by a greater osmotic tolerance. Perhaps even greater than early species belonging to the Dorylaimia. However, lack of resolution in the molecular phylogenies makes it unclear whether enoplian freshwater lineages arose earlier or later than their dorylaimian counterparts (De Ley *et al.* 2006).

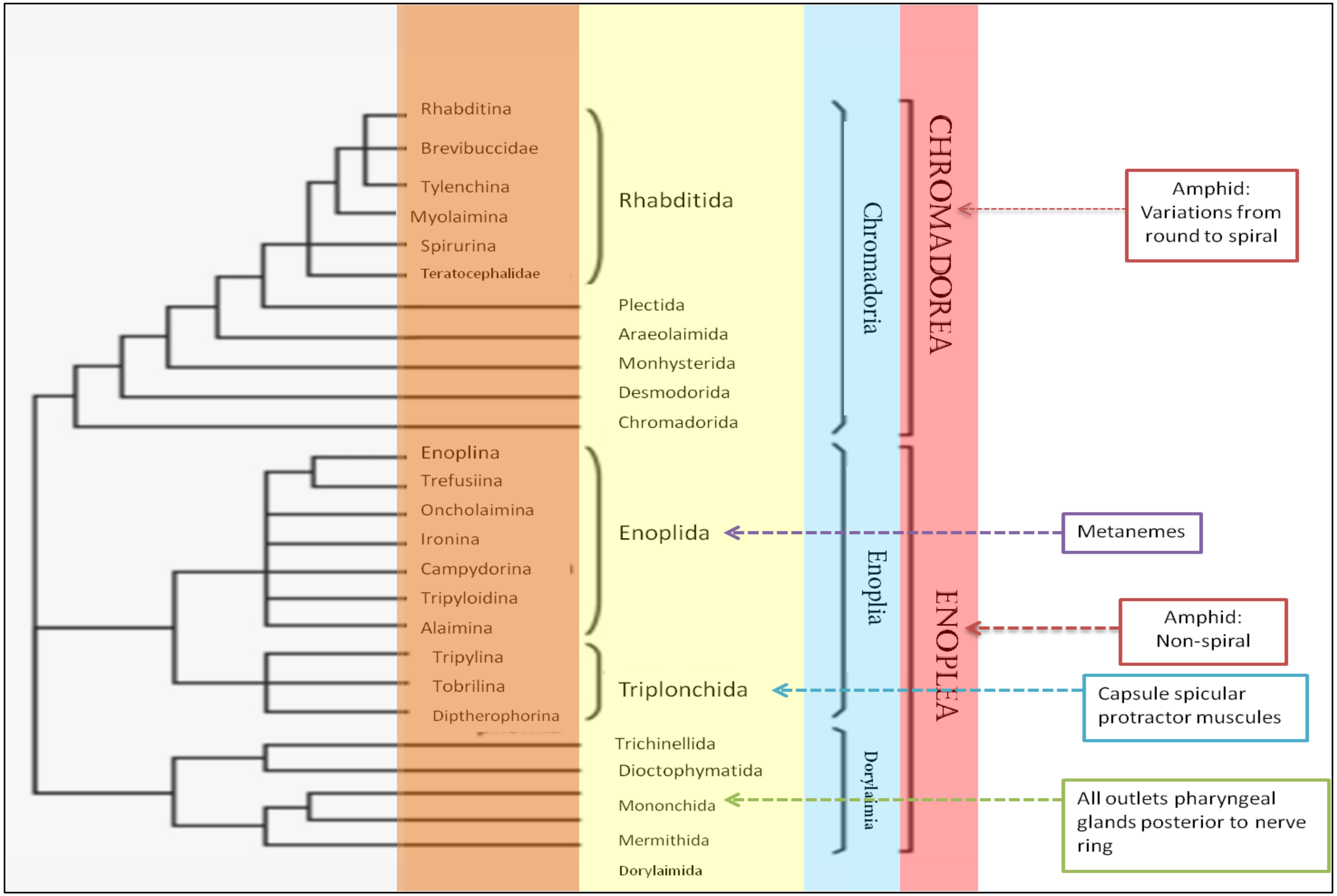

Figure 2.2: Phylogenetic relationships derived from analysis of SSU rDNA (redrawn from De Ley & Blaxter 2002).

Some characteristics which differentiate the taxa from each other are represented in Figure 2.2. The amphids in members of the class Chromadorea vary from round to spiral in contrast to the non-spiral amphids in members of the class Enoplea. Metanemes are present in members of the Enoplida and capsule spicular muscles in members of the Triplonchida. The subclass Chromadoria includes half of the currently known freshwater nematode families. Members of the Monhysterida and Plectida are among the most widely reported freshwater nematodes. Members of the subclass Enoplia are primarily marine although they do include some exclusively freshwater taxa with extreme endemism (Abebe *et al.* 2008). According to De Ley *et al.* (2006), one enoplian order which stands out as having radiated extensively in freshwater and terrestrial habitats is the order Triplonchida. This order includes not only plant parasitic trichodorids, but also the morphologically very incongruent free-living nematodes: Tobrilids and Prismatolaimids. Although excluded from marine habitats, the Dorylaimia are the most common and successful nematode group in freshwater habitats. Over sixty percent of all known freshwater families belong to this subclass.

NEMATOLOGY

Nematology, which is the youngest of the zoological disciplines, is fractured along taxonomic lines into plant, insect, animal and human-parasitic, and free-living nematode factions (Gaugler & Bilgrami 2004). Nematodes may be found as internal parasites of annelids, molluscs, arthropods and vertebrate animals and feed on protozoan's, oligochaetes and other small soil animals, bacteria, fungi, algae, mosses, ferns and higher plants. Alternatively, they are also parasitized or preyed upon by viruses, bacteria, fungi, crustaceans, insects, mites and other nematodes (Kleynhans *et al.* 1996). Thus, they are representatives of virtually all trophic levels. A food web is represented in Figure 2.3 illustrating where nematodes are found at different trophic levels (USDA Natural Resources Conservation Service 2013).

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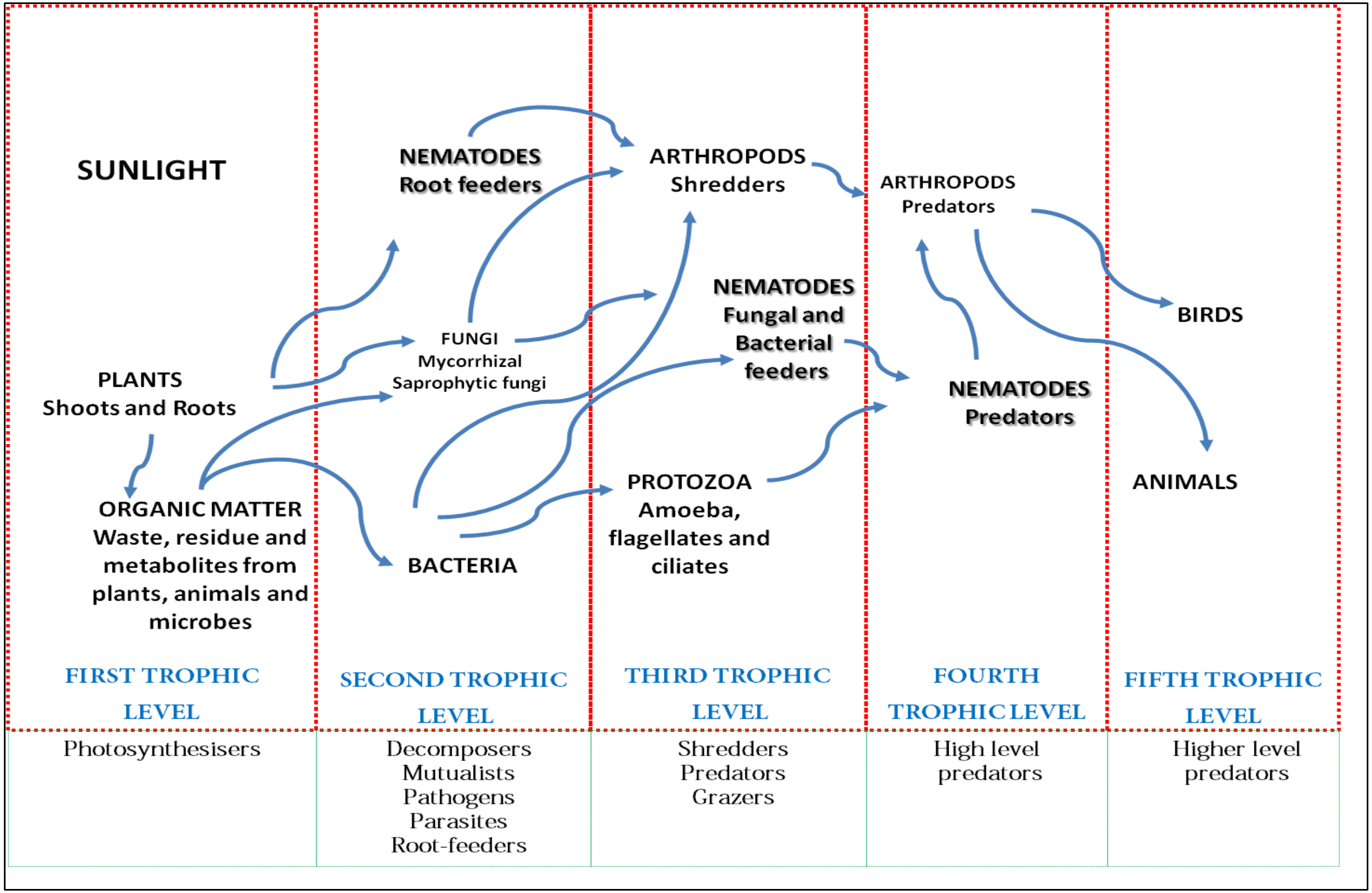


Figure 2.3: A generalised soil food web depicting nematodes at different trophic levels (redrawn and edited from USDA Natural Resources Conservation Service 2013).

Nematodes occur in water, sediment and soil ecosystems (Antofica & Poiras 2009). Freshwater sediments teem with nematodes and their task is exceptionally significant (Abebe 2006; Antofica & Poiras 2009). These include both described and undescribed nematode species. Free-living nematodes from freshwater habitats have received relatively less attention than marine and terrestrial forms (Abebe *et al.* 2008). According to Abebe (2006), freshwater nematology remains the least studied of the nematology branches. Current research in nematology follows the trend depicted in Figure 2.4 Parasitic nematodes are more studied than free-living terrestrial nematodes, free-living terrestrial nematodes are more studied than marine aquatic nematodes; and marine nematodes are more studied than freshwater forms (adapted from Abebe 2006)

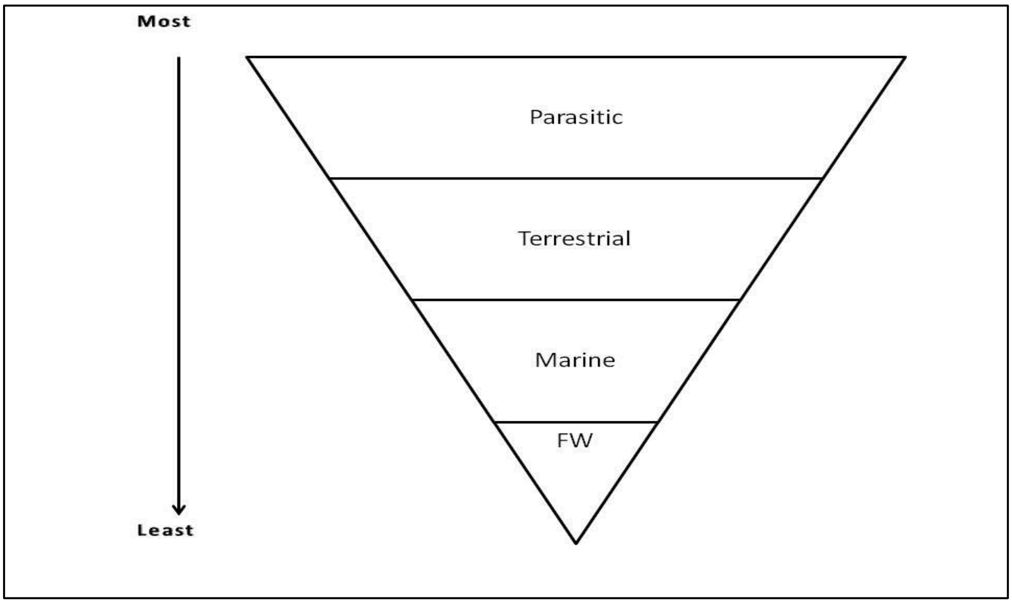


Figure 2.4: Trend of current research in Nematology (Adapted from text Abebe 2006); FW = Freshwater.

FRESHWATER NEMATOLOGY

According to Abebe *et al.* (2008), the current nematode species conservative estimates seem to stabilise at approximately one million species. Over 97% of these are currently unknown. Of the 27000 species known, large proportions are free-living nematodes and 7% have been documented from freshwater habitats. The classes Chromadorea and Enoplea, all three subclasses (Chromadoria, Dorylaimia and Enoplia), two-fifths of the nematode families and one fifth of the nearly 1800 nematode genera known are recorded from freshwater habitats.

According to Abebe *et al.* (2008), studies on freshwater nematodes show extreme regional bias. Those from the southern hemisphere are extremely underrepresented in comparison with the northern hemisphere. Data on freshwater nematodes has a patchy geographic distribution and this is due to the low number of specialists in the field of freshwater nematology and their corresponding presence in study locations (De Ley *et al.* 2006). A compressed timeline of literature available for freshwater nematology worldwide is presented in Figure 2.5 and following that, is discussion on freshwater nematology in southern Africa specifically. In figure 2.5, it can be seen that the book: *Soil and Freshwater Nematodes* by T. Goodey (1951) and the second edition by J.B. Goodey in 1963, were the first books to provide a comprehensive account of free living nematodes, including freshwater taxa. It can also be seen that up until the mid-90's, most literature belonged to European and Asian freshwater bodies only.

For Asia and South America, several taxonomic articles have become available in relatively recent years. Jacobs (1984) provided a checklist of the free-living aquatic fauna in Africa. Several articles were also written by Abebe & Coomans (1996a; 1996b; 1996c) concerning freshwater nematodes of Ethiopia.

1950	
1951	****Soil and Freshwater Nematodes*** - GOODEY, T.
1952	
1953	
1954	
1955	
1956	
1957	
1958	
1959	
1960	
1961	
1962	
1963	****Soil and Freshwater Nematodes*, 2nd Edition** - GOODEY, J.B.
1964	
1965	
1966	
1967	
1968	
1969	
1970	
1971	
1972	
1973	The Bremerhaven Checklist of Aquatic Nematodes Part 1– GERLACH, S. & RIEMANN, F.
1974	The Bremerhaven Checklist of Aquatic Nematodes Part 2– GERLACH, S. & RIEMANN, F.
1975	
1976	
1977	
1978	
1979	**Limnofauna Europaea – ANDRASSY, I.*
1980	**Free-living nematodes of Lake Baikal (Svobodnozhivuschchi nematody Baikala)– TSALOLIKHIN, S.J.*
1981	*Freshwater Nematodes of the European Part – GAGARIN 1981
1982	*Nematodi (Nematoda) *Guide per il riconoscimento delle specie animali delle acque interne Italiane* – ZULLINI, A.
1983	
1984	**Klasse Nematoda (Ordnungen Monhysterida, Desmosclecida, Araeolaimida, Chromadorida, Rhabditida) – ANDRASSY, I.* #The free-living inland aquatic nematodes of Africa- A review – JACOBS, L.
1985	*Nematodes of Fresh & Brackish Waters of Mongolia – TSALOKHIN, S.J.
1986	
1987	
1988	*De nematoden van Nederland – BONGERS, T.*
1989	*Free-living Nematodes of Lakes Issy-Kul and Son-Kul - LEMZINA
1990	
1991	
1992	
1993	

Figure 2.5: A compressed timeline of books, articles and literature available for freshwater nematology from different continents (Adapted from text De Ley *et al*. 2006).

1994	*A compendium of our knowledge of the free-living nematofauna of ancient lakes – DECRAEMER, W. & COOMANS, A.
1995	#*Freshwater nematodes of the Galapagos* – EYAULEM, A. & COOMANS, A.
1996	*Aquatic nematodes from Ethiopia I, II & III – EYAULEM, A. & COOMANS, A.
1997	
1998	# On nematode fauna of high mountain lakes in the Pamir and Himalayas – TSALOLIKHIN, S.J.
1999	#Supplement to the list of soil and freshwater nematodes recorded in continental Argentina – DOUCET, M.E. & DOUCET, M.M.A. *Nematoda, Adenophorea (Dorylaimida) In: *Subwasserfauna von Mitteleurpa* – LOOF, P.A.A.
2000	#New species of free-living freshwater nematodes from Eurasia – GAGARIN, V.G.
2001	#Some species of freshwater nematodes from Singapore and Japan – TSALOLIKHIN, S.J. *Nematoda, Secernentea (Tylenchida, Aphelenchida) In: *Subwasserfauna von Mitteleurpa* – LOOF, P.A.A. *Ecology & Classification of Norh American Freshwater Inverterbrates, 2nd edition. – THORPS, J.H. & COVICH, A.P. *Penaks Fresjwater Onvertebrates of the United States: Porifera to Crustacea –SMITH, D.G.
2002	#Free-living nematodes from nature reserves in Costa Rica – ZULLINI, A., LOOF, P.A.A. & BONGERS, T. * Checklist of free-living nematodes recorded from freshwater habitats in Southern Africa – HEYNS, J.
2003	#Three new species of free-living nematodes from freshwater bodies of Vietnam – GAGARIN, V.G., NGUYEN, V.T. & NGUYEN, D.T.
2004	# Nematode survey in Costa Rican conservation areas – ESQUIVEL, A. & ARIAS M.
2005	
2006	*** FRESHWATER NEMATODES: ECOLOGY & TAXONOMY –ABEBE, W. , TRAUNSPUNGER, W. & ANDRASSY, I.**

LEGEND
- •Universal
- •North America
- •South America
- •Europe
- •Asia
- •Africa

*→ JOURNAL ARTICLE
→BOOK

Figure 2.5 (continued): A compressed timeline of books, articles and literature available for freshwater nematology from different continents (Adapted from text De Ley *et al.* 2006).

FRESHWATER NEMATODES ACROSS THE GLOBE

When studying the distribution of freshwater nematodes from different biogeographic regions (Fig. 2.6) it is clear that the limnetic fauna of Antarctic is restricted to 10 species. Whilst important orders such as the Dorylaimida and Rhabditida have not been reported from Antarctic freshwaters, there are records of them being recorded in Antarctic soil. This may be a result of species being seasonally aquatic and collection was not in the right spatio-time frame especially considering the extreme environmental conditions and brief summer in Antarctica. Thirteen families were recorded from the Pacific and Oceanic Islands. All orders of Enoplea and Chromadorea are represented in the other geographic regions. Overall, the proportion of representatives of the seven orders of Chromadorea varies little between the regions. The majority of families belong to Rhabditida. The largest number of families was recorded from the Palaearctic region with 89% of the total number of freshwater nematode families. The Australasia region represents only 44% of the freshwater families (Abebe *et al.* 2008).

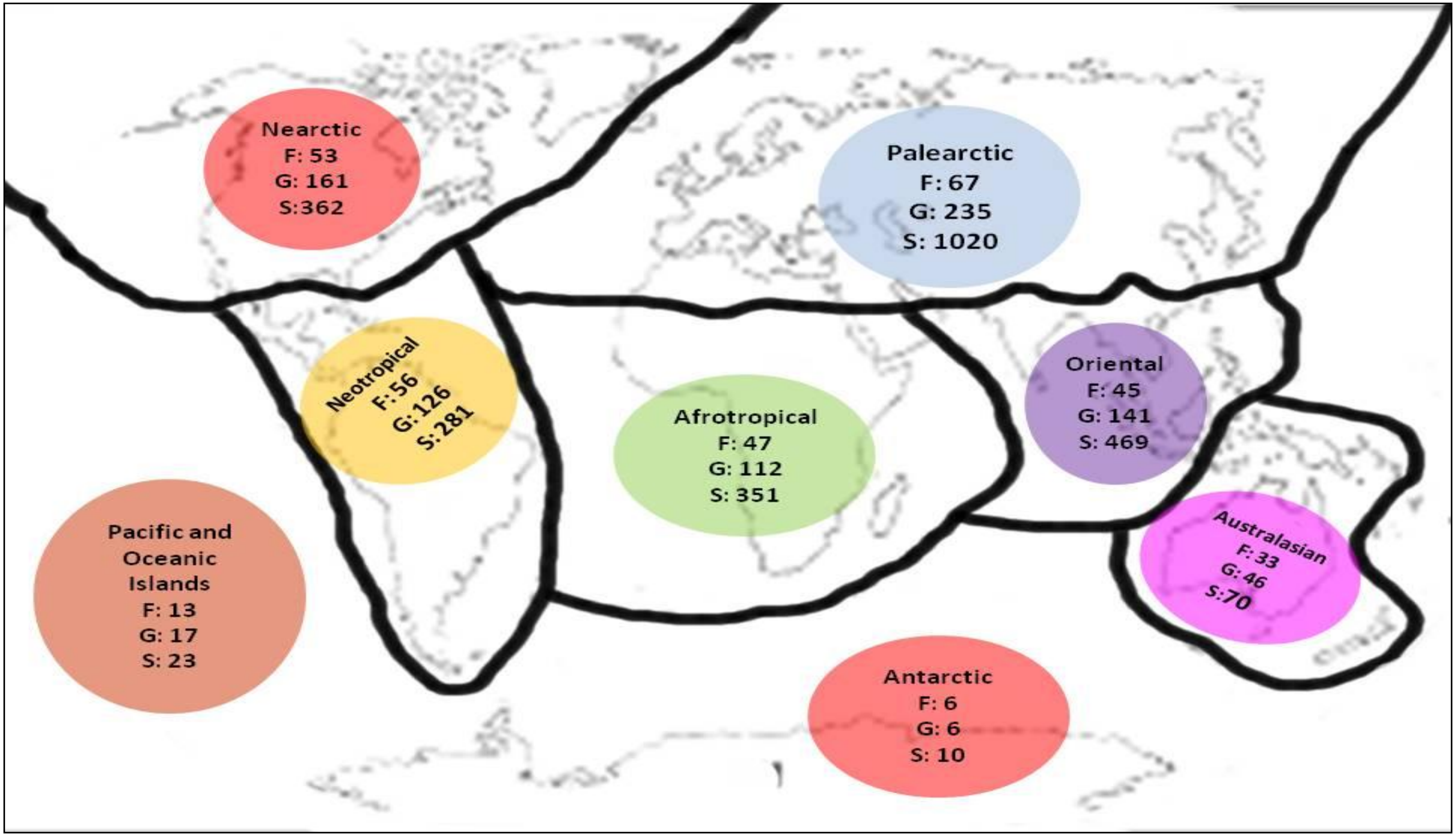


Figure 2.6: Distribution of the number of freshwater nematode species, genera and families in each biogeographic region. F = Families, G = Genera, S = Species (Redrawn and edited from Abebe *et al*. 2008).

FRESHWATER NEMATOLOGY IN SOUTHERN AFRICA

In 1976, Heyns published the preliminary results of freshwater nematodes in South Africa. In 2002, he produced a chapter in the World Research Commission publication titled: *Guides to Freshwater Invertebrates of Southern Africa*. A key to some of the genera of freshwater nematodes in Southern Africa was presented in here (Heyns 2002a). His checklist of free living nematodes from freshwater habitats in Southern Africa (Heyns 2002b) was completed and published posthumously by Annermariè Avenant-Oldewage and serves as a supplement to the key.

The earliest work done on freshwater nematodes in South Africa was by Coetzee between 1965 and 1968 (refer to table 2.1). The current literature available for freshwater nematodes from southern Africa is limited, with a bulk of work done by Heyns and co-workers during the 20th century. This included the publication of new and known species of nematodes from Skinnerspruit with Dassonville in 1984, following Dassonville's thesis publication in 1981. A range of articles have been published regarding nematodes from the rivers in the Kruger National Park between 1992 - 1993. Heyns was also involved in the publication of work from other parts of Southern Africa including freshwater bodies in Namibia and some predatory nematodes from the Okavango Delta in Botswana. More recent publications include work by van den Berg *et al*. (2009) for a study on the KwaZulu-Natal (KZN) Midlands in which plant-parasitic nematodes from wetlands were collected to identify those species most likely to be associated with different wetland conditions.

Table 2.1. Chronological listing of some of the literature available on freshwater nematology in southern Africa.

REFERENCE: YEAR AND AUTHOR	**TITLE**
(1965) Coetzee	South African species of the genus *Cobbonchus* Andrássy, 1958 (Nematoda: Mononchidae).
(1966) Coetzee	Species of the genera *Granonchulus* and *Cobbonchus* (Mononchidae) occurring in southern Africa
(1967a) Coetzee	Species of the genus *Mylonchulus* (Nematoda:Mononchidae) occurring in southern Africa.

Table 2.1. (continued): Chronological listing of some of the literature available on freshwater nematology in southern Africa.

(1967b) Coetzee	Species of the genus *Iotonchus* (Nematoda: Mononchidae) occurring in southern Africa
(1968a) Coetzee	Southern Africa species of the genera *Mononchus* and *Prionchulus* (Mononchidae)
(1968b) Coetzee	Mononchidae (Nematoda) of southern Africa
(1972) Argo & Heyns	Four new species of the genus *Ironus* Bastian, 1865 (Nematoda: Ironidae) from South Africa
(1975) Basson & Heyns	The genus *Mesodorylaimus* in South Africa (Nematoda: Dorylaimidae)
(1977) Heyns & Coomans	Freshwater nematodes from South Africa. 2. *Oncholaimus deconincki* n.sp.
(1979) Joubert & Heyns	Freshwater nematodes from South Africa. 3. *Tobrilus* Andrássy, 1959
(1980) Joubert & Heyns	Freshwater nematodes from South Africa. 4. The genus *Monhystera* Bastian, 1865
(1980) Heyns & Coomans	Freshwater nematodes from South Africa 5. *Chronogaster* Cobb, 1913
(1981) Dassonville	An taxonomic and ecological study on freshwater nematodes in Skinnerspruit, South Africa
(1983) Heyns & Kruger	Freshwater nematodes from South Africa. 6. *Mesodorylaimus* Andrássy, 1959
(1984) Dassonville & Heyns	Freshwater nematodes from South Africa. 7. New and known species collected in Skinnerspruit

Table 2.1. (continued): Chronological listing of some of the literature available on freshwater nematology in southern Africa.

(1986) Coomans & Heyns	*Oncholaimus jessicae* n. sp. (Nematoda: Oncholaimidae) from freshwater in the Transvaal
(1988) Swart & Heyns	Redescription of *Eutobrilus heptapapillatus* (Joubert and Heyns, 1979) Tsalolikhin, 1981 with notes on its morphology and a possible excretory system (Nematoda: Tobrilidae)
(1989) Heyns & Coomans	A new freshwater species of *Theristus* from South West Africa/Namibia (Nematoda: Xyalidae)
(1990) Rashid, Geraert & Heyns	Description of *Tobriloides loofi* n.sp. from Natal, South Africa (Nematoda: Onchulidae)
(1990) Heyns & Coomans	Three *Monhystrella* species from inland waters in South West Africa – Namibia (Nematoda: Monhysteridae)

Table 2.1. (continued): Chronological listing of some of the literature available on freshwater nematology in southern Africa.

(1990) Rashid, Heyns & Coomans	*Paracrobeles* and *Acrobeles* species from South West Africa/Namibia with description of a new *Acrobeles* species (Nematoda: Cephalobidae)
(1990) Swart & Heyns	Description of *Tobriloides loofi* n.sp. from Natal, South Africa (Nematoda: Onchulidae)
(1991) Swart *et al*.	A review of the genus *Euteratocephalus* Andrássy, 1958, with description of *E. punctatus n.sp.*
(1991a) Swarts & Heyns	*Lenonchium frimbricaudatum* n.sp. from South Africa, with a key to the species of *Lenonchium* (Nematoda: Nordiidae)
(1991b) Swart & Heyns	*Desmodora (Sibayinema) natalensis* subg. nov., spec. nov. from Lake Sibayi, South Africa (Nematoda: Desmodorida)
(1992a) Botha & Heyns	Freshwater nematodes of the genera *Thornenema* and *Mesodorylaimus* from the Kruger National Park with a diagnostic species compendium for South African species of the genus *Mesodorylaimus* (Nematoda: Dorylaimida)
(1992a) De Bruin & Heyns	Mononchida (Nematoda) of southern Africa: genera *Mononchus* Bastian, 1865, Clarkus Jairajpuri, 1970 and Coomansus Jairajpuri & Khan, 1977
(1992b) De Bruin & Heyns	Mononchida (Nematoda) from southern Africa: genus *Iotonchus* (Cobb, 1916) Altherr, 1950
(1992b) Botha & Heyns	Species of *Tyleptus*, *Proleptonchus*, *Aquatides* and *Afractinolaimus* from rivers in the Kruger National Park (Nematoda: Dorylaimida)
(1992c) Botha & Heyns	Further records and descriptions of nematodes from rivers in the Kruger National Park (orders Enoplida, Chromadorida, Monhysterida, Mononchida and Araeolaimida)

Table 2.1. (continued): Chronological listing of some of the literature available on freshwater nematology in southern Africa.

(1993) Swart & Furstenberg	A description of two new species of nematodes belonging to the genera *Onchulus* and *Limonchulus* from Southern Africa
(1993) Swart & Heyns	Description of two new species of the genera *Onchulus* and *Limonchulus* from Southern Africa (Nematoda: Enoplida, Onchulinae)
(1993a) Botha & Heyns	Account of species belonging to the genera *Oxydirus, Dorylaimellus (Axodorylaimellus), Laimydorus & Rhabdolaimus* from rivers in the Kruger National Park
(1993a) Botha & Heyns	Species of the genera *Oxydirus*, *Dorylaimellus (Axodorylaimellus), Laimydorus* and *Rhabdolaimus* from rivers in the Kruger National Park (Nematoda: Dorylaimida and Araeolaimida)
(1993b) Botha & Heyns	New records of Tylenchida, Araeolaimida and Enoplida from the Kruger National Park, with an addendum to the checklist of nematode species in the park
(1994) Swarts & Heyns	Description of *Aetholaimus trochus* n.sp. and the male of *Ironus ignavus* Bastian, 1865 (Nematoda) from Caprivi, Namibia
(1995) Coomans *et al.*	On some predatory nematodes from the Okavango Delta, Botswana
(2007) Van den Berg *et al*.	Information and description of two new *Criconemoides* species (Nematoda: Criconematidae) from the KZN midlands
(2009) Van den Berg *et al.*	*Hirschmanniella kwazuna* sp.n. from South Africa with notes on a new record of *H.spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964 (Nematoda: Pratylenchidae) and on the molecular phylogeny of *Hirschmanniella* Luc & Goodey, 1964

MORPHOLOGY AND BIONOMICS

A generalised nematode body illustrating some of the key morphological characteristics is presented in Figure 2.7A. The outer layer is called the cuticle and its surface is thought to consist of a thin lipid layer (Lee 1965). Most freshwater nematodes are members of the subclass Enoplia and may be characterised by the presence of setae, adhesive glands and prominent amphids (Abebe *et al.* 2008) (Fig. 2.7A). Amphids are paired sense organs on or near the lip region (Lee 1965). Photoreceptor organs are present in a few freshwater taxa in the form of ocelli in the pharyngeal region (Abebe *et al.* 2008). The pattern of cephalic setae is consistent with three concentric rings of sensilla on the anterior end surrounding the mouth which De Coninck (1965), considered a primitive arrangement.

Except for the adults belonging to the family Mermithidae, all freshwater nematodes possess a continuous digestive tract. The wide range of food sources and different methods of ingestion is reflected in the structure of the digestive system and especially in the morphology of the anterior feeding apparatus (Figs 2.7B-F) which differs from nematode to nematode. The buccal cavity and pharynx which include the median bulb and basal bulb, may assume many different shapes (Abebe *et al.* 2008; Heyns 2002a) . Food is transported through the intestine and excreted via the anus. The circumpharyngeal nerve ring and associated structures, forms part of the nervous system (Lee 1965). The secretory-excretory system in most free-living freshwater taxa consists of a ventral gland or renette cell connected to a ventral pore by a duct. This system may play a role in the excretion of nitrogen as ammonia/urea. It also contributes to osmotic regulation and locomotion (Abebe *et al.* 2008). The spinneret at the tip of the tail extrudes mucoid secretions which facilitate the attachment of aquatic nematodes temporarily to the substratum (Traunspurger 2000).

The nematode life span may vary from several days to several years. Their life cycle is direct and uncomplicated. It consists of an egg stage, four juvenile stages and an adult stage. Each juvenile moults once and the adult develops after the last moult. Females generally outnumber males. In many groups males are rare or unknown (Kleynhans *et al.* 1996). The female reproductive system consists of a vulva opening leading to the vagina and further consists of one (monodelphic) or two ovaries (didelphic), a uterus and eggs (Lee 1965) (Fig. 2.7A). Females are usually oviparous. In some cases however, the eggs hatch inside the body of the female (ovoviparity).

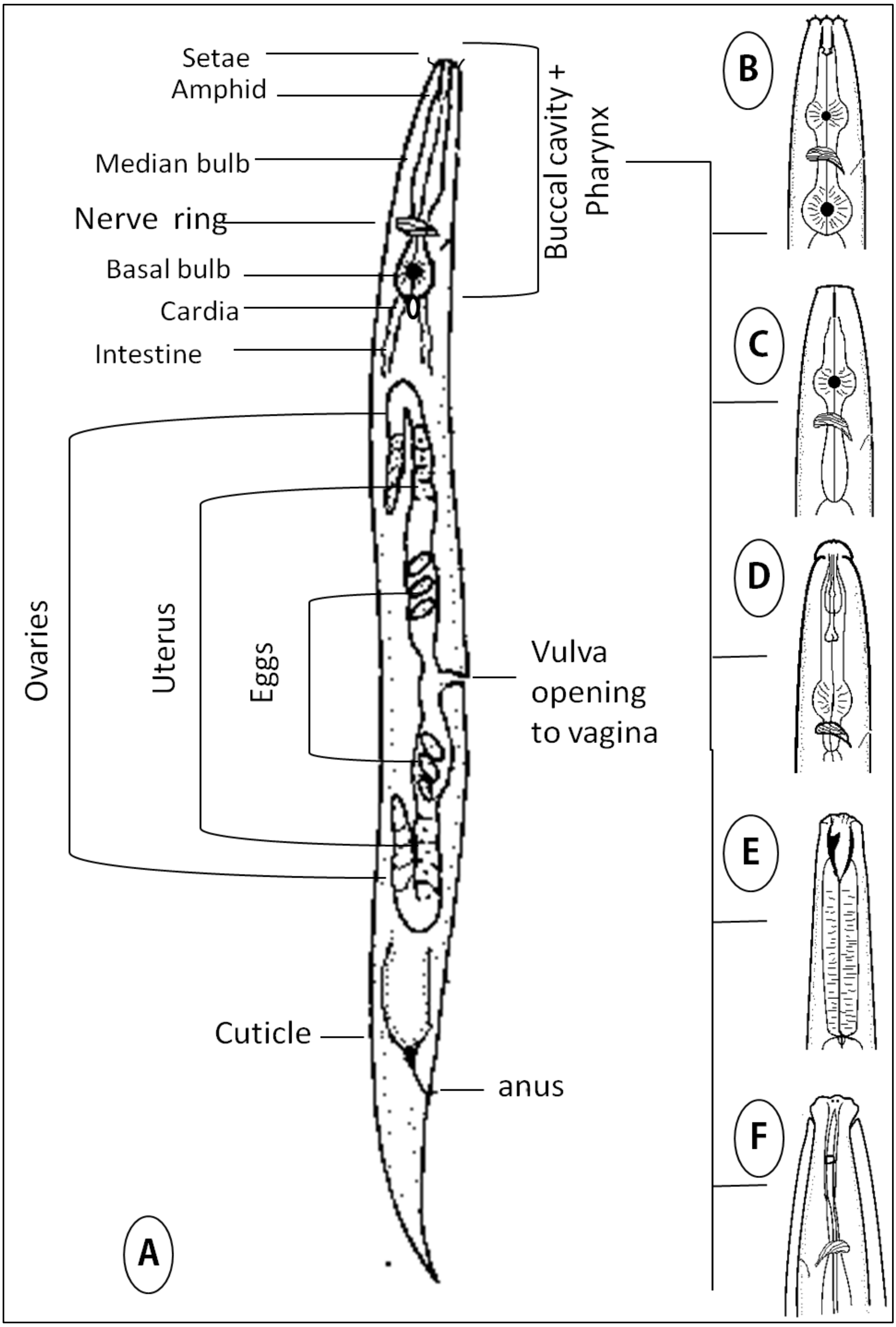


Figure 2.7: (A) Generalised nematode morphology. Redrawn from Heyns (2002a). **B-F:** Various nematodes mouthparts: **(B)** Bacterial feeder **(C)** Fungal feeder **(D)** Plant feeder**, (E)** Predator**, (F)** Omnivore **(**Redrawn from Zaborski 2014).

Nematodes are omnipresent and are the most diverse and numerically dominant metazoans in freshwater habitats. These properties bestow exceptional significance to their position and array of functional roles has been attributed to them:

- They are the main catalysts of some water, sediment and soil processes (Antofica & Poiras 2009).
- Studies show that burrowing by nematodes may result in new spaces for bacteria. Bacteria may also use nematode excreta as a substratum (Traunspurger 2000).
- They are of major energetic importance in benthic systems and occupy positions at the base of food chains that ultimately sustain and form a significant part of the diet of many other organisms (Antofica & Poiras 2009).
- They facilitate the mineralization of organic matter and humification of dead organic matter (Antofica & Poiras 2009).
- Nematodes enhance the carbon mineralisation rate by stimulating microbial activity through predation and bioturbation as well as consumption of detritus by larger deposit-feeding invertebrates (Traunspurger 2000).
- In addition, they influence the physical stability of sediments and the exchange of materials between the sediment and water column and are responsible for cycling of sediment and soil nutrients and self-purification of water due to their interaction with bacteria, algae and fungi (Wasilewska 1997; Abebe 2006).

Very little is known about resistant stages, dispersal and survival of freshwater nematodes (Abebe *et al.* 2008). It can be said that aquatic nematodes disperse by rafting, drifting or dispersal by suspension in the water column following mechanical removal from the substratum by current waves. There is still only speculation about the importance of nematodes as food for young fish (Traunspurger 2000).

Today, our knowledge of nematode biodiversity and ecosystem functioning are becoming intertwined because we need to know the taxa within freshwater sediments that are most vulnerable to global changes (Abebe 2006).

NEMATODES AS BIO-INDICATORS

Of the various functions of soils, its ecology is the most vulnerable to pollutants and other forms of disturbance (Bongers 1990). Soil sediments have a high potential for accumulation of contaminants and are particularly sensitive to anthropogenic impacts. The presence of contaminated sediments may hinder a water body from achieving a good ecological status (Heininger *et al.* 2007).

The universal parameters used to describe water quality, including River Pollution Index (RPI) are: dissolved oxygen concentration, pH and temperature, amongst others. Whilst the advantages of RPI are accuracy, standard and reliability, it only provides information about water quality at the time of measurement. It cannot determine the impact of previous events on the ecology (Spellman & Drinan 2001). Bio-indicators on the other hand, could offer information about past and episodic pollution (Wu *et al.* 2010).

(McGeoch, 1998) described biological indicators as being:

> "A species or group of species that readily reflects the biotic state of an environment, represents the impact of environmental change on a habitat, community or ecosystem, or is indicative for the diversity of a subset of taxa or of the wholesale diversity, within an area"

Wilson & Kakouli-Duarte (2009) stated that in order for an organism to be considered an effective bio-indicator, it needs to possess the following ideal characteristics (amongst others):

- ✓ Highly abundant and easily manipulated
- ✓ Easily sampled and sorted
- ✓ Cheap and easy to husband in the laboratory
- ✓ Be representative of their habitat
- ✓ Known to exhibit well-defined responses to environmental challenges

Meiobenthic organisms are more suitable for bio-monitoring than macrofauna as a result of meiobenthic taxa being more abundant and richer in species than macrofauna taxa. Meiobenthic communities also respond faster to disturbances because of their relatively short generation cycle of component species (Heininger *et al.* 2007). Nematode assemblages offer several advantages for assessing the quality of freshwater, marine and terrestrial ecosystems and in effect, constitute a potential instrument for determining the quality of: submersed, temporarily submersed and terrestrial soils. It is believed that no other group of organisms offer as much competence as nematodes do (Wilson & Kakouli-Duarte 2009). According to Bongers (1990) nematodes exhibit the following characteristics:

1. Their diversity is high.
2. They occur in high numbers.
3. They are easily sampled and identified.
4. Their permeable cuticle allows them to be in direct contact with solvents in the soil capillary water.
5. Nematodes represent a trophically heterogeneous group.
6. Nematodes are present in submersed soils, even where macrofauna is sparse.
7. Numerous species of nematodes can withstand anaerobic conditions.
8. Nematodes have high colonisation ability.
9. They can be sampled in all seasons.
10. Numerous species are able to be frozen or dehydrated.

According to Wu *et al.* (2010), using nematodes as a bio-indicator could complement conventional monitoring. It could offer a more accurate and precise assessment of the real ecological impact of contamination in water bodies. According to Bremez *et al.* (2008), changes that can be revealed by nematode community structure include: Agro-ecosystem conditions, organic adding, heavy metal compounds, soil tillage system, air and river pollution, natural disturbance (e.g. climatic changes that affect water systems of soil) and anthropogenic disturbances (e.g. chemical inputs that alter soil structure). There are many records of specific nematode species showing a preference for certain environmental factors. In addition to this, nematodes can also show a range of reactions to pollutants and other disturbances in various ecosystems including soil and rivers (Bongers 1990).

THE MATURITY INDEX

Successional changes are observed in nematode fauna in soil, following a disturbance. If it is possible to give an increasing value to each of the taxa that subsequently colonises a disturbed habitat, then the weighted mean of those values could give an indication of recovery. An index - the Maturity Index (MI) - is proposed as a semi-quantative value which indicates the condition of an ecosystem based on the composition of the 'nematode community' (Bongers 1990). Colonisers and persisters are extremes on a scale from 1 to 5 respectively. Their differences are represented in Table 2.2.

Table 2.2: Differences between colonisers and persisters [(adapted from Bongers (1990)].

COLONISERS	PERSISTERS
Rapidly increase in number under favourable conditions	Low reproduction rate
R-strategists (in the loose sense)	K-strategists (sensulato)
Short life-cycles	Long life-cycles
High colonisation ability and their tolerance to disturbances, eutrophication and anoxybiosis	Low colonisation ability and are sensitive to disturbances
Numerically dominant in samples	Never belong to the dominant species in a sample
Show high fluctuations in population densities	Hardly fluctuate during the year
Release large numbers of small eggs and are often viviparous	They have few offspring, small gonads
Live in ephemeral habitats	Live in habitats with a long durational stability
Species of the families Rhabditidae, Panagrolaimidae, Diplogasteridae and Monhysteridae	Species of the families Nygolaimidae, Thornematidae and Belondiridae

Based on life history strategies, Bongers (1990) assigned a coloniser-persister (c-p) value for different nematode families. Examples given in Table 2.3.

Table 2.3: C-P values for some nematode families: 1 = coloniser, 5 = persister (Values obtained from Ferris 2013a).

C-P	FAMILY	C-P	FAMILY
5	Actinolaimidae	3	Hoplolaimidae
2	Anguinidae	3	Leptolaimidae
3	Aphanolaimidae	1	Monhysteridae
2	Aphelenchidae	1	Panagrolaimidae
2	Aphelenchoididae	2	Plectidae
5	Aporcelaimidae	3	Pratylenchidae
3	Belonolaimidae	1	Rhabditidae
2	Cephalobidae	3	Rotylenchulidae
3	Chronogasteridae	5	Thornenematidae
3	Criconematidae	3	Tobrilidae
4	Dorylaimidae	2	Tylenchidae

The Maturity index (MI) is calculated as the weighted mean of the c-p values assigned to individuals in a representative soil sample using the equation:

$$\mathrm{MI} = \sum_{i=1}^{n} v(i) - f(i)$$

where *v(i)* is the c-p value of the taxon and *f(i)* the frequency of that taxon in a sample. In practice, MI values for soil subjected to varying levels of disturbance range from less than 2.0 in nutrient-enriched disturbed systems to ± 4.0 in undisturbed pristine environments (Bongers & Ferris 1999).

There is a growing awareness of loss of biodiversity and environmental degradation. However, discussion on nematode ecology is conspicuously lacking in most limnological treatises. In 2002, a checklist of free living nematodes from Southern Africa, by Prof. Juan

Heyns was compiled and thereafter completed and published posthumously by Prof. Annemarie Avenant-Oldewage of the University of Johannesburg. This checklist presented all freshwater species recorded from South Africa, Botswana & Namibia. From this checklist, it can be noted that very little work has been done in the Free State Province, South Africa. Ecological work for nematology includes that done on the general ecology of the nematodes by Dye (1977). A case study on nematodes as indicators of pollution was accomplished by Gyedu-Ababio et al. (1999). Gyedu-Ababio also published a paper on the pollution status of two river estuaries in the Eastern Cape, South Africa in 2011. Further articles on work on the meiobenthos of freshwater systems in South Africa (Nozaisa *et al*. 2005; Pillay 2009) includes some work on freshwater nematodes, but does not focus specifically on the subject.

SOUTH AFRICAN WETLANDS: THE SEEKOEIVLEI NATURE RESERVE

Wetlands are considered unique (Mitsch & Gosselink 1993) because of their hydrological conditions and their role as ecotones between terrestrial and aquatic systems. It is therefore understandable that the global importance of wetlands, as well as the scale and extent of disturbance, calls for assessments and monitoring programmes on a national, regional and local scale. The Biodiversity Convention signed during the United Nations Conference on Environment and Development in 1992, identified two of the major threats to the ecological integrity in the conservation of wetlands, as being:

1. Wetland functionality and
2. The role of wetlands as reservoirs of biodiversity, specifically adapted to these ecosystems.

Over two decades ago, Walmsley (1988) already reported that approximately 50% of South Africa's wetland ecosystems have been lost mainly through agricultural development and poor land management. It is likely that the loss of wetlands has increased since then. South Africa is considered a water-scarce country (Mathipa & Le Roux 2009) and this has an immense impact on our precious resource, in a water-scarce country. It requires every effort and hence optimal use of appropriate environmental management tools to ensure maximum compliance with the legal as well as Ramsar requirements (Sandham *et al.* 2008). South Africa, as a signatory to the Ramsar convention on wetlands, has an obligation to promote the conservation and responsible use of wetlands which will include a commitment to the assessment and monitoring of wetland conditions (Ramsar Convention 2002).

18 735 wetlands are mapped within the Free State Province in South Africa and are estimated to cover at least 2129 km^2 (about 1.7% of the Province total area). However, considering the level of accuracy of technology, this may be underestimated (DEAT 2010). It is estimated that there are approximately 23000 wetlands in the Free State at present. The wetlands of the wetter north eastern Free State, especially the Vaal Dam catchment are typically well watered, marshy typed and are known as vleis. In the drier west, seasonal pans predominate. According to the River Health Program (RHP), about 13750 pans that are greater than one hectare in size are located throughout the province (RHP 2003).

The Senqu sub-basin contains the highland sources of springs and rivers, in wetlands commonly called sponges. Stagnant water bodies like marshes, and exposed water bodies, occur in the gentle slope sections of the various rivers, dams in the main stem of the Orange-Senqu River and at the river mouth (Ramsar 2013). The wetlands along the upper Klip River are typical of many that occur in the sub humid to semiarid eastern interior of South Africa (FS DEEAT IMP 2005). The Seekoeivlei Nature Reserve (SKVNR) in the North East, near Memel, is a registered Ramsar site in terms of the Ramsar Convention of Wetlands (Ramsar 2013). This wetland was chosen as the study site for this project.

Chapter 3

STUDY SITE: THE SEEKOEIVLEI NATURE RESERVE

The environmental, economic, social and aesthetic benefits provided by wetlands are becoming increasing appreciated by scientists, engineers and the public at large. According to McCarthy *et al.* (2010), the Seekoeivlei Nature Reserve (SKVNR) is a specifically important wetland in South Africa because:

1. It is the only protected area in the Free State Province covering the Amersfoort Highveld Clay Grassland and the Eastern Temperate Freshwater Wetland veld type.
2. It is the largest protected area of wetlands on the Highveld and in South Africa.
3. A large number of threatened flora and fauna have established a habitat here.
4. Several endangered avian species have set up a breeding site in this area.

GEOLOGICAL EXTENT AND GEOLOGICAL ORIGINS

The Nature Reserve covers an area of approximately 4 500 ha (45 km^2) and is situated in the Drakensberg mountain foothills, on the north-eastern boundary of the Free State Province near the town of Memel (Fig 3.1). It lies between the following co-ordinates: 27˚32′ to 27˚39′ South and 29˚34′ to 29˚36′ East (FS DTEEA IMP 2005). A conglomerate of complex ecosystems and habitats are contained in the area and according to Du Preez & Marneweck (1996), the World Conservation Strategy recommended that the region be recognised as a priority biogeographic region in which major protected areas should be established.

The SKVNR is made up of a flat floodplain which is surrounded by an undulating landscape interspersed with small koppies (Fig. 3.2A & B). The wetland consists of a unique aquatic habitat with a floodplain up to 1.5 km wide that is comprised of numerous oxbow lakes, abandoned channels and backswamps (Fig. 3.3) (Tooth & McCarthy 2007). Approximately 220 oxbow lakes (Fig. 3.4A & B) have formed over centuries by the meandering course of the Klip River (Fig. 3.4C & D). This marshland is a very important sponge area for the Vaal River (Fig. 3.4E & F) and it has high conservation priority since it provides water to the highly industrialised and densely populated Gauteng Province (Ramsar 2013). The Klip River arises at an elevation of approximately 1950 m in the Drakensberg Mountains, and flows 230 km north and northwest to the Vaal River. The wetland is of natural origin and remains constant at approximately 1700 meters above sea level, from the reserves southern to its northern boundary, a distance in a straight line of 14 km. The high altitude of the wetland plays a critical role in regulating water flow as well as maintaining the highest water quality standards (Du Preez & Marneweck 1996).

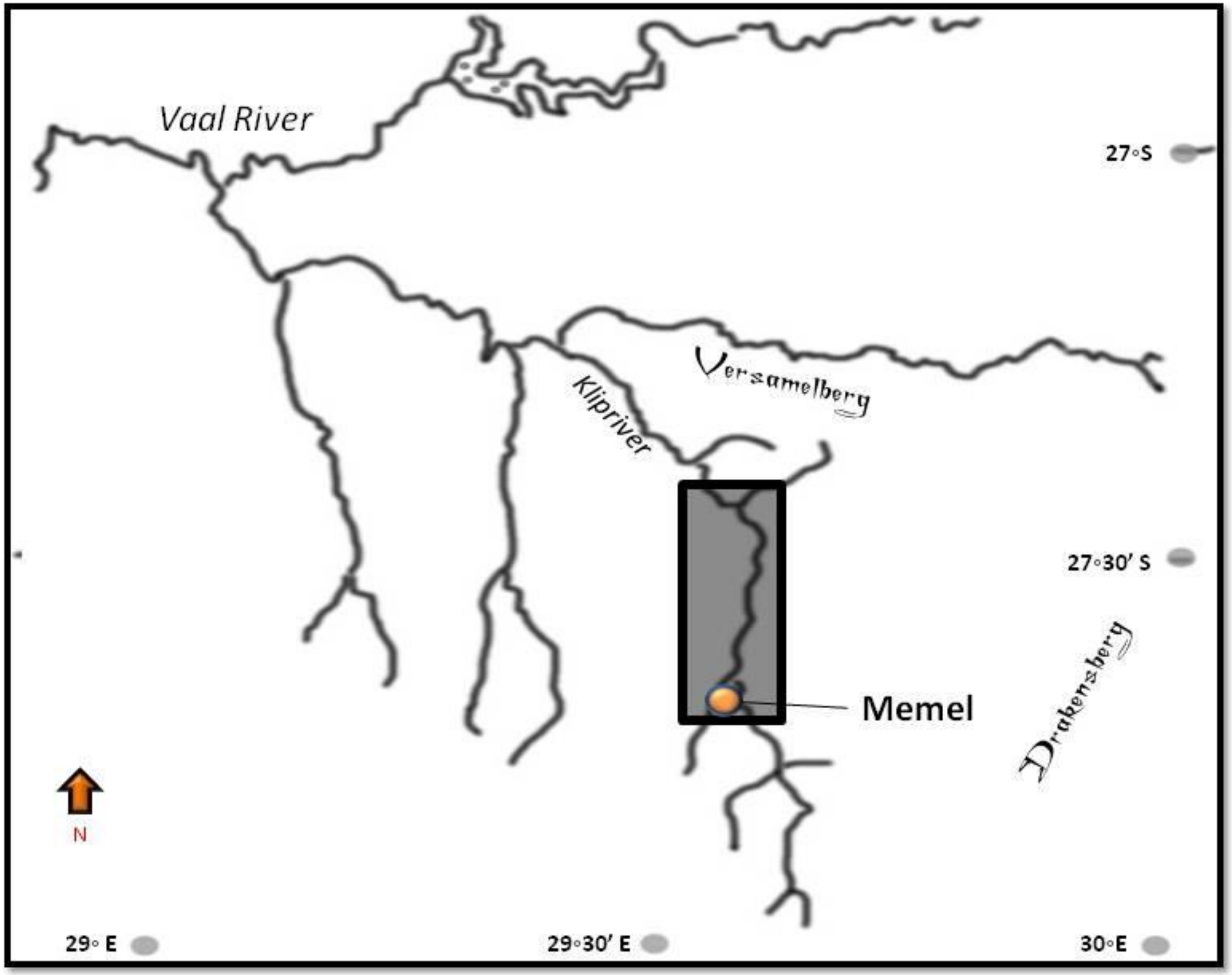


Figure 3.1: The Seekoeivlei Nature Reserve of 4500ha (45 km^2) is situated in the Drakensberg mountain foothills, on the north-eastern boundary of the Free State Province near the town of Memel (Redrawn from Tooth *et al.* 2009).

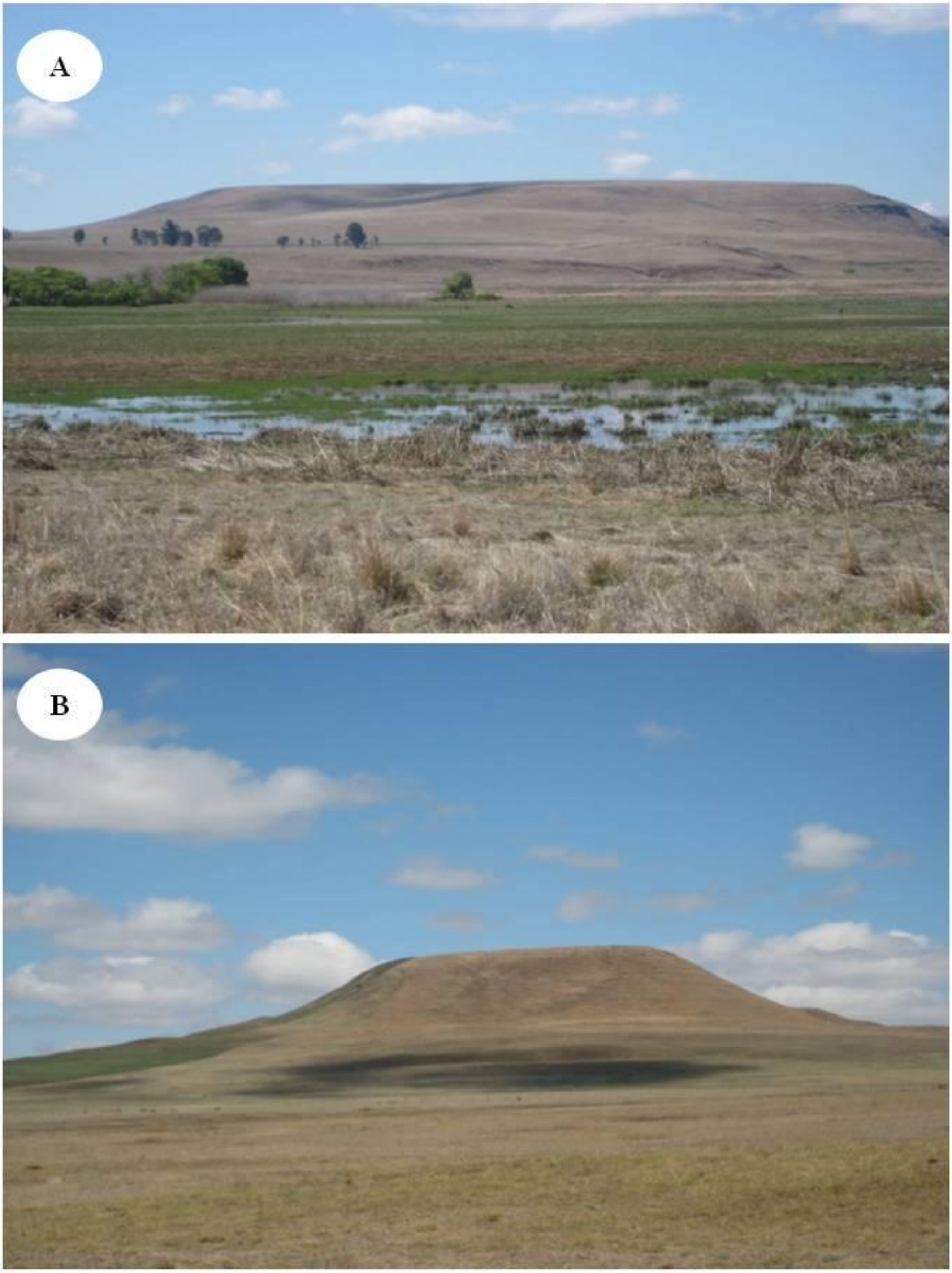


Figure 3.2: (A) & (B) Wetland flat floodplain surrounded by an undulating landscape interspersed with small koppies at the Seekoeivlei Nature Reserve.

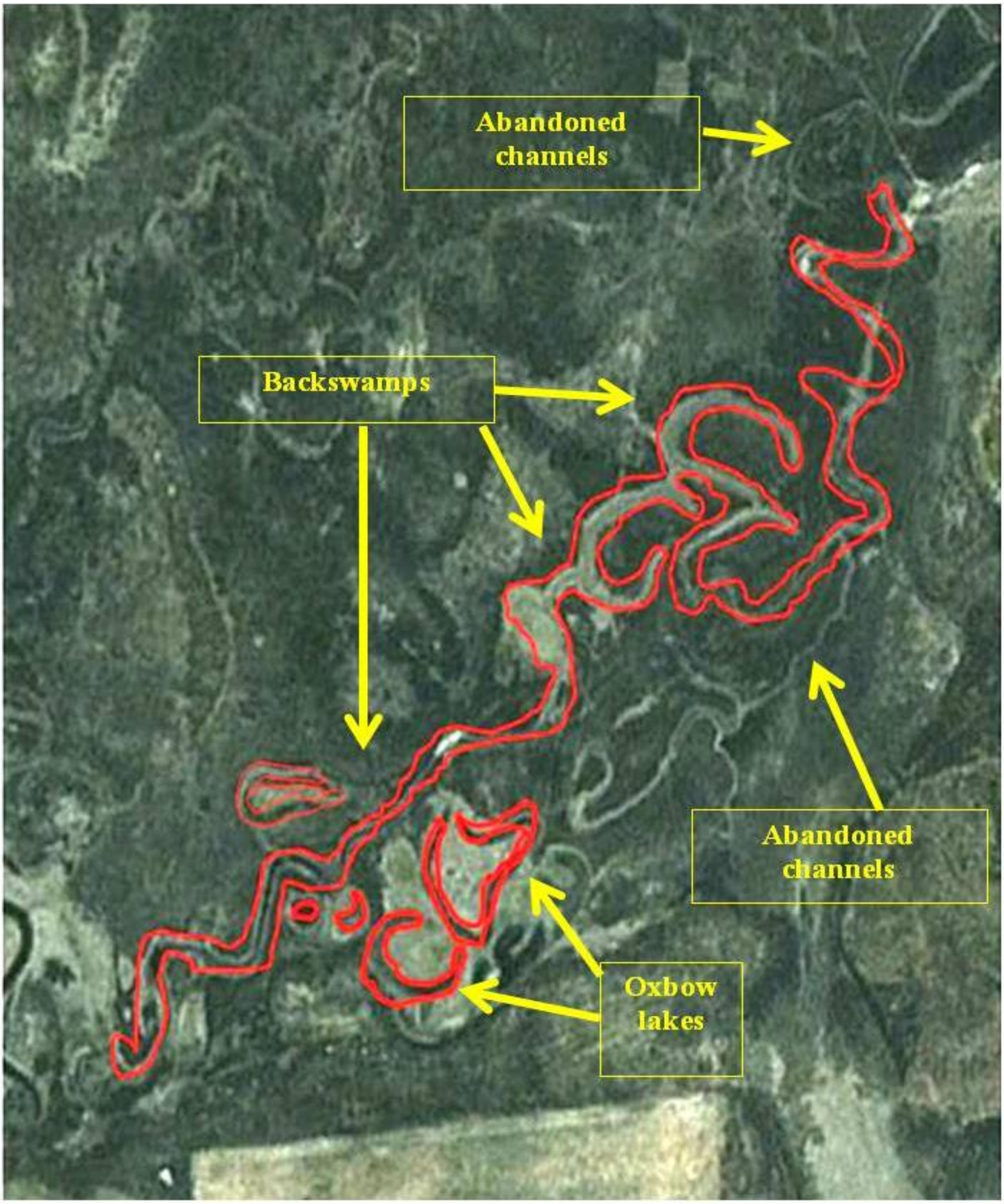


Figure 3.3: Aerial view portraying oxbow lakes, abandoned channels and backswamps of the wetland within the Seekoeivlei Nature Reserve. (Edited image (Google Earth 2013).

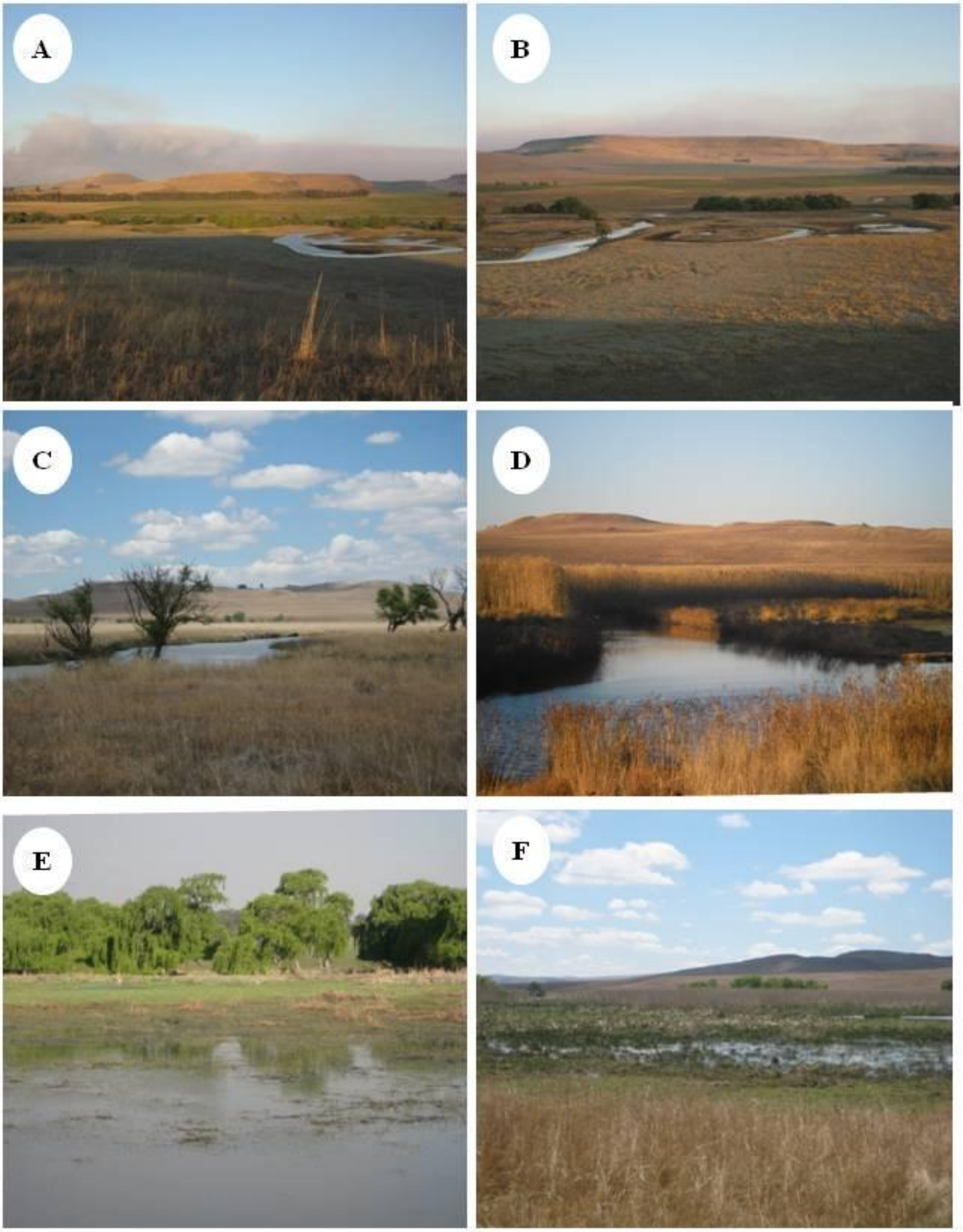


Figure 3.4: (A) & (B) Oxbow lakes. **(C) & (D)** Meandering course of the Klip River**. (E) & (F)** Floodplains and marshlands of the wetland in the Seekoeivlei Nature Reserve.

The wetland in the reserve covers 3000 ha (30 km^2) of the 12000 ha (120 km^2) floodplain. This wetland is the largest on the Highveld and considered one of the most important natural wetlands in South Africa (FS DTEEA IMP 2005). It was designated as a Ramsar site on the 21 January 1997, Ramsar site 888 (Ramsar 2013).

GEOLOGY

The SKVNR is underlain by sediments of the lower Beaufort and upper Ecca Groups of the Karoo Sequence. The Normandien formation of the Beaufort group consists of various mudstones (shales) and sandstones. Alluvium has been deposited over a layer of shale (approximately 205 meters in depth). The alluvium consists of unconsolidated grey-coloured fine and clay-rich sand and silt. Dolerite dykes and sills cut through the sediments and occur throughout the reserve. The area is generally flat to slightly undulating, but becomes more jagged in the mountainous catchment area south-east of the floodplain (Du Preez & Marneweck 1996). According to McCarthy *et al*. (2010), the flow and sediment regime have been altered as a result of channel modifications coupled with faunal and floral changes. This initiated major changes to erosional and depositional patterns.

SOILS

Some of the soils in this area are very erodible and dispersive, resulting in erosion being a great concern and is imperative that a good vegetation basal cover is maintained (FS DTEEA IMP 2005). The soils vary from deep (>500 mm) vertic Rensburg and Arcadia forms to exposed rocky gravel deposits in the stream beds. The soils are seasonally waterlogged in the marshy areas. Peat does occur in some areas and consists of loosely compacted, half decayed plant materials which can consist of up to 97 % water (Ramsar 2013). Bank materials coarsen downstream, being dominated by silt and clay in the upper part of the reach, and by sand in the lower part (Marren *et al.* 2006). Overbank flooding and precipitation result in floodplains being submersed during summer months, whilst progressively desiccating during winter months. Oxbows, abandoned channels and backswamps retain water year round especially in the upper muddier part (Tooth & McCarthy 2007).

HYDROLOGY

The Seekoeivlei wetland can be classified as being a valley bottom floodplain wetland, implying that the wetland receives water and is drained through various water transfer mechanisms (Fig. 3.5).

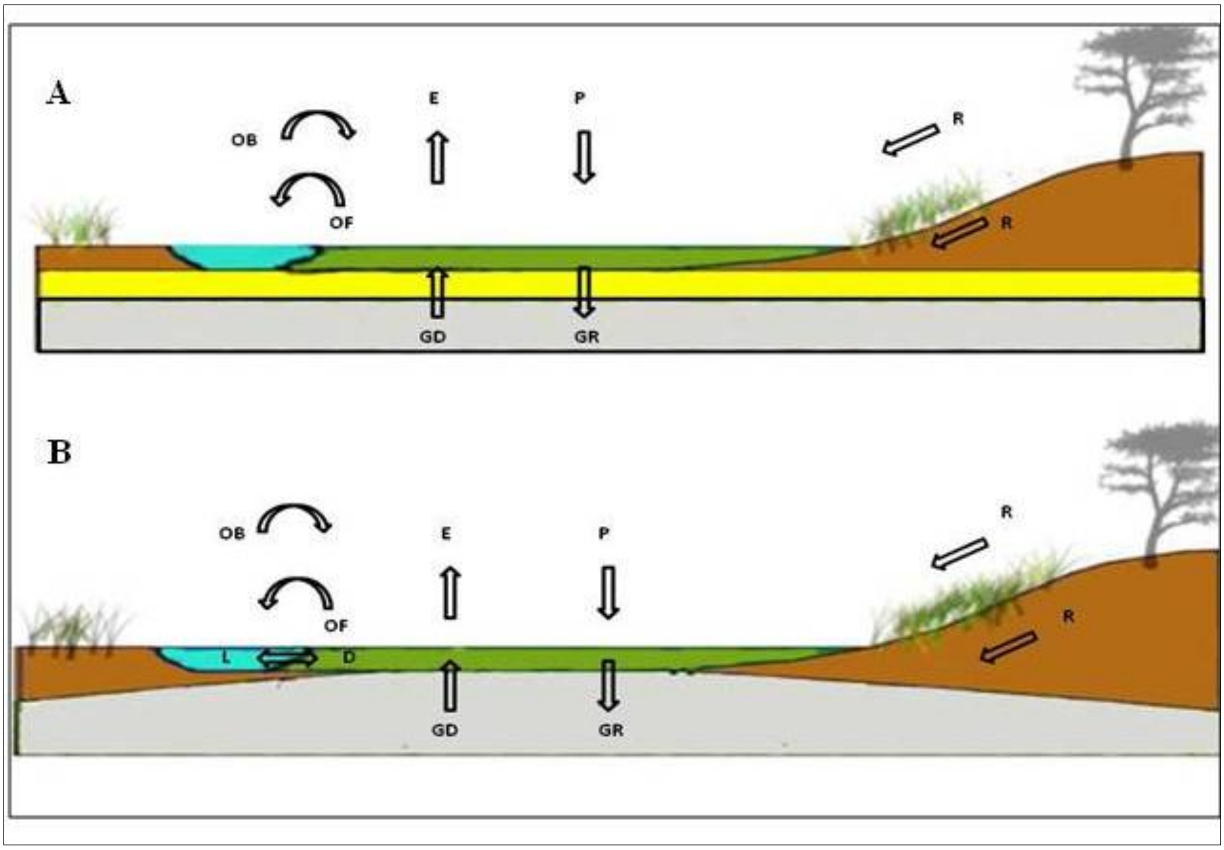


Figure 3.5: Water transfer mechanisms in valley bottom wetlands **(A)** Surface and groundwater-fed **(B)** Groundwater-fed (edited from Collins 2005). D = Drainage, E = Evaporation, GD = Groundwater Discharge, GR = Groundwater Recharge, L = Lateral inflow, OB = Overbank Flow, OF = Overflow, P = Precipitation, R = Runoff.

Following over a century of direct and indirect anthropogenic impacts, faunal and floral changes in addition to channel modifications have altered the flow and sediment regime of the wetland. This initiated major changes to erosional and depositional patterns, including promoting rapid headward growth of a new channel and abandonment of a former channel (McCarthy *et al.* 2010).

RAINFALL

Annual rainfall in the upper Klip River catchment may reach 1200 mm (Du Preez & Marneweck 1996). The mean annual rainfall of the reserve is approximately 800 mm

(McCarthy *et al.* 2010), with the catchment producing an average annual flow of 46 000 000 m^3 (Du Preez & Marneweck 1996). The site consists of seasonal freshwater lakes, riverine floodplains and seasonally flooded grasslands, marshes and pools and peatlands (Ramsar 2013). Flow gauging records show that peak flows occur during the austral summer months i.e. November to March (McCarthy *et al.* 2010). Lower flows occur during the winter months (Tooth *et al.* 2009). Floodplains are generally submersed by a combination of overbank flooding and local rainfall. Although they progressively desiccate during winter, many abandoned channels, oxbows, and backswamps are able to retain water year-round. Precipitation is mostly in the form of thunderstorms which occur between November and March. However, toward the end of December to the middle of January, mid-summer droughts do occur (Du Preez & Marneweck 1996; McCarthy *et al.* 2010).

CLIMATE

The mean annual temperature of the SKVNR is 15.2˚C. Wind occurs mostly from a south-westerly and easterly direction (FS DTEEA IMP 2005). Temperature ranges are typical of a high-altitude plateau climate with fluctuation in seasonal temperatures ranging from an average winter temperature of -2.3˚C to an average summer temperature of 26.5˚C. The absolute minimum temperature was measured at -15.3˚C and absolute maximum temperature at 37.0˚C (FS DTEEA IMP 2005).

ECOSYSTEM STRUCTURE

VEGETATION

Tiner (1999) defines a hydrophyte as:

> "An individual plant adapted for life in water or periodically flooded and/or saturated soils (hydric soils) and growing in wetlands and deepwater habitats; it may represent the entire population of a species or only a subset of individuals so adapted".

It is important to note from this definition that there is a specific reference to "an individual plant" which "may represent the entire population of a species or only a subset of individuals so adapted". This wording implies that not all individuals of a species need to occur within wetlands for that species to be considered a hydrophyte. Thus, even if only a single individual of a species occurs within a wetland, then that species may be considered a hydrophyte (Collins 2005).

The vegetation of the reserve is defined as grassland biome. According to O'Conner & Bredenkamp (1997), this biome occupies 349 174 km^2 and is centrally located in southern Africa. It is a vegetation type (Fig. 3.6A, B & C) that covers 24 percent of the country and dominates the central plateau. The reserve is the only formally protected area preserving this veld type which occurs mostly between an altitude of 1700 and 1850 meters (FS DTEEA IMP 2005).

According to Du Preez & Marneweck (1996), the vegetation of the SKVNR area can be characterised as: Grassland, woodland and thicket and hygrophilous communities. The vegetation found on the sandy loam soils in the eastern Free State may be classified as: *Aristida junciformis* Trin. & Rupr. – *Eragrostis plana* (Schrad.) Nees, grassland. The poorly drained floodplains form typical examples of seasonally moister habitats within drier western grasslands (O'Conner & Bredenkamp 1997). Whilst generally poor in species. *E. plana* is usually dominant and *Hyparrhenia hirta* (L.) Stapf, is often prominent species in this area (O'Conner & Bredenkamp 1997).

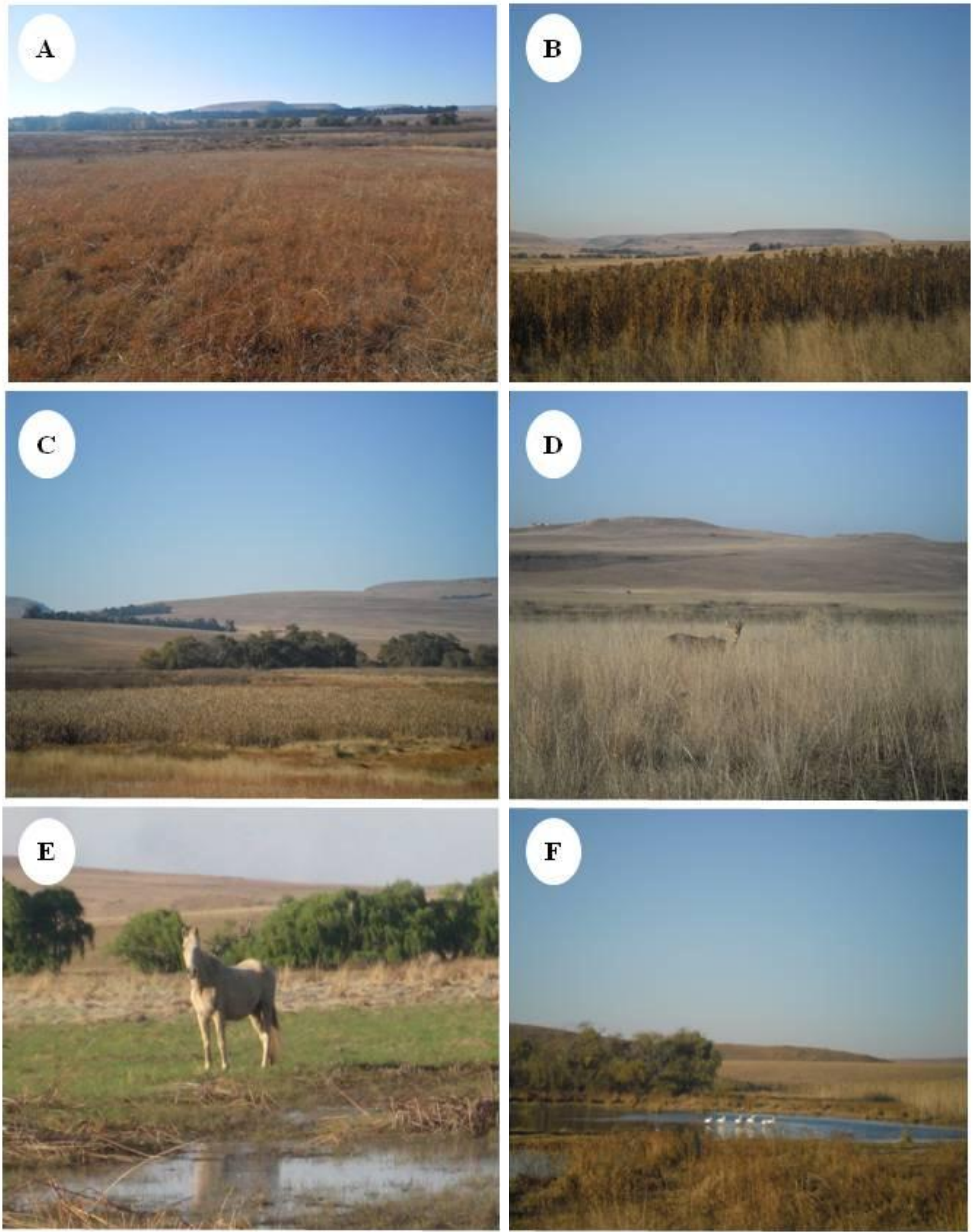


Figure 3.6: (A), (B) & (C): Grassland Biome which is comprised of various grasses and veld vegetation. **(D)** Antelope present at reserve. **(E)** Resident horse, Mamba. **(F)** The Seekoeivlei Nature Reserve supports a large number of birds.

FAUNA

The area supports an appreciable assemblage of rare, vulnerable or endangered species/subspecies of animals. One red data mammal, five red data birds and one red data-listed fish species, are all partially or wholly dependent on the wetland.

MAMMALS

A total of 31 mammal species, including antelope (Fig. 3.6D) have been recorded at the SKVNR. The most important species in terms of their conservation status and importance include roan antelope, *Hippotragus equinus* Desmarest, 1804; buffalo, *Syncerus caffer* (Sparrman, 1779) and black wildebeest, *Connochaetes gnou* (Zimmermann, 1780). According to McCarthy *et al.* (2010), ungulates and hippopotami (*Hippopotamus amphibious* L.) were eradicated from the wetland and replaced by cattle for farming in the early twentieth century. Hippopotami were reintroduced to the reserve in 1999. Local bank erosions and the formation of extensive networks of trails in some areas have resulted from the movement of hippopotami along the length of the wetlands, especially along the channel as well as surrounding areas for nocturnal grazing

Besides the reintroduction of hippopotami (the name of the wetland has been deduced from the Afrikaans translation ‘seekoei’), the reserve has also been restocked with certain game species and currently supports ten species of game. South African endemics are present and include the black wildebeest and grey rhebok, *Pelea capreolus* (Forster, 1790). Rare and endangered species are represented by roan antelope and oribi, *Ourebia ourebi* Zimmerman, 1782. Species sought after by tourists include *H. amphibious*, roan antelope and buffalo, although currently present in low numbers. From a tourism perspective, the hippopotamus is the most important species. This is as a result of their limited distribution. The extensive wetland is also suitable for the establishment of reedbuck, *Redunca arundinum* (Boddaert, 1785), a species that has declined rapidly throughout the country and is now found in reasonable numbers only in the KwaZulu-Natal Province (FS DTEEA IMP 2005). Also present at the reserve is a resident horse named Mamba (Fig. 3.6E).

BIRDS

The SKVNR supports a large number of local and migratory birds. It is a world renowned sanctuary rich in birdlife (Fig. 3.6F) and supporting several species of rare or endangered birds (Ramsar 2013).

Five Red Data bird species are partially or wholly dependent on the wetland, these being:

- Little bittern, *Ixobrychus minutes* (Linnaeus, 1766),
- Yellowbilled stork, *Mycteria ibis* (Linnaeus, 1766),
- Grass owl, *Tyto capensis* (Smith, 1834),
- Wattled crane, *Bugeranus carunculatus* (Gmelin, 1789), and the
- White-winged flufftail, *Sarothrura ayresii* (Gurney, 1877)

Of the 102 South African birds that are listed in the Red Data Book, 29 species occur in the Free State. The wattled crane is particularly important as its nesting site within the reserve is one of only three in the Free State Province. The wattled crane has been listed as "critically endangered'' in the Red Data listings. The white-winged flufftail is another extremely rare bird that has been recorded in the wetland. These birds are restricted to high altitude marshes where sedges and aquatic grasses can be found growing in shallow water. The white-winged flufftail has been reported from only nine localities throughout the country and its ultimate survival is entirely dependent on effective wetland conservation (FS DTEEA IMP 2005).

The introduction of exotic trees provided perching, roosting and nested sites for bird species that would not normally have been resident, as a result contributing to the biodiversity of the reserve (McCarthy *et al.* 2010).

FISH

A total of seven fish species have been recorded within the reserve. The large-mouth yellowfish, *Labeobarbus kimberleyensis* Gilchrist & Thompson, 1913, and small-mouth yellowfish, *Labeobarbus aneus* (Burchell, 1822), have both been declared red data species and are indigenous to the Orange River System.

REPTILE & AMPHIBIAN

Whilst at least 47 reptile and 20 amphibian taxa are expected to occur in the reserve (FS DTEEA IMP 2005), Bates (1997) listed only two reptile and two amphibian taxa.

The Giant bullfrog *Pyxicephalus adspersus* Tschudi, 1838, is known to be widespread and not known to be severely threatened in the Free State. However, economic activity and pollution are of concern as this may lead to the disappearance of water bodies which are required for breeding. No monitoring programs are being undertaken at this time on any amphibians in the Free State. Thus, no information is available with respect to population numbers or decline in particular areas (FSDETEA SOER 2008).

INVERTEBRATES

Although 46 insect species have previously been recorded at the reserve, very little is known about the invertebrates. The list of nematodes collected and identified to genus level for this project can be found in Table 5.1 and are discussed in chapters 5 and 6.

OTHER FACTORS

HUMAN ACTIVITIES

Human activities at the SKVNR include livestock grazing and tourism (Ramsar 2013)

GRAZING OF UNDEVELOPED WETLANDS BY DOMESTIC STOCK

Commercial farming in the upper Klip River valley started in the late nineteenth century and resulted in the establishment of the town of Memel by the early twentieth century. The development of farming caused modifications to the wetlands ecosystem process. Cultivation took place which included mainly maize, wheat and animal winter feed crops. The primary land use was however cattle farming (McCarthy *et al.* 2010).

Grazing has both positive and negative effects on the indirect benefits of wetlands. In wetlands, some grazed areas are short and some are left tall, thereby increasing diversity of habitats. However those which are completely grazed, decreases the diversity of habitats. Some wetlands erode easily when disturbed by trampling and grazing. In these situations, the erosion can cause the channel to cut into the wetland and dry it out destroying most of its functions and values (Collins 2005). Although the wetland does not have a very high plant diversity, part of the catchment area is used for farming and in areas where the soil is arable, maize and wheat are cultivated. Cattle and sheep are grazed on the typical short dense grassland (Du Preez & Marneweck 1996).

When wetlands are converted to cropland, most of their indirect benefits are lost, especially when they are drained. Drained wetlands are less effective at regulating stream flow and purifying water as the drainage channels speed up the movement of water through the wetland. Hydrological changes resulting from wetlands have negative effects on the soil, such as reduced soil organic matter and moisture levels. Erosion is less effectively controlled as crops are planted which do not bind soil as well as natural wetland vegetation. Adding pesticides further reduces effectiveness of the wetland and purifying of water (Collins 2005).

TOURISM

The fact that the wetland is a sanctuary that supports large numbers of local and migratory birdlife is well known amongst professional and amateur ornithologists as well as photographers (Ramsar 2013).

ALIEN SPECIES

Exotic species introduced to the wetland in the late nineteenth and early twentieth century, include:

- Willows - *Salix* spp.,
- Bluegum - *Eucalyptus* spp.,
- Pines - *Pinus* spp. and
- Poplars - *Populus* spp.

The removal of exotic trees and erosion control structures would in fact further reduce habitat and biodiversity permanently, in the case of some avian species as well as some aquatic species. This is because of the very slow natural rates of channel and floodplain change (McCarthy *et al.* 2010).

EROSION

As previously stated, wetlands are characteristically areas where movement of surface water is slowed down and sediment is deposited. When wetlands vulnerable to erosion, do erode, more sediment is removed than is deposited. The result is deep gullies forming which drain water from the wetland, making it less wet. This greatly reduces the values of the wetland. The susceptibility of a wetland to erosion depends on several factors including the stability of the soil, the slope and landform setting. Other influences are vegetation cover and disturbances such as those by cattle or farm machinery (Collins 2005).

Chapter 4

MATERIAL & METHODS

A permit was obtained from the Free State Department of Economic Development, Tourism and Environmental Affairs (FSDETEA) for collection of nematodes at the Seekoeivlei Wetland (Appendix 1). Field trips were made to the Seekoeivlei Nature Reserve (SKVNR) on three separate occasions:

- The Preliminary Study: 03 - 06 October 2011
- A Summer Survey: 14 - 17 February 2012
- A Winter Survey: 14 - 16 May 2012

FIELD PROCEDURES

COLLECTION LOCALITIES

Three different localities were chosen within the reserve for the collection of soil samples. These sites were casually labelled:

1. The Bird Lookout Point - as a result of a bird lookout point situated on the site (Fig. 4.1A)
2. The Hippo Pool - hippotami were spotted in the pool (Fig. 4.1B),
3. The Mamba Pool - a resident horse named Mamba resided in the area (Fig. 4.1C).

The GPS locations and elevation were measured at each locality (Table 4.1). Water parameter measurements including: temperature, pH and dissolved oxygen concentration were taken at each site during the summer and winter surveys using the H19023 Multiparameter instrument.

Figure 4.1: **(A)** The Bird Lookout Point **(B)** The Hippo Pool **(C)** The Mamba Pool at the Seekoeivlei Nature Reserve (SKVNR).

Table 4.1: Name, GPS coordinates elevation and description of three sites at the Seekoeivlei Nature Reserve (SKVNR).

NAME	GPS CO-ORDINATES	ELEVATION	DESCRIPTION
Bird Lookout Point	27˚36′677″S 029˚34′641″E	1706 m	Consisted of a grassland, marsh and pool area hence a lentic environment. An abundance of birds were present at this site, as well as small and large mammals roaming in the area.
Hippo Pool	27˚37′270″ S 029˚39′914″E	1712 m	*Included a riverine floodplain and considered a lotic environment.* The hygrophilous vegetation which is restricted to stream banks and streambeds can be broadly described as *Eragrostis plan - Agrostis lachnantha* wetland community. Also present at this site was a weeping willow tree (*Salix babylonica*), which is an invasive alien woody perennial.
Mamba Pool	27˚38′302″S 029˚34′942″E	1681 m	Made up of a grassland, peatland, marsh and pool area forming a lentic environment similar to that at Bird Lookout. This water body is closest to the nearby town of Memel. The area is restricted from larger wild land animals by a fence. Domesticated animals including a horse and cows were also present.

Collection of samples

Collection of samples at the SKVNR (Fig. 4.2A & B), were made using twelve 60 ml syringes which were cut at suction point (Fig. 4.2C). Soil samples were collected in replicates of three, at a depth of 20 cm using the core syringe method (Fig. 4.2D & E). A new syringe was used for each individual collection and thoroughly washed before being used again. Soil samples were placed in bottles (Fig. 4.2F) and kept in the refrigerator. Bottled samples were placed in cooler boxes for transportation back to the nematology laboratory at University of the Free State (UFS) campus in Bloemfontein.

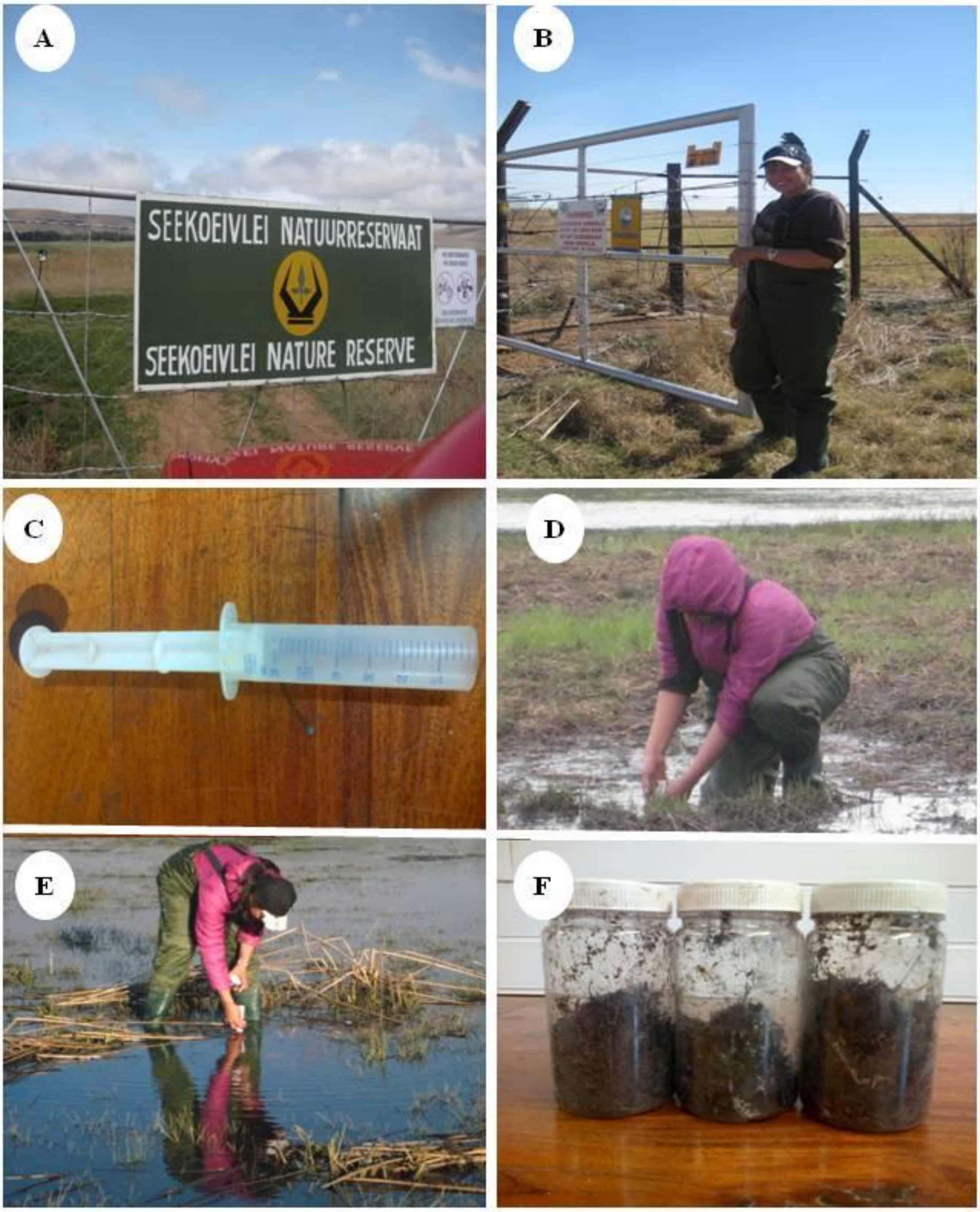


Figure 4.2: (A) & (B) Fieldwork at the Seekoeivlei Nature Reserve. **(C)** 60 ml syringes cut at suction point. **(D) & (E)** Soil samples collected at two different localities using the core syringe method. **(F)** Bottled soil samples were stored at the nematology laboratory, UFS.

Figure 4.3: Laboratory work at the nematology laboratory, UFS, Bloemfontein **(A)** Refrigerated soil samples. **(B)** Sieves and bucket used for extraction of nematodes. **(C)** Centrifuge used for adapted sugar centrifugal method for extraction of nematodes. **(D)** Dessicator filled containing watch glasses with nematodes ready for mounting. **(E)** Bunsen burner, wax, steel tube and stand used for making Cobb slides. **(F)** Nematode specimens mounted on Cobb slides.

LABORATORY WORK

Once transported back to the nematology laboratory at UFS, soil samples were refrigerated (Fig. 4.3A) before nematodes were extracted, fixed and mounted.

EXTRACTION OF NEMATODES FROM SOIL SAMPLES

Nematodes were extracted from soil by an adapted decanted and sieving method (Fig. 4.3B) (Hooper *et al.* 2005; Khan 2008) as originally described by Cobb (1918).

Principle:

This method is based upon the density and size of nematodes. When the soil samples were stirred and allowed to settle for 30 seconds, about 90% of the nematodes remained in suspension. Approximately 20% of the nematodes originally present in the soil sample would have been lost during this method.

Procedure:

After the soil sample was soaked in water, soil particles with a diameter of more than 1mm was removed by passing the sample through a 1mm kitchen sieve which had been placed above a five litre bucket. The residue on the sieve was washed out for about two minutes and then discarded. Thereafter, the bucket was filled with tap water up to five litres. The soil sample was thoroughly mixed with the water and the mixture was allowed to settle for approximately 30 seconds. The mixture was then decanted through a 38 µm mesh sieve, resulting in the sediment settling at the bottom of the bucket.

The procedure was repeated twice more with settling times of 20 and ten seconds respectively. The nematodes and fine soil particles retained on the sieves were then washed into centrifuge tubes.

This was followed by an adapted sugar centrifugal method (Fig. 4.3C) (Hooper 1970) as originally described by (Caveness & Jensen 1955).

Principle:

This method is based on the specific gravity of nematodes.

Procedure:

Water centrifugation

The soil was washed into 50 ml tubes using tap water and a teaspoon of kaolin powder added. Thereafter centrifuged for seven minutes at 1750 rpm (revolutions per minute). After centrifugation, the supernatant was carefully decanted and discarded.

Sucrose centrifugation

A sucrose solution with a specific gravity of 1.15 was added to the centrifuge tubes containing the nematodes at the bottom. The sucrose solution was prepared by adding 625g sugar to 1 litre tap water. This solution and the sediment with nematodes in the centrifuge tubes were thoroughly mixed and centrifuged for three minutes again at 1750rpm. After the centrifugation was completed, the supernatant (with nematodes in suspension) was decanted on a 38 µm mesh sieve and gently rinsed with tap water in order to remove the sucrose as quickly as possible. The nematodes were washed into a sample beaker for examination and counting purposes.

PRESERVATION OF NEMATODES

Nematodes were individually picked using a fine picking needle, under a dissection microscope and placed in an embryo dish. Nematodes were heat killed and fixed using the glycerol-ethanol method [Seinhorst (1959) as modified by De Grisse (1969)].

Procedure

Nematodes were transferred from the 4% formaldehyde solution into an embryo dish containing a Glycerine-I solution (99 ml 4% formaldehyde + 1 ml glycerine). The embryo dish was placed in a dessicator that contained 96% ethanol. The dessicator was placed into an oven at 30°C for 12 hours. In this way, the alcohol was replaced by formaldehyde. After 12 hours, the embryo dish containing the nematodes was removed from the dessicator and placed in an oven at 30°C. Following this, two drops of Glycerine-II solution (95 ml 96% ethanol + 5 ml glycerine) was added every two hours. At the end of day two Glycerine-III solution was added and left overnight. The following day the embryo dish containing the nematodes was placed in a dessicator (Fig. 4.3D) containing silica gel (SiO_2) until they were mounted.

MOUNTING OF NEMATODES ON COBB SLIDES

Specimens were mounted on Cobb slides (Cobb 1917), a steel tube (diameter = 1.5 cm) was heated and pressed in paraffin wax and touched on the surface of a glass coverslip. As a result a paraffin ring formed on the coverslip. A small drop of glycerine was placed in the middle of the wax ring. Nematodes were individually fished from the embryo dish using a fine needle and transferred to the drop of glycerine and sealed off by slipping a round glass coverslip over the paraffin ring. The slide was heated over an open flame to melt the paraffin wax (Fig. 4.3E) and in this way a permanent nematode slide was made (Fig. 4.3F).

NEMATODE COMMUNITY ANALYSIS

Nematode specimens once placed on Cobb slides were examined under a Nikon compound light microscope and identified to genus level. For nematode community analysis, nematode genera were classified according to their feeding types (Yeates *et al*. 1993) and assigned c-p (coloniser-persister) values on scale from 1 to 5 according to their R-or-K life strategies (Bongers 1990; 1999). The Maturity Index (MI), Plant Parasitic Index (PPI) and MI2-5, was calculated using these c-p values (Bongers 1990; 1999). Nematode genera data was also used to calculate species richness (S), Shannon-Wiener diversity (H'), Hills N_1 and Simpsons Dominance Index (D) (Neher & Darby 2009).

SPECIES DESCRIPTIONS

Once all nematodes were identified to genus level, key specimens were selected. These included specimens from the following genera: *Chronogaster* Cobb 1913; *Tobrilus* Andrássy, 1959; *Brevitobrilus* Tsalolikhin, 1981 and *Eutobrilus* Tsalolikhin, 1981. The species from these genera were selected for this the taxonomic study because: 1) these were one of the most numerous in all samples found; 2) they are free-living freshwater genera and 3) Two of the species selected had additional male specimens, which were are usually rare in samples.

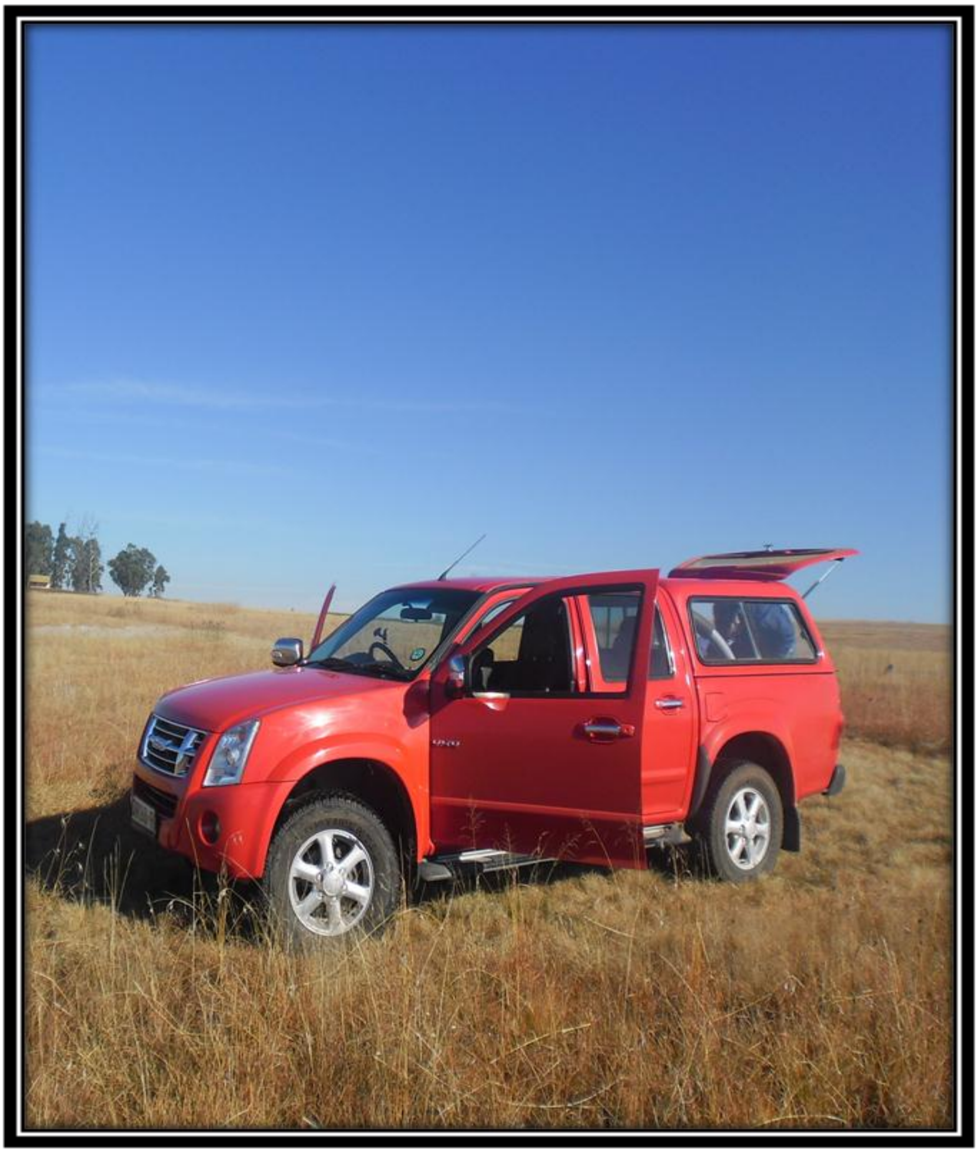
Specimens were drawn using a Nikon 80i compound light microscope with an attached drawing tube. Light micrographs were taken using a Zeiss camera system. Nematodes were identified to species level using literature and taxonomic keys (including: Zullini 2006; Andrássy 2005; Andrássy 2007; Andrássy 2009) for the specific species as referenced in Chapter 6, as well as referring to reference material from the National Collection of Nematodes of the Agricultural Research Council-Plant Protection Research Institute Nematology Unit.

ABBREVIATIONS USED FURTHER IN THE TEXT:

ABBREVIATION	
a	body length divided by greatest body width **(**De Man,1880)
ABW	anal body width
ABE	Anterior Body End
b	body length divided by distance from anterior end to junction of pharynx and intestine **(**De Man,1880)
c	body length divided by tail length (anus or cloaca to tail terminus) **(**De Man,1880)
c'	tail length divided by body width at anus or cloaca **(**De Man,1880)
CSL	cephalic setae length
G_1L	length of anterior genital branch (from vulva to terminal cell of ovary via flexures)
G_2L	overall posterior genital branch length
L	total body length
LM	Light microscope
LRW	lip region width
LSL	labial setae length
n	number of specimens
Nerve ring ABE	distance of nerve ring from anterior body end
Ph. L	Pharyngeal length
Ph. Bulb ABE	Pharyngeal bulb distance from anterior body end
S	supplement expressed with a number indicating supplement position from anterior proximity

Stoma L	length of stoma
Stoma W	width of stoma
Tooth ABE	distance of tooth from anterior body end
Tooth 1 ABE	distance of first tooth from anterior body end
Tooth 2 ABE	distance of second tooth from anterior body end
Vulva ABE	Vulva distance from anterior body end
V%	distance of vulva from anterior end expressed as a percentage of total body length

Chapter 5



ECOLOGICAL RESULTS

NEMATODE GENERA: COMPOSITION, RICHNESS, DIVERSITY AND DOMINANCE

A total of 43 genera belonging to 20 families and 7 orders were identified from the three localities (Table 5.1). Most of the genera (37) were collected from the Bird Lookout Point and the least number of genera were found at the Hippo Pool (25). Eighteen genera were present at all three sites. Four species from four different genera were identified and are described in chapter 6.

Table 5.1: Nematode genera collected from the three sampling localities at the Seekoeivlei Nature Reserve, Memel, with indication of their c-p value. (Key species are described in chapter 6)

ORDER	FAMILY & GENUS	SAMPLING LOCALITY			C-P VALUE
		BIRD LOOKOUT	HIPPO POOL	MAMBA POOL	
ENOPLIDA FILIPJEV, 1929	**Tobrilidae De Coninck, 1965**				**3**
	**Brevitobrilus* sp.1	✓	✓	✓	
	**Eutobrilus* sp.1	✓	✓	✓	
	Neotobrilus sp.1	✓		✓	
	Semitobrilus sp.1	✓		✓	
	**Tobrilus* sp.1	✓		✓	
MONHYSTERIDA FILIPJEV, 1929	**Monhysteridae de Man, 1876**				**2**
	Diplolaimelloides sp.1	✓			
	Eumonhystera sp.1	✓		✓	
	Monhystera sp.1		✓	✓	
PLECTIDA MALAKHOV, 1982	**Aphanolaimidae Chitwood, 1936**				**2**
	Aphanolaimus sp.1			✓	
	Chronogasteridae Gagarin, 1975				**2**
	**Chronogaster* sp.1	✓	✓	✓	
	Leptolaimidae Örley, 1880				**2**
	Leptolaimus sp.1		✓		
	Plectidae Örley, 1880				**2**
	Plectus sp.1	✓		✓	
RHABDITIDA CHITWOOD, 1933	**Cephalobidae Filipjev, 1934**				**2**
	Acrobeles sp.1		✓	✓	
	Acrobeloides sp.1	✓	✓		
	Cephalobus sp.1	✓	✓	✓	
	Eucephalobus sp.1	✓	✓	✓	
	Pseudoacrobeles sp.1	✓	✓		
	Panagrolaimidae Thorne, 1937				**1**
	Panagrolaimus sp.1	✓	✓		

Table 5.1 (continued): Nematode genera collected from the three sampling localities at the Seekoeivlei Nature Reserve, Memel, with indication of their c-p value.

DORYLAIMIDA PEARSE, 1942	**Aporcelaimidae Heyns, 1965**				**5**
	Aporcelaimellus sp.1	✓	✓	✓	
	Dorylaimidae de Man, 1876				**4**
	Dorylaimus sp.1	✓	✓	✓	
	Mesodorylaimus sp.1	✓	✓	✓	
	Prodorylaimus sp.1	✓	✓	✓	
	Qudsianematidae Jairajpujri, 1965				**4**
	Allodorylaimus sp.1	✓		✓	
	Eudorylaimus sp.1	✓	✓	✓	
	Labronema sp.1	✓		✓	
	Microdorylaimus sp.1	✓		✓	
	Actinolaimidae Thorne, 1939				**5**
	Parastomachoglossa sp.1	✓	✓	✓	
APHELENCHIDA SIDDIQI, 1980	**Aphelenchoididae Skarbilovich, 1947**				**2**
	Aphelenchoides sp.1	✓	✓	✓	
TYLENCHIDA THORNE, 1949	**Anguinidae Nicoll, 1935**				**2**
	Nothotylenchus sp.1	✓			
	Ditylenchus sp.1	✓	✓	✓	
	Belonolaimidae Whitehead, 1959				**2**
	Paratrophurus sp.1	✓		✓	
	Tylenchorynchus sp.1	✓	✓	✓	
	Criconematidae Taylor, 1936				**3**
	Criconema sp.1	✓	✓	✓	
	Hoplolaimidae Filipjev, 1934				**2**
	Helicotylenchus sp.1	✓	✓	✓	
	Rotylenchus sp.1	✓		✓	
	Scutellonema sp.1	✓		✓	
	Pratylenchidae Thorne, 1949				**2**
	Hirschmaniella sp.1	✓	✓	✓	
	Radopholus sp.1		✓		
	Zygotylenchus sp.1	✓			
	Rotylenchulidae Husain & Khan, 1967				**2**
	Rotylenchulus sp.1	✓		✓	
	Tylenchidae Orley, 1880				**2**
	Psilenchus sp.1	✓		✓	
	Tylenchus sp.1	✓	✓	✓	

Overall, 1979 individual nematodes were collected from the three localities during the three sampling surveys. The majority of nematodes, almost sixty percent (59.83%) were collected during the summer survey. Close to thirty percent (28.25%) were collected during the winter occasion and 11.93% during the preliminary survey.

The highest numbers of nematodes (718) were collected from the Mamba Pool during the summer collection. The most relatively abundant family at the Mamba Pool during all three occasions was the family Belonolaimidae (dark blue) (Fig. 5.1)). Members belonging to this family at the reserve include: *Tylenchorynchus* Cobb, 1913 and *Paratrophurus* Arias, 1970. During the preliminary survey, members of the family Tylenchidae (light purple) were found to be the most numerous at the Hippo Pool. However, during the summer and winter collection, members of the family Criconematidae (dark purple) proved to be the most relatively abundant (Fig. 5.1). The most prominent family found at the Bird Lookout during the winter survey was Tobrilidae (light green). Tylenchidae (light purple) and Hoplolaimidae (dark orange) showed the highest relative abundance at this location during the preliminary and summer survey, respectively (Fig. 5.1). Members of the family Cephalobidae (light red) were also found to be present in relatively high proportions at all three sites during the summer survey. Members of the family Dorylaimidae (turquoise) were found on all three sites and all three locations, but were in highest proportion at the Mamba Pool during the summer survey.

Members of the family Tobrilidae (light green) and Chronogasteridae (green) were regarded as key families for this study. Tobrilidae is a true freshwater family and was highly abundant during the winter survey. The discovery of a male *Chronogaster* Cobb, 1913 specimen was a rare find and thus, this genus was regarded as an important genus. Even though the members belonging to the genus *Tylenchorynchus* from the family Belonolaimidae were highly abundant, this is not a free-living nematode, but rather a plant parasitic one and it was therefore not regarded as a key genus for the current study.

Table 5.2: Comparison of nematode species richness (S), Shannon-Wiener diversity (H'), Hills N_1 and Simpsons Dominance Index (D) between the different sampling localities per survey at the Seekoeivlei Nature Reserve

SURVEY	LOCATION	SPECIES RICHNESS (S)	SHANNON'S DIVERSITY (H')	HILLS N_1	SIMPSONS DOMINANCE INDEX (D)
PRELIMINARY	**Bird Lookout**	10.0	1.65	5.21	0.29
	Hippo Pool	5.0	1.32	3.75	0.33
	Mamba Pool	8.0	0.75	2.11	0.69
SUMMER	**Bird Lookout**	16.0	2.18	8.78	0.15
	Hippo Pool	13.0	1.60	4.95	0.30
	Mamba Pool	14.0	1.73	5.66	0.26
WINTER	**Bird Lookout**	11.0	1.60	4.95	0.32
	Hippo Pool	11.0	1.56	4.74	0.34
	Mamba Pool	15.0	1.66	5.26	0.29

Even though the highest number of nematodes were found at the Mamba Pool during the summer survey, the Bird Lookout point proved to be highest in terms of species richness during the summer survey i.e. S = 16.0 (Table 5.2). It was however closely followed by that of the Mamba Pool during both the winter survey (S = 15.0) and the summer survey (S=14.0) (Table 5.2). The Hippo Pool during the preliminary survey (S = 5.0) had the lowest species richness overall.

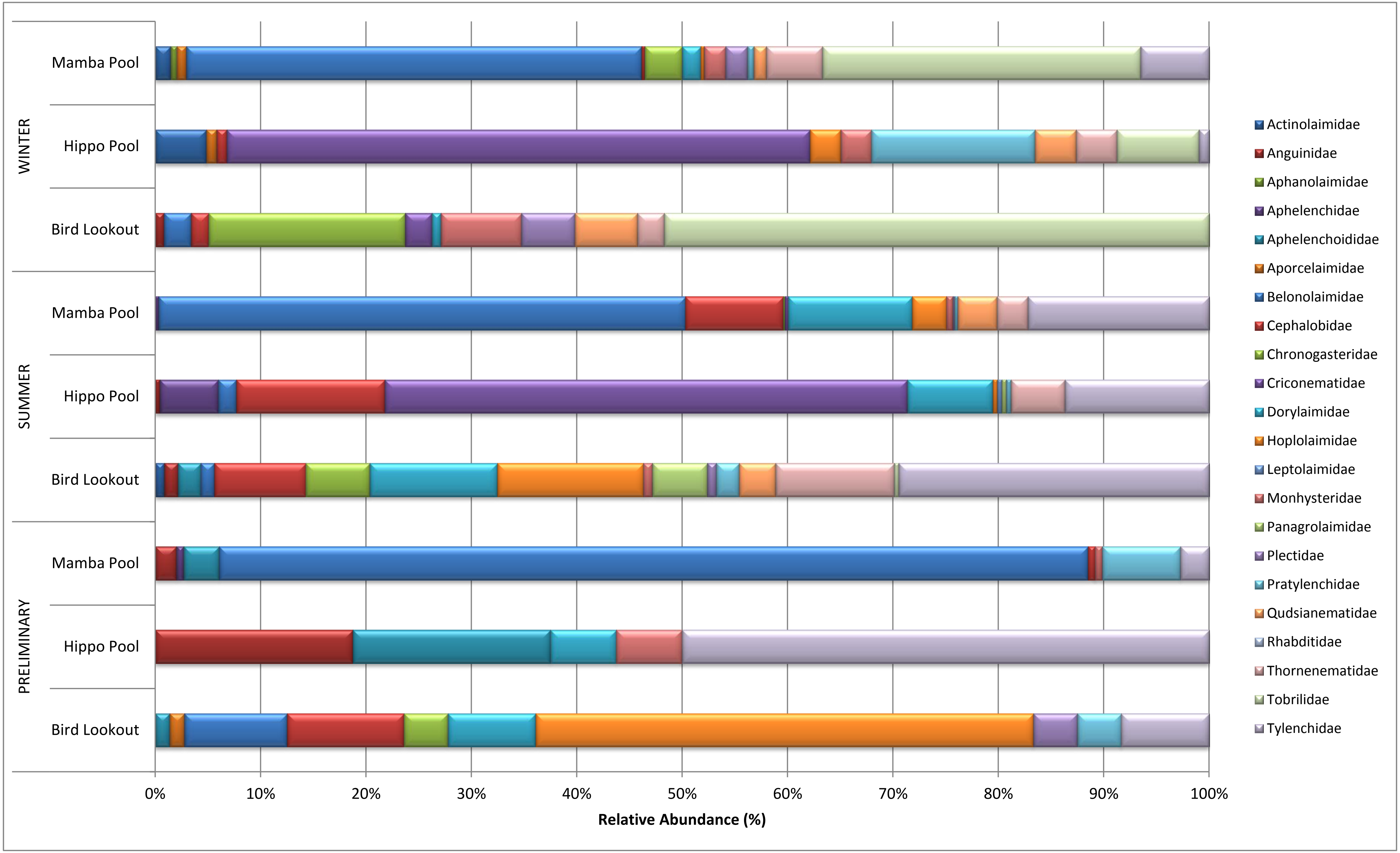


Figure 5.1: Proportion of nematode families from the Seekoeivlei Nature Reserve collected at Bird Lookout Point, Hippo Pool and Mamba Pool during the preliminary, summer and winter sampling survey.

Whilst species richness refers to the number of species collected from a sample, evenness refers to the equitable distribution of proportions of species within a sample. Diversity is therefore the combination of both richness and evenness elements (Neher & Darby 2009). The Shannon-Wiener diversity index (H') showed that diversity was highest at the Bird Lookout point during the summer survey (H' = 2.18) and lowest at the Mamba Pool during the preliminary survey (H' = 0.75). Corresponding results were obtained using Hills diversity (Hills N_1) which was highest at the Bird Lookout Point during the summer survey (Hills N_1 = 8.78) and lowest at the Mamba Pool during the preliminary survey (Hills N_1 = 2.11) (Table 5.2)

Simpson Dominance Index [$D = \sum Pi^2$ (Simpson 1949)] increases as species are distributed more unevenly i.e. an increase in dominance (Neher 2001). D = 0.69 was the highest at the Mamba pool during the preliminary survey in which the family Belonolaimidae proved to be the dominant family (Fig. 5.1). Tylenchidae was most prominent at the Bird Lookout during the summer survey, but with this locality had the lowest dominance overall (D = 0.15). The Hippo Pool had an almost equal Simpson index of D = 0.33, D = 0.30 and D = 0.34, during all three surveys. The family Criconematidae (dark purple) was the most prominent at the Hippo Pool during the summer and winter survey and Tylenchidae the most dominant during the preliminary survey at the same site. Tobrilidae (light purple) was the dominant genera at the Bird Lookout during the winter survey (D = 0.32). It was also found in a large proportion at the Mamba pool during the winter survey.

TROPHIC STRUCTURE

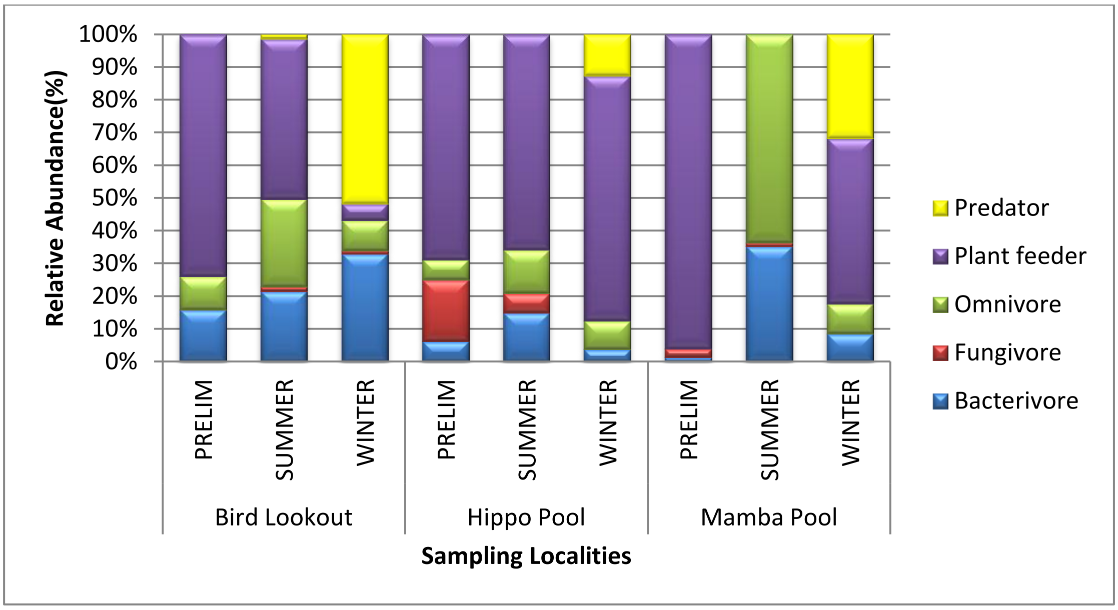


Figure 5.2: Nematode trophic structure from the three sampling localities and sampling surveys from the Seekoeivlei Nature Reserve.

With the exception of the Bird Lookout during the winter collection and Mamba Pool during the summer collection, it was found that all localities were dominated by plant feeders (purple) (Fig. 5.2). Omnivores (green), which are represented by members of the families: Aporcelaimidae, Dorylaimidae, Qudsianematidae and Thornenematidae were most prominent at the Mamba Pool during the summer season. Predators (yellow) which are represented mainly by members of the family Tobrilidae, but also Actinolaimidae were most prominent at the Bird Lookout Point during the winter season. Bacterivores (dark blue) which were second only to plant feeders, were present at all three sites during the winter and summer survey with the highest being at the Mamba Pool during the summer collections. Fungivores (red) were limited and highest at the Hippo Pool during the preliminary survey, but even so, it was at a relatively low percentage in comparison to the other trophic groups during other season collections.

C-P VALUES AND ENVIRONMENTAL DISTURBANCE INDICES

The general opportunists (c-p 1, blue) were represented by only one family Panagrolaimidae and were found at both the Bird Lookout and Hippo Pool, but in relatively low numbers (Fig. 5.3). Enrichment opportunists (c-p 2 -, red) and c-p 3 (green) proved dominant during the summer survey and overall.

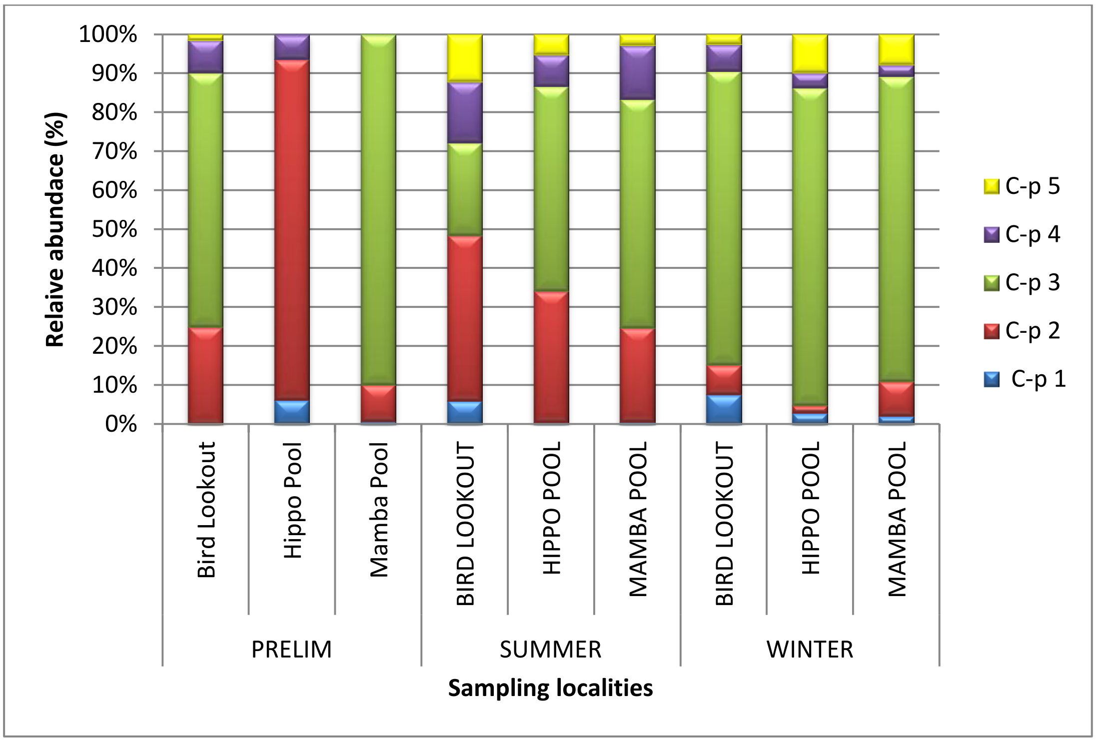


Figure 5.3: C-p values of nematodes at Bird Lookout Point, Hippo Pool and Mamba Pool per sampling survey from the Seekoeivlei Nature Reserve (Prelim = Preliminary).

C-p class 2 were represented by 13 families (Monhysteridae, Aphanolaimidae, Chronogasteridae, Leptolaimidae, Plectidae, Cephalobidae, Aphelenchoididae, Anguinidae, Belonolaimidae, Hoplolaimidae, Pratylenchidae, Rotylenchulidae and Tylenchidae) and c-p class 3 represents two families, namely Tobrilidae and Criconematidae. C-p class 4 (purple) and c-p class 5 (yellow) were each represented by two families. The families Dorylaimidae and Qudsianematidae are assigned c-p 4 values and were more prominent during the summer survey, being highest at the Mamba Pool. The families Aporcelaimidae and Actinolaimidae are assigned c-p 5 values and were virtually absent during the preliminary collection. They were found in relatively low numbers during the summer and winter survey being highest at the Bird Lookout during the summer survey.

It is clear that the highest MI value was at the Hippo Pool during the preliminary survey (MI = 4.30), followed by the Bird lookout point during the winter survey (MI = 2.32) indicating pristine conditions during these spatial-temporal periods (Table 5.3).

Table 5.3. MI, MI2-5 and PPI values at Bird Lookout Point, Hippo Pool and Mamba Pool per sampling survey from the Seekoeivlei Nature Reserve

SURVEY	INDEX	BIRD LOOKOUT	HIPPO POOL	MAMBA POOL
PRELIMINARY	**MI**	1.16	4.30	0.05
	MI2-5	4.20	14.22	1.98
	PPI	2.82	8.59	1.90
SUMMER	**MI**	0.74	0.43	0.12
	MI2-5	1.37	1.22	0.41
	PPI	0.50	0.79	0.29
WINTER	**MI**	2.32	0.90	0.47
	MI2-5	2.79	3.22	0.94
	PPI	0.13	2.17	0.43

As can be seen in Table 5.3., the MI (Maturity Index) values were lowest during all three surveys at the Mamba Pool. The MI2-5 (Maturity Index obtained from cp class 2 to 5 values) is identical to the MI but excludes enrichment opportunists. Results of the MI2-5 showed a virtually similar pattern to the MI overall, with the highest MI2-5 at the Hippo Pool during the summer survey (MI2-5 = 14.22). The Plant-Parasitic Index (PPI) is comparable to the MI but computed only for plant-feeding nematodes. It is with the underlying principle that their large quantity is a result of the vigour of their host plants which in turn is determined by system enrichment. The PPI value was lowest at the Bird Lookout during the winter collection (PPI = 0.13) and highest at the Hippo Pool during the preliminary survey (PPI = 8.59).

PHYSICAL PARAMETERS

The pH remained relatively constant overall with an average of 6.66 in summer and 6.33 in winter (Table 5.4). This indicates a slightly acidic ecosystem. The temperature was highest at the Hippo Pool during summer survey (23.98˚C) and coldest at the Bird Lookout during winter (13.38˚C) (Table 5.4).

Table 5.4: Physical parameters measured during the summer and winter survey using the H19023 Multiparameter instrument at the Seekoeivlei Nature Reserve

	Bird lookout		**Hippo Pool**		**Mamba Pool**		**Average**	
	Summer	*Winter*	*Summer*	*Winter*	*Summer*	*Winter*	*Summer*	*Winter*
pH	6.25	5.24	7.02	6.63	6.71	7.11	6.66	6.33
Temperature (°C)	21.83	13.38	23.98	14.13	23.71	17.73	23.17	15.08
Sal µS/cm	0.05	0.18	0.05	0.07	0.05	0.08	0.05	0.11
DO%	5.50	22.80	39.90	79.00	29.30	73.60	24.90	58.47

The average temperature for summer was 23.17˚C and for winter 15.08˚C. Salinity peaked during winter with an average of 0.11 **µS/cm** and was lowest during summer with an average of 0.05 **µS/cm**. There was a major difference in the dissolved oxygen concentrations (DO%) between the two seasons, with a low concentration in summer (DO = 24.90%) and high concentration in winter (DO = 58.47%).

Chapter 6

TAXONOMIC RESULTS

Four species belonging two four genera and two families were selected for the purpose of this study. The first family was the family Tobrilidae which may be classified as follows:

Kingdom: Animalia

Phylum: Nematoda

Class: Adenophorea

Subclass: Enoplia

Order: Enoplida

Family: Tobrilidae

The genus *Tobrilus* Andrássy 1959

Syn. *Trilobus* Bastian, 1865 nec Brünnich, 1781, (*Paratobrilus*) Micoletzky, 1921

Bastian (1865) originally erected this genus under the name *Trilobus* in which *Tri* = three and *lobus* = a lobe, thus referring to the cardiac glands. Since it was a junior homonym, Andrássy (1959) renamed it *Tobrilus*. The genus became a collecting group for all tobrilids. A total of 110 species were placed under the genus *Tobrilus* or placed into it from Bastian's *Trilobus* (Andrássy 2007). In 2006, Zullini listed 21 species as belonging to this genus. However, in a more recent publication by Andrássy (2007), 23 species were retained in the genus. Species of the genus *Tobrilus* are predominantly freshwater nematodes although they do occur in moist soil. Their diet consists of algae, diatoms and a variety of microfauna including ciliates and rotifers (Goodey 1963; Heyns 1971).

In their article, Joubert and Heyns (1979) indicated that the first reference to *Tobrilus* from South Africa was by Andrássy (1970). In his checklist of freshwater nematodes from southern Africa, Heyns (2002b) listed seven species of *Tobrilus.* However, many of the previously described species from South Africa have now been placed in other genera within the family Tobrilidae. The amount of available information existing for species from the genus *Tobrilus* from South Africa is very limited and therefore the species from the Seekoeivlei Nature Reserve are compared with known species from the available literature. One of the key diagnostic characteristics for specimens being regarded as members of the genus *Tobrilus* for this study, was classified by the stoma being an interior large buccal cavity and containing two overlapping adjacent pockets with a single tooth or two teeth being 0 - 6 µm apart (Zullini 2006). Here, *Tobrilus* sp. 1. is described from South Africa.

Tobrilus sp. 1

(Figs 6.1 - 6.2)

Measurements

(See Table 6.1)

Description

Female

Body straight to slightly or completely ventrally curved in fixed specimens. Cuticle smooth, slightly thickened. Somatic setae absent. Length of longer cephalic setae ¼ of lip region diameter. Shorter setae 1 - 5 µm. Cephalic diameter 13.0 - 25.5 µm. Stoma with two indistinct overlapping pockets and two teeth. Distance of teeth from anterior end 12.0 - 19.0 µm. Amphideal fovea opening at level of anterior tooth or slightly lower, not always visible. Cardiac glands circular to ovoid, width 11.0 - 24.0 µm. Reproductive system amphidelphic-didelphic. Gonads well developed. Vulva at 49% body length from anterior end; transverse slit; vaginal muscular slightly sclerotised; vagina extending inwards one half of mid-body diameter. Tail long, convex-conoid then slender, nine anal body widths long. Three caudal glands present, open through spinneret.

Male

Not found.

Material Examined

Twenty eight females (n = 28) extracted from sediments collected at three different localities at the Seekoeivlei Nature Reserve, Memel.

Diagnosis and Relationships

The species from the Seekoeivlei Nature Reserve is characterised by body length 1000 – 1681 µm, cephalic setae ¼ of lip region diameter, circular to ovoid cardiac glands, vulva at mid body (49%), each genital tract of equal size, tail long, cylindrical 216 - 295 µm, spinneret present.

The species from the present study are similar to *Tobrilus helveticus* (Hofmänner in Hofmänner & Menzel, 1914) Andrássy, 1949 and *Tobrilus brevisetosus* (W. Schneider, 1925) Andrássy, 1959.

The species from the present study is similar to *T. helveticus*, as was described by Andrássy (1958) and Tsalolikhin (2009), in that the species from the present study has no caudal setae and the longer cephalic setae are about ¼ of the labial diameter. It agrees with the population of *T. helveticus* from Denmark as described by Tsalolikhin (2009) in having similar body length and tail length and b-ratio (L = 1468.5 µm *vs.* 1370 - 2200 µm; tail length = 216 µm - 295 µm *vs.* tail length = 276 µm, ratio b = 5.0 *vs.* b = 5.1 - 6.5). Differences between the two populations occur in the body width and pharyngeal length (body width = 26.0 µm *vs.* 54.0 µm and pharyngeal length = 253 - 338.4 µm *vs.* 368 µm). Similarities between the population from the SKNVR and Andrássy's European population include vulva position, as well as b and c' ratios (V% = 43 – 50, ratio b= 4.8 – 6.2, ratio c' = 6.5 – 8.5). The main differentiating characteristic of *T. helviticus* remain labial setae and tail length. According to Tsalolikhin (2009) specific characters may have high degree of intraspecfic variability such as tail length and supplements.

When compared to *T. brevisetosus* it can be seen that the two populations have similar body lengths and vulval positions (L = 1468.5 µm *vs.* L = 1300 - 1700 µm, V% = 49 current study *vs.* V% = 45 - 52), however, significant differences were found in the a, c and c' ratios (ratio a = 51 *vs.* 24 - 36, ratio c = 6 *vs.* 7 - 12 and ratio c' = 9 *vs.* 6-8).

Therefore considering the above mentioned remarks with respect to the differences of species from the SKVNR to the known species we preliminary assign the name *Tobrilus* sp. 1 to the population of *Tobrilus* specimens collected from Seekoeivlei Nature Reserve, South Africa.

Table 6.1: Morphometrical data of *Tobrilus* sp. 1 collected from the Seekoeivlei Nature Reserve, Memel and known species. All measurements in µm and in format mean±stdev (range) for the current morphometrics.

SPECIES	*Tobrilus* sp.1	*Tobrilus helviticus* (Hofmänner & Menzel, 1914) Andrássy, 1959	*Tobrilus helviticus* (Hofmänner & Menzel, 1914) Andrássy, 1959	*Tobrilus brevisetosus* (W. Schneider, 1925) Andrássy 1959)
LOCATION	SKVNR, Memel	Denmark	Europe	Europe
n	28♀	♀	♀	♀
L	1468.5±180.0 (1000.0-1681.0)	2070 (1370-2200)	1500-2200	1300-1700
a	51.0± 9.5 (35.0-74.0)	38.9 (29-52)	28-40	24-36
b	5.0 ± 0.5 (3.5-6.0)	5.7 (5.1-6.5)	4.8-6.2	4.3-5.6
c	6.0 ± 1.0 (4.0-7.0)	7.4 (6.3-8.3)	4.9-9.7	7 -12
c'	9.0 ± 2.0 (6.0-13.0)	-	6.5-8.5	6-8
V%	49.0 ± 4.9 (33.0 - 58.0)	44 (42-45)	43-50	45-52
Stoma L	11.0±1.5 (8.0-13.0)	-	-	-
Stoma W	5.0±1.0 (3.0-7.0)	-	-	-
Nerve Ring ABE	106.0±7.5 (106.0-200.0)	-	-	-

Table 6.1 (continued): Morphometrical data of *Tobrilus* sp. 1 collected from the Seekoeivlei Nature Reserve, Memel and known species. All measurements in µm and in format mean±stdev (range) for the current morphometrics.

SPECIES	*Tobrilus* sp.1	*Tobrilus helviticus* (Hofmänner & Menzel, 1914) Andrássy, 1959	*Tobrilus helviticus* (Hofmänner & Menzel, 1914) Andrássy, 1959	*Tobrilus brevisetosus* (W. Schneider, 1925) Andrássy, 1959)
LOCATION	SKVNR, Memel	Denmark	Europe	Europe
Ph. L	311.0±19.0 (254.0-338.0)	368	-	-
Body width: Neck base	29.0±9.0 (21.0-39.0)	-	-	-
Mid-body	35.0±6.0 (22.0-40.0)	-	-	-
Anus	26.0±3.0 (20.8-32.1)	-	-	-
Vulva ABE	722.0±90.0 (538.0-866.0)	-	-	-
G_1L	92.1±12.5 (73.5-111.5)	-	-	-
G_2L	93.0±22.0 (70.0-165.0)	-	-	-
Tail length	258.0±22.0 (216.0-295.0)	276	-	-

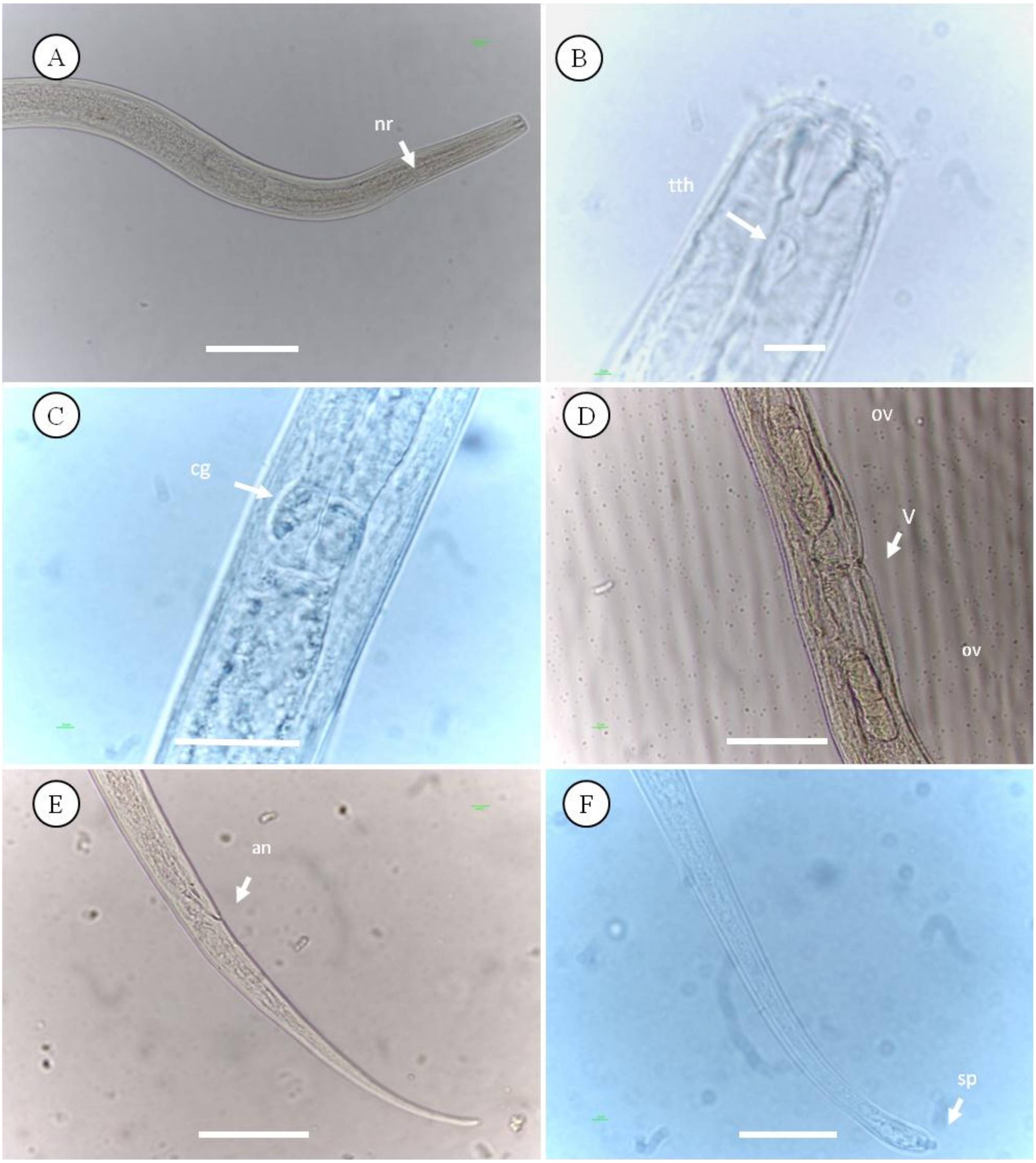


Figure 6.2: ***Tobrilus* sp. 1** collected from the Seekoeivlei Nature Reserve, Memel. (LM) **A**. Female neck region showing nerve ring. **B**. Anterior end focussed on stoma and tooth. **C**. Pharyngo-intestineal junction showing cardiac glands. **D**. Female reproductive system. **E**. Tail showing rectal region. **F.** Spinneret. [an - anus, cg - cardiac glands, nr - nerve ring, OV -ovaries, sp - spinneret, tth - tooth, V - vulva opening] **Scale bars**: A, D, E, F = 50 µm, B = 10 µm, C = 20 µm.

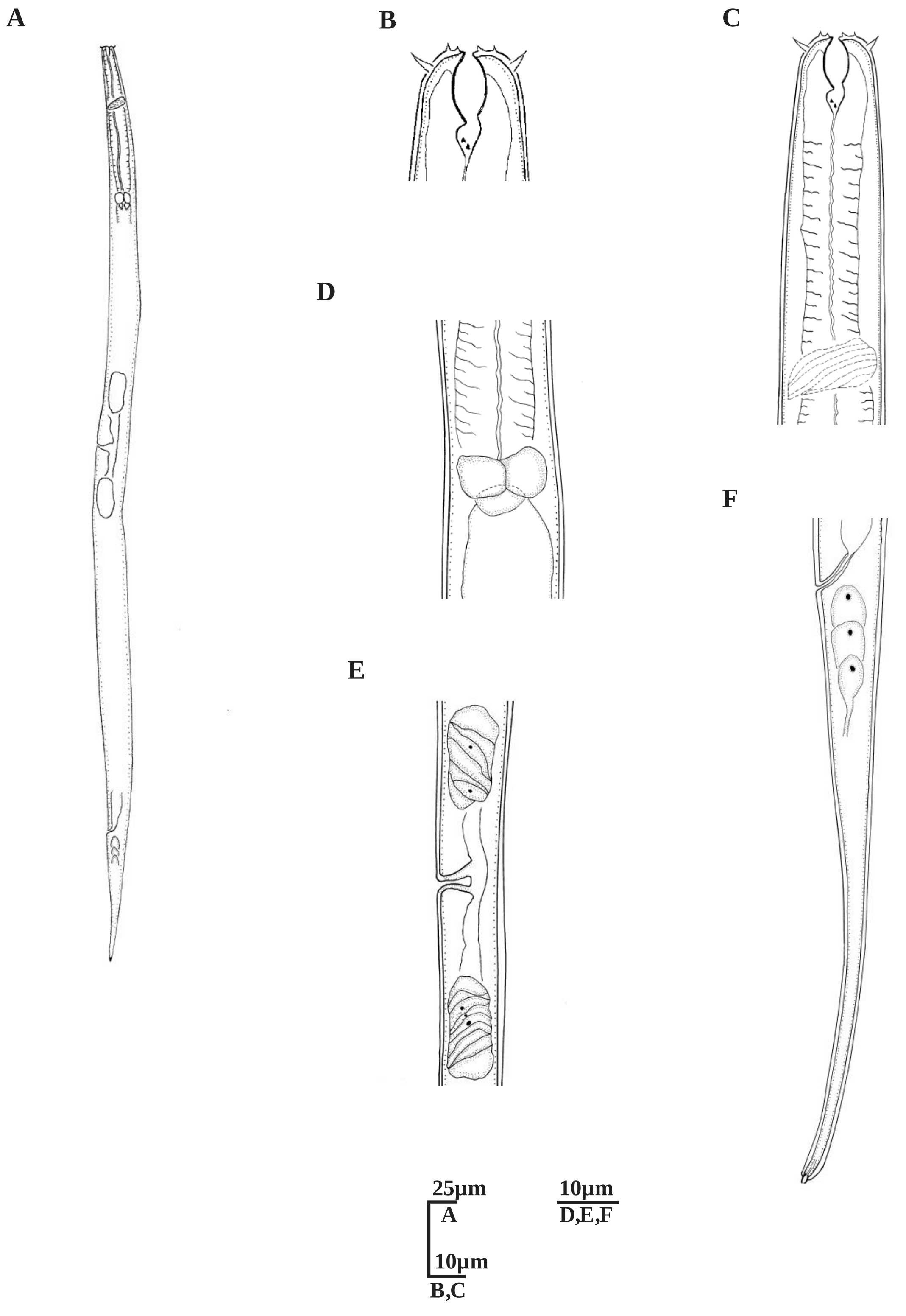


Figure 6.1: Line drawing: *Tobrilus* sp.1. A) Entire female. **B)** Anterior end at level of stoma. **C)** Median view of pharyngeal region. **D)** Pharyngo-intestinal junction showing cardiac glands. **E)** Reproductive system of mature female. **F)** Female tail showing rectal region and spinneret.

The genus *Eutobrilus* Tsalolikhin, 1981

Syn. *Peritobrilus* Gagarin, 1993; *Raritobrilus* Tsalolikhin, 1981;

Setsalia Shoshina, 2003

In 1981, Tsalolikhin revised the family Tobrilidae and as a result, the genus *Eutobrilus* was separated from the genus *Tobrilus*. Over the years the genus *Eutobrilus* was subjected to many revisions [(Tsalolikhin (1983); Gagarin (1991); Tsalolikhin (1992); Gagarin (1993); Tsalolikhin (2001)]; Tsalolikhin (2005); Zullini (2006)). *Eutobrilus* was separated into the genera *Eutobrilus* and *Peritobrilus* Gagarin, 1993, on the basis of the location of supplements (Gagarin 2009). *Eutobrilus* included species with the 0 supplement in the supplementary row of males. In contrast, the 0 supplement is absent in *Peritobrilus*. Included in the genus *Eutobrilus*, was two species of the genus *Raritobrilus* Tsalolikhin, 1981, namely: *Raritobrilus steineri* (Micoletzky, 1925) and *Raritobrilus scallensis* (Schneider, 1925) whose males possess the 0 supplement. Tsalolikhin (2001) regarded *Peritobrilus* Gagarin, 1993 as a subgenus of *Eutobrilus*. He excluded the above-mentioned species of *Raritobrilus* from the genus *Eutobrilus*, without any substantiation. On the other hand Zullini (2006) also synonymised *Peritobrilus* and *Eutobrilus*, but brought the genus *Raritobrilus* Tsalolikhin, 1981 to the synonymy of the genus *Eutobrilus* as well. The genus *Setsalia* Shoshina, 2003 is also virtually identical to *Eutobrilus*, except for the presence of a third pocket in the buccal cavity in comparison to the two pockets present in *Eutobrilus* (Shoshina 2003). However, according to Andrássy (2007), Shoshina herself wrote that "In tobrilids, the third dorsal pocket of the stoma is always present, but reduced."

There is a wide discrepancy existing in the opinions on both the volume and structure of this genus (Gagarin 2009). Andrássy (2007) stated that due to the high number of species described and/or placed in this genus, it is not easy to state the exact sum of current species, but regard 27 as probably valid. In an article by Gagarin and Naumova (2012) two new species were reported and they stated that the current global number of species for this genus is 23. In his checklist, of free-living nematodes recorded from habitats in southern Africa, Heyns (2002b) listed two species of nematodes belonging to the genus *Eutobrilus*, namely: *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981 and *Eutobrilus heptapapillatus* (Joubert & Heyns, 1979) Tsalolikhin, 1981.

The genus *Eutobrilus* is present in Europe, Asia, North America, Australia, Antarctica and Africa (Andrássy 2007). Here *Eutobrilus* sp. 1 is reported from South Africa.

Eutobrilus sp. 1

(Figs 6.3 - 6.4)

Measurements

(See Table 6.2)

Description

Female

Body slightly or completely ventrally curved in fixed specimens. Cuticle smooth or finely annulated. Lips well developed. Somatic setae sparse or not visible under compound microscope. Labial sensillae papilliform ranging from 0.7 - 3.0 µm. Cephalic setae up to 5.4 µm long, just over one third of labial region diameter. Dimension of stoma 11.0 x 7.0 µm. Pockets overlap, occasionally reduced, teeth close to each other. Amphidial fovea cup shaped in stoma region, level with pockets, not always visible. Pharynx muscular, cylindroid, comparatively long, enveloping stoma. Cardia large, three to four round to ovoid glands, 18.0 x 7.5 µm. Nerve ring 60 µm from anterior end. Female genital system amphidelphic-didelphic with reflexed ovaries, anterior genital branch slightly shorter than posterior genital branch. Vulva at 50% body length from anterior end. Vaginal musculature slightly sclerotised. Vagina extending inwards, a third of body diameter. Tail elongate tapering, 6.5 anal body widths long. Three caudal glands present, open through spinneret at tail tip.

Male

Not found.

Material Examined

Thirty four females (n = 34) were extracted from sediments collected at three different localities at the Seekoeivlei Nature Reserve, Memel.

Diagnosis and Relationships

The species from the SKVNR is characterised by a body that is ventrally curved, 907.5 - 1660 µm long, well developed lips, labial sensillae papilliform, cephalic setae just over one third labial region diameter, stoma with two overlapping pockets with a tooth in each, vulva at midbody (50%), tail 6.5 anal body widths long, spinneret present.

Eutobrilus annetteae was originally placed into the genus *Tobrilus* by Joubert & Heyns (1979) and then moved to the genus *Eutobrilus* by Tsalolikhin (1983). Yunliang & Coomans (2000) described *E. annetteae* from the Li River, China. Heyns & Botha (1992) described a population of *E. annetteae* from the Crocodile River, South Africa. The specimens from the current study were compared to both the above-mentioned populations and showed slight similarities in body length (L = 907.5 - 1660.0 µm *vs.* 1400 - 1710 µm) as well as b ratios (ratio b = 5.5 *vs.* 5.2). However, the specimens from the current study showed a number of differences to the other populations (V% = 50 *vs.* 43; ratio a = 27 *vs.* 31; tail length 164 µm *vs.* 239 µm, ratio c' = 5.0 - 8.0 *vs.* 7 - 10).

Similar to *E. annetteae, E. heptapapillatus* (Joubert & Heyns, 1979) Tsalolikhin, 1981 was also initially placed into the genus *Tobrilus* and later moved to the genus *Eutobrilus* by Tsalolikhin (1981). The species from the present study show similar vulva positions (V% = 37.8 - 58.3 *vs.* V% = 39 - 48), as well as similar a and c ratios (ratio a = 20.1 - 41.2 *vs.* 28.0 - 41.0; ratio c = 7.0 *vs.* 7.1) to *E. heptapapillatus*. A significant difference occurs in the body length as specimens from the present study have a shorter average length in comparison with *E. heptapapillatus* (L = 1183.5 µm *vs.* L = 2040 µm), as well as vulva position (V% = 49 *vs.* V% = 42) and tail length (165 µm *vs.* 228-337 µm).

There are too many differences among the species from the present study to the known species, therefore we preliminary assign the name *Eutobrilus* sp. 1 to the population from Seekoeivlei Nature Reserve, Memel, South Africa.

Table 6.2: Morphometrical data of *Eutobrilus* sp. 1 collected from the Seekoeivlei Nature Reserve (SKVNR), Memel and known species. All measurements in µm and in format mean±stdev (range) for the current morphometrics.

SPECIES	*Eutobrilus* sp. 1	*Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1983	*Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1983	*Eutobrilus heptapappilatus* (Joubert & Heyns, 1979) Tsalolikhin, 1981
LOCATION	SKVNR, South Africa	Crocodile River, South Africa	Ling River, Guiling, China	South Africa
n	34 ♀	♀	♀	♀
L	1183.5±212.0 (907.5-1660.0)	1590 (1400-1710)	1550	2040 (1720-2280)
a	27.0±6.0 (17.5-34.0)	34 (30-38)	31.4	34.2 (28.0-41.0)
b	5.5±1.0 (6.0-8.0)	5.2 (4.9-6.0)	6.8	5.3 (4.8-5.9)
c	7.0±1.5 (4.5-10.5)	6.3 (5.9-7.1)	5.9	7.1 (6.1-9.0)
c'	6.5±1.0 (5.0-8.0)	9.1(7-10)	9.5	8.7 (6.8-10.0)
V%	50.0±4.9 (37.8-58.3)	43 (42-46)	43.1	42 (39-48)
Tooth 1 ABE	14.5±2.5 (8.5-22.0)	19	-	-
Tooth 2 ABE	17.5±2.0 (11.0-21.0)	22.5	-	-
Ph L	226.5±36.0 (118.0-280.5)	-	229.5	-
Body width: neck base	30.5±8.5 (20.0-42.0)	-	-	-
mid body	44.5±7.5 (34.0-58.5)	-	49.5	-

Table 6.2 (continued): Morphometrical data of *Eutobrilus* sp. 1 collected from the Seekoeivlei Nature Reserve (SKVNR), Memel and known species. All measurements in µm and in format mean±stdev (range) for the current morphometrics.

SPECIES	*Eutobrilus* sp. 1	*Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1983	*Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1983	*Eutobrilus heptapappilatus* (Joubert & Heyns, 1979) Tsalolikhin, 1981
	SKVNR, South Africa	Crocodile River, South Africa	Ling River, Guiling, China	South Africa
anus	25.0±2.5 (20.0-32.0)	-	27.5	-
Vulva ABE	587.5±99.5 (430.0-786.0)	-	-	-
G_1L	81.0±12.0 (49.0-115.0)	-	148.5	-
G_2L	83.0±13.5 (44.5-107.5)	-	178	-
Tail length	164.5±14.0 (135.5-192.5)	-	-	-

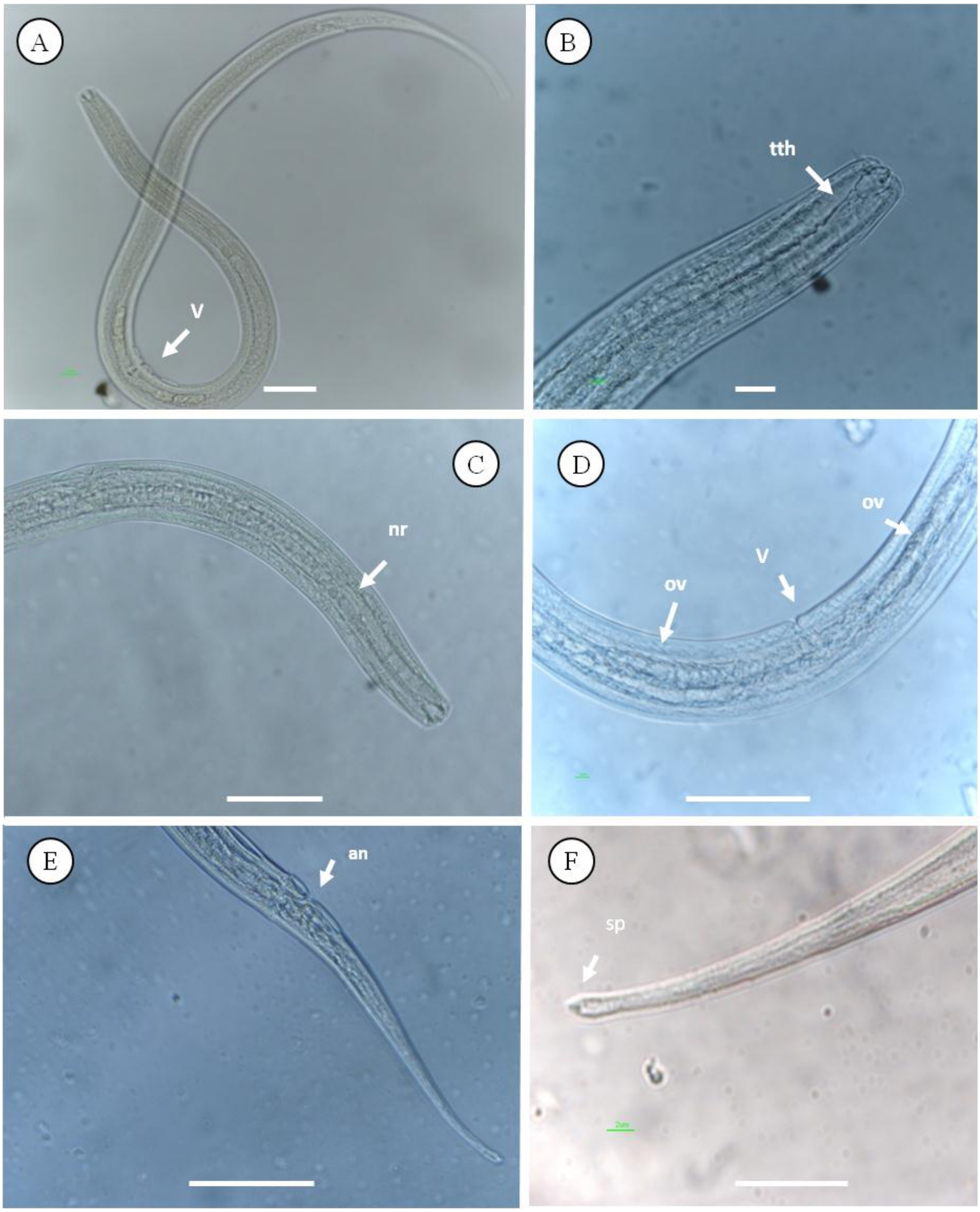


Figure 6.4: ***Eutobrilus* sp. 1** collected from the Seekoeivlei Nature Reserve, Memel. (LM) **A**. Entire female showing vulva. **B**. Anterior end showing position of teeth in pockets. **C.** Female neck region showing position of nerve ring. **D.** Female reproductive system. **E.** Female posterior end. **F.** Spinneret. [an - anus, nr - nerve ring, ov - ovaries, sp - spinneret , tth - tooth, V - vulva] **Scale bars:** A, C, D, E, F = 50 µm, B = 20 µm

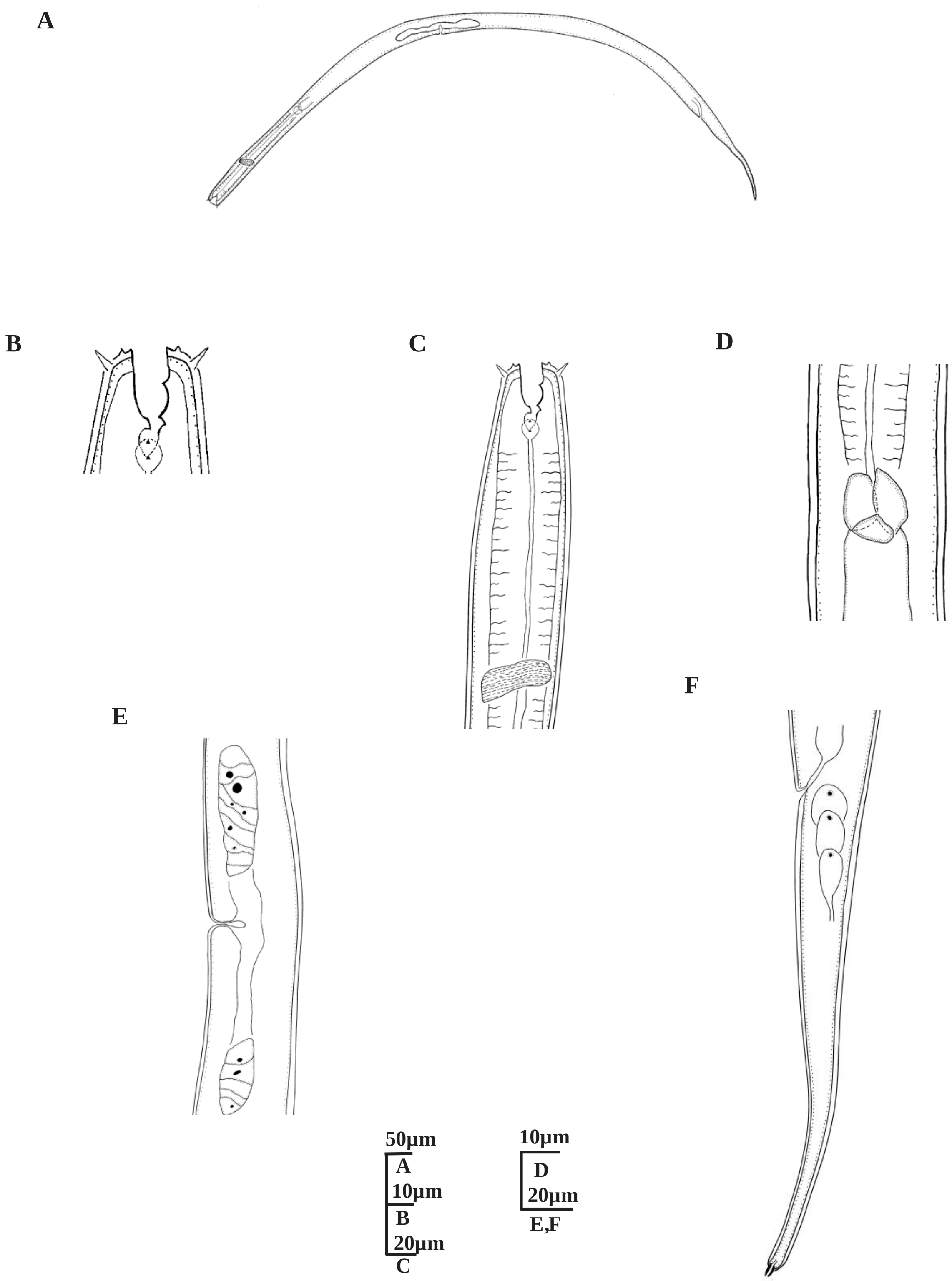


Figure 6.3. Line drawings: *Eutobrilus* sp. 1. **A)** Entire female. **B)** Anterior end at level of stoma. **C)** Median view of pharyngeal region. **D)** Pharyngo-intestinal junction showing cardiac glands. **E)** Reproductive system of mature female. **F)** Female tail showing rectal region and spinneret.

The genus *Brevitobrilus* Tsalolikhin, 1981

Syn. *Raritobrilus* Tsalolikhin, 1981; *Epitobrilus* Tsalolikhin, 1981

Thirteen genera were proposed by Tsalalokhin (1981) to accommodate the 72 species of the genus *Tobrilus* Andrássy, 1959, with eight of these species belonging to the genus *Brevitobrilus*. De Ley *et al.* (2006) had a new scheme of classification and placed Tobriilina as a suborder of Triplonchida. The genus *Brevitobrilus* is similar to the genus *Epitobrilu*s Tsalolikhin, 1981, with the main difference between them being the position of the anterior pocket (Andrássy 2007). According to Zullini (2006) the two genera were placed into different tribes: *Brevitobrilus* belongs to the tribe Neotroblini, characterised by a stoma with a buccal cavity distinctly separated from the anterior pocket. *Epitobrilus* belongs to the tribe Epitobrilini Tsalolikhin, 2001 which is characterised by a stoma with a buccal cavity either joint to the anterior pocket or not distinctly separated from it. Other differentiating features includes: the length of setae which is short in *Brevitobrilus* and long in *Epitobrilus* and male supplements being echinate in *Epitobrius* whilst not so in *Brevitobrilus*. Based on this, the two genera are distinctly different in the author's (of this thesis) opinion. In reality, there are all sorts of transitions in the male reproductive structure (Andrássy 2007). Zullini (2006) listed eight species as members of the genus *Brevitobrilus* and seven species under the genus *Epitobrilus*.

Although individual species have a limited distribution, the genus as a whole is known to be cosmopolitan. The genus has been recorded from Europe, Asia, North America, South America and Africa. Heyns (2002b) listed no species belonging to this genus in his checklist on free-living nematodes from freshwater habitats in southern Africa Here, *Brevitobrilus* sp. 1 is reported from South Africa.

Brevitobrilus sp. 1

(Figs 6.5-6.6)

Measurements

(See Table 6.3)

Description

Female

Body straight or slightly to fully curved. Somatic setae scattered. Lip region not offset, flattened, continuous with adjoining body. Labial sensillae papilliform. Cephalic setae up to 3.5 µm long (one third of lip region diameter), shorter setae 1.5 µm. Cephalic diameter

30.5 µm. Amphidial fovea not always visible. Cheilostome arched weakly sclerotised. Rest of stoma divided into a funnel shaped buccal cavity followed by an anterior and posterior pocket with dimension 16.2 x 10 µm. Each pocket armed with well developed tooth, separated by narrow duct approximately 10 µm long. Pharynx muscular. Nerve ring envelopes pharynx at 460 µm from anterior end. Cardiac glands circular to ovoid, 26.49 x 38.30 µm. Reproductive system amphidelphic-didelphic. Ovaries reflexed laterally, with anterior and posterior genital tracts of almost equal length. Vulva a transverse slit at 57% of body length from anterior end.. Vagina extending inwardshalf of body diameter. Tail slightly ventrally curved, elongate uniformly tapering, five anal body widths long. Three caudal glands open through common duct at well developed spinneret on tail tip.

Male

Male similar to female in morphology, but with tail slightly longer, more curved ventrally. Ductus ejaculatorus weakly muscular. Narrow constriction at junction of ductus ejaculatorus and vas deferens. Six precloacal supplements; internal part globular externally terminating in a crown of three to four projections. Distance between supplements variable. Supplements S5 and S6 widely separated. Spicule length 21.6 µm, bifurcate gubernaculum. Male tail three anal body widths long. Subterminal setae present.

Material Examined

Three females (n = 3) and six males (n = 6) extracted from sediments collected at three different localities at the Seekoeivlei Nature Reserve, Memel.

Diagnosis and Relationships

The species from the SKVNR is characterised by body length (1019 - 1792 µm female; 1123 - 1526 µm male), labial sensillae papilliform, and cephalic setae one third of lip region diameter. Stoma divided into funnel shaped buccal cavity followed by an anterior and posterior pocket separated by an isthmus, each pocket with a tooth, cardiac glands circular to ovoid, vulva at 46% (mean value) body length, tail ventrally curved, five anal body widths long, spinneret present. Males characterised by spicule 21 µm (20 - 23 µm), tail three anal body widths long, subterminal setae present.

Specimens from the current study showed similarity to three known species namely, *Brevitobrilus fesehai* Abebe & Coomans 1997; *Brevitobrilus graciloides* (von Daday, 1908) Tsalolikhin, 1983: syn. *Tobrilus africanus* Zullini, 1988; *Trilobus graciloides* Daday, 1908 and *Brevitobrilus sardus* (Vincigerra & Zullini, 1991) Tsalolikhin, 2002. Specimens from the present study are similar to *B. fesehai* in b and c' ratios (ratio b = 5.3 *vs.* 5.2; ratio c' = 5.0 *vs.* 5.3). Differences occur in body length (L = 1405.5 µm *vs.* 1700 µm), vulva position (V% = 46.7 *vs.* 44.2) and the absence of terminal setae which is especially prominent in male specimens of the current study. Furthermore, even though supplements are morphometrically similar, distances between supplements differ greatly.

The SKVNR specimens are similar to *B. graciloides* in vulva position (V% = 46.7 *vs.* 45.6), as well as b and c' ratios (ratio b = 5.3 *vs.* 5.3 and ratio c' = 5.0 *vs.* 5.3). In both, intestinal cell walls are filled with brown granules, setae are present on the tail, supplements are bulb like and the largest distance between supplements is between S5 and S6. Differences include body length, and c ratios (L = 1405.5 µm *vs.* L = 1236 µm; c = 6.0 *vs.* 7.9).

However, using the taxonomic key compiled by Tahseen *et al*. (2009), the species from the present study may be identified as *B.sardus* Tsalolikhin, 2002. Characters used in this key to distinguish *B. sardus* from other species are the supplements that are unequal, one supplement (S6) that is smaller than the other supplement and the supplements being globose and spicules deformable. Specimens from the current study differ from *B. sardus* in the distances between supplements which are proportionally larger in the current specimens when compared to *B. sardus*. Furthermore, there is a large difference in the a-ratio (ratio a = 17.1 *vs.* 32 - 43). Other ratios show effectively comparable results (ratio b = 4.7 - 5.6 *vs.* 4.7 - 5.3, ratio c = 6.23 *vs.* 8.5 - 12.3, ratio c' = 3.0 - 4.0 *vs.* 4.5 - 6.0).

Therefore, the species from the present study are nearest to *B. graciloides* with only the body length and c ratio differing. There are also too many differences with *B. sardus* to assign this name to the specimens from the current study and therefore we assign the name *Brevitobrilus* sp. 1.

Table 6.3: Morphometrical data of *Brevitobrilus* sp. 1 collected from the SKVNR, Memel and known species. Measurements in µm and in format mean±stdev (range)

SPECIES	*Brevitobrilus* sp. 1		*Brevitobrilus fesehai* Abebe & Coomans 1997	*Brevitobrilus graciloides* (von Daday, 1908) Tsalolikhin, 1981	*Brevitobrilus sarduss* (Vincigerra & Zullini, 1991) Tsalolikhin, 2002	
LOCATION	SKVNR, Memel, South Africa		Lake Tana, Gedero	Lake Tana, Gedero	Flascio river, Sicily,Italy	
n	3 ♀	6 ♂	♀	♀	♀	♂
L	1405.5±546.3 (1019.2-792.0)	1328.5±151.0 (1123.6-1526.3)	1700	1236	1100 - 1260	1020 - 1380
a	17.1±7.2 (12.5-22.2)	20.70±1.8 (18.7-22.6)	23.2	23.1	25-30	32-43
b	5.3±0.5 (5.0-6.0)	5.3±0.4 (4.7-5.6)	5.2	5.3	4.5-4.8	4.7-5.3
c	6.00±2.0 (4.5-7.5)	6.23±7.7 (4.29-8.1)	9	7.9	8.3-9.6	8.5-12.3
c'	5.0±0.5 (4.7-5.5)	3.0±0.1 (3.0-4.0)	5.3	5.3	46-52	2.5-4.3
V%	46.7±7.2 (41.5-51.8)	-	44.2	45.6	4.5-6.0	-
LRW	30.5±0.5 (30.3-30.8)	26.80±4.78 (21.26-32.35)	-	-	-	-
LSL	0.4±0.6 (0.8-1.5)	0.5±0.6 (0.8-1.54)	-	-	-	-

Table 6.3 (continued): Morphometrical data of *Brevitobrilus* sp. 1 collected from the SKVNR, Memel and known species. All measurements in µm and in format mean±stdev (range)

SPECIES	*Brevitobrilus* sp. 1		*Brevitobrilus fesehai* Abebe & Coomans 1997	*Brevitobrilus graciloides* (von Daday, 1908) Tsalolikhin, 1981	*Brevitobrilus sarduss* (Vincigerra & Zullini, 1991) Tsalolikhin, 2002	
LOCATION	SKVNR, Memel, South Africa		Lake Tana, Gedero	Lake Tana, Gedero	Flascio river, Sicily,Italy	
CSL	3.5±2.7 (1.5-5.4)	3.5±2.7 (0.8-5.4)	5.5-7.5	-	5-6	-
Tooth 1 ABE	16.0±1.5 (14.6-16.9)	16.2±1.2 (14.5-17.5)	-	-	12-15	-
Tooth 2 ABE	27.0±3.0 (24.6-29.2)	25.6±1.3 (23.6-26.9)	-	-	-	-
Ph L	262±82.0 (204-320)	251.5±26.5 (226.0-282.0)	-	-	238-274	-
Body width: neck base	66.5±4.5 (63.5-70.0)	65.00±7.3 (50.0-70.0)	-	-	-	-
Mid-body	83.0±2.7 (81.0-85.0)	70.00±5.3 (69.0-74.0)	-	-	-	-
Anus	46.5±5.0 (43.0-50.0)	82.0±2.2 (78.6-85.0)	-	-	-	-
Vulva ABE	742.0±37.5 (738.3-745.3)	-	-	-	-	-
G_1L	252.0±45.5 (200.0-304.0)	-	-	-	-	-

Table 6.3 (continued): Morphometrical data of *Brevitobrilus* sp. 1 collected from the SKVNR, Memel and known species. All measurements in µm and in format mean±stdev (range)

SPECIES	*Brevitobrilus* sp. 1		*Brevitobrilus fesehai* Abebe & Coomans 1997	*Brevitobrilus graciloides* (von Daday, 1908) Tsalolikhin, 1981	*Brevitobrilus sarduss* (Vincigerra & Zullini, 1991) Tsalolikhin, 2002	
LOCATION	SKVNR, Memel, South Africa		Lake Tana, Gedero	Lake Tana, Gedero	Flascio river, Sicily,Italy	
G_2L	255.0±48.0 (200.0-310.0)	-	-	-	-	-
tail length	234.0±4.5 (230.2-236.5)	243.5±13.0 (222.3-256.6)	-	-	120-150	-
Spicule length	-	21.6±1.00 (20.6-23.2)	-	48.0	-	-
Number of supplements	-	6	-	-	-	-
S1-S2	-	26.0±0.8 (24.6-26.9)	35	-	-	37
S2-S3	-	19.0±1.2 (17.0-20.0)	37	-	-	35
S3-S4	-	19±.1.0 (16.9-20.0)	34	-	-	41
S4-S5	-	27±0.9 (25.4-27.7)	30	-	-	29
S5-S6	-	51.73±1.0 (50.0-53.0)	48	-	-	30

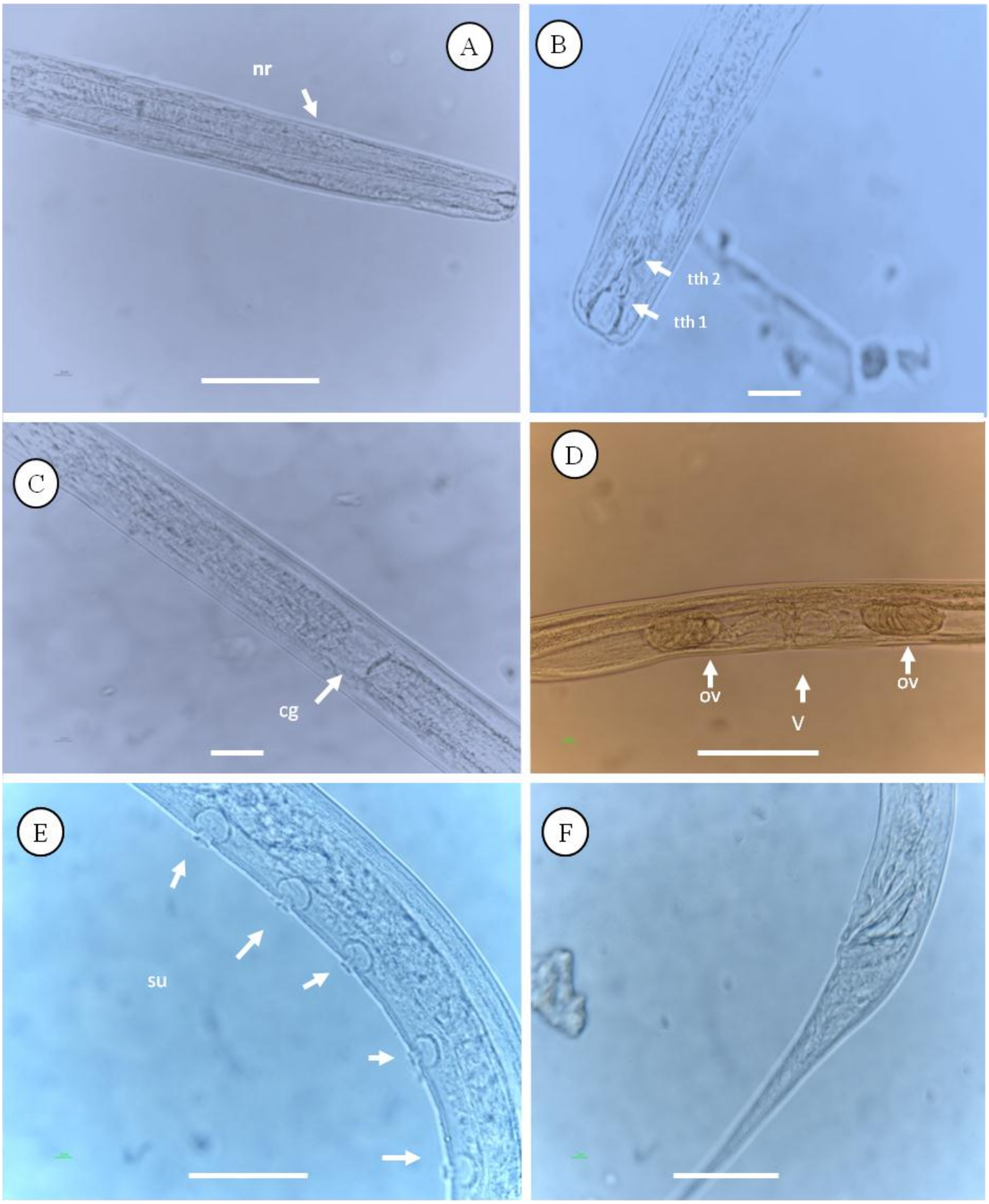


Figure 6.6: ***Brevitobrilus*** **sp. 1** collected from the Seekoeivlei Nature Reserve, Memel (LM) **A.** Neck region. **B**. Anterior end of stoma showing position of teeth. **C**. Pharyngo-intestinal junction with cardiac glands. **D**. Female reproductive system. **E.** Supplements. **F.** Male posterior end, copulatory apparatus. [cg - cardiac glands, nr - nerve ring, ov - ovaries, tth – tooth, su – supplements, V - vulva] **Scale bars**: A, D, F = 100 µm, B = 20 µm & C & E = 50 µm

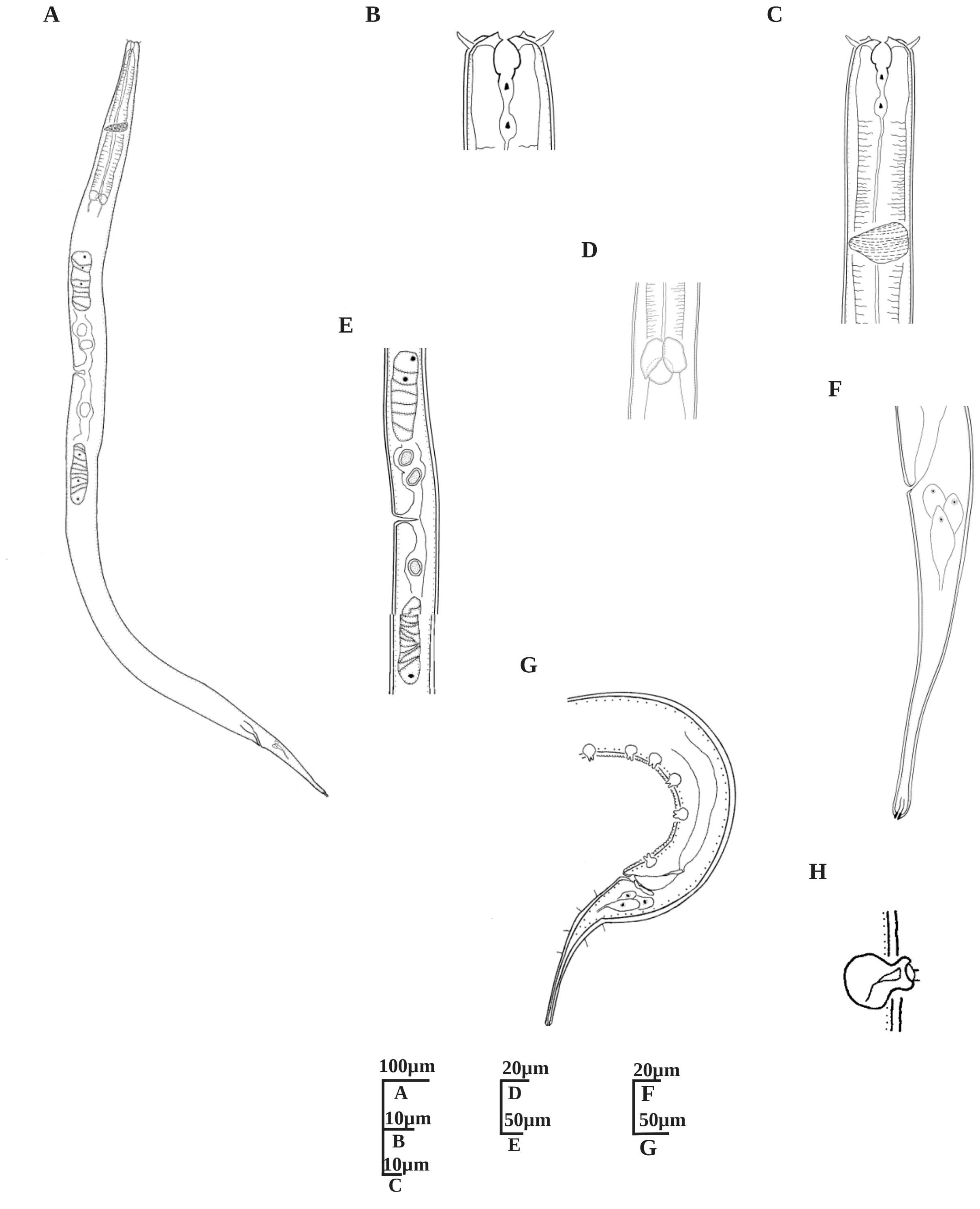


Figure 6.5. Line drawings: *Brevitobrilus* sp. 1. **A)** Entire female. **B)** Anterior end at level of stoma. **C)** Median view of pharyngeal region. **D)** Pharyngo-intestinal junction showing cardiac glands. **E)** Reproductive system of mature female. **F)** Female tail showing rectal region and spinneret. **G)** Male posterior end showing spicules and supplements. **H)** Male supplement showing projections.

The last species belongs to the family Chronogasteridae which is classified as follows

Kingdom: Animalia

Phylum: Nematoda

Class: Adenophorea

Order: Plectida

Family: Chronogasteridae

The genus Chronogaster Cobb 1913

Syn. *Walcherenia* de Man, 1921

The genus *Chronogaster* was established by Cobb 1913 to accommodate the newly found freshwater species *Chronogaster gracilis* Cobb 1913. Andrássy (1958) synonymised Cobb's species with von Daday's (1899) *Cephalobus longicollis*. The correct contemporary nomenclature designation for the type species is therefore *Chronogaster longicollis* (von Daday, 1899) Andrássy, 1958; syn. *Chronogaster gracilis* Cobb (1913).

Heyns & Coomans (1980) reviewed the genus and several species were added. The genus name is derived from the Greek words *Chronos* = clock and *Gaster* = stomach as a result of the bulb being similar to the hands of a clock. This limnoterrestrial genus is cosmopolitan with most species reported from tropical regions. In his checklist of free-living nematodes recorded from freshwater habitats in Southern Africa, Heyns (2002b) listed four species belonging to this genus, namely: *Chronogaster africana* Heyns & Coomans, 1980, *Chronogaster glandifera* Heyns & Coomans, 1980, *Chronogaster longicauda* Heyns & Coomans, 1980 and *Chronogaster multispinata* Heyns & Coomans, 1980. Males are rare suggesting that amphimictic reproduction occurs. There are 46 valid species in this genus (Sultana & Bohra 2011). Here *Chronogaster* sp. 1 is reported from South Africa.

Chronogaster sp. 1

(Figs. 6.7 - 6.8)

Measurements

(See Table 6.4)

Description

Female

Body medium sized, cylindrical, c-shaped or completely ventrally curved; tapers towards both extremities. Cuticle annulated. Lip region dome-shaped, not annulated. Lip papillae not always visible. Cephalic setae 4 µm equal to or longer than lip region diameter originating from the base of non annulated lip region. Stoma cylindrical. Pharynx cylindrioid anteriorly, expanding posteriorly to an oval basal bulb with denticulated valve; cardia long. Nerve ring encompasses pharynx at six body widths from anterior end. Female reproductive system monodelphic-prodelphic. Ovary reflexed. Vulva at 45% body length from anterior end, transverse slit. Vagina perpendicular to main body axis extending inwards just more than half body diameter. Vulva to anus distance three times tail length. Tail elongate, conoid, ventrally arcuate, eleven anal body widths long. Spinneret and caudal glands absent.

Male

Male similar to female in morphology. Male habitus slight ventral curve, J-shaped. Fifteen supplements precede cloacal opening. Supplements differ morphologically; unequal distances apart. Shortest distance between S13 - S14, 9.4 µm. Longest distance between S2 - S3 20.8 µm. Cloacal opening 825 µm from anterior extremity. Spicule 13.2 µm long. Male tail shorter than female tail. Tail conoid: nine anal body widths long.

Material Examined

Nineteen females (n = 19) and one male (n = 1) extracted from sediments collected at three different localities at the Seekoeivlei Nature Reserve, Memel.

Diagnosis and Relationships

The SKVNR specimens can be recognised by body length (999 - 1384 µm female, 932 µm male), lip region not annulated, cephalic setae as long or longer than lip region diameter, cylindroid pharynx with denticulated valve in basal bulb, female reproductive system monodelphic prodelphic, vulva at 42 - 47% body length from anterior end, tail eleven anal body widths long. Male characterised by spicule length (13.2 µm), fifteen dissimilar supplements, tail conoid, nine anal body widths long.

Specimens from the current study are similar to *Chrongaster laxus* Sultana & Bohra, 2011 in a, b and c' ratios (ratio a = 42.2-75.0 current study *vs.* 44.2; and ratio c' = 9.0-16.0 *vs.* 9.9). Differences occur in body length, vulva position and tail length (L = 1131 µm *vs.* L = 1151 µm; V = 45.3% *vs.* 53.8% and tail l = 126 µm *vs.* 159 µm).

Chronogaster neotypica Tahseen, 1994 was described from a sewage slurry in Aligarh, India. Specimens from the current study are similar to *C. neotypica* in vulva position (V = 45.3 % *vs.* 45.8%) as well as a and c – ratios (ratio a = 42.0 - 75.0 *vs.* 37 - 64; ratio c = 7.4 -10.7 *vs.* 6.5 - 9.4. Differences between the two species include body length, b and c' ratio (L = 999.9 - 1384.6 µm *vs.* 950-1300 µm; ratio b = 4.7 - 7.5 *vs.* 3.9 - 4.8; and ratio c' = 11.9 *vs.* 7).

Chronogaster troglodytes Poinar & Sarbu 1994, was originally described from Movile cave in Romania by Poinar & Sarbu (1994). Species from the current study are similar to *C. troglodytes* in a and c-ratios (a = 53 *vs.* a = 55; c = 9.0 *vs.* c' = 9.3). Significant differences occur in body length, vulva position, pharyngeal length and tail length (L = 1131 µm *vs.* 1290 µm; V% = 45 *vs.* 49; Ph. L = 199.0 µm *vs.* 254 µm; Tail = 126.0 µm *vs.* 141 µm)

Species from the current study were also compared to *Chronogaster* species found by Heyns & Coomans (1980). Differences occurred between *Chronogaster africana* and *Chronogaster andrassyi* Loof & Jairajpuri, 1965 in that each possessed a mucro, which was absent in species from the current study. *Chronogaster glandifera* had large glandular bodies within the body which were absent in the species at hand.

There are too many differences among the species from the present study to the known species, therefore we preliminary assign the name *Chronogaster* sp. 1 to the population from Seekoeivlei Nature Reserve, Memel, South Africa.

Table 6.4: Morphometrical data of *Chronogaster* sp. 1 collected from the Seekoeivlei Nature Reserve, Memel and known species. All measurements in µm and in format mean±stdev (range) for current morphometrics

SPECIES	*Chronogaster* sp. 1		*Chrongaster laxus* Sultana 2011	*Chronogaster neotypica* Tahseen 1994	*Chronogaster troglodytes* Poinar 1994
LOCATION	SKVNR Memel, South Africa		Pichola lake, Rajasthan	Sewage slurry, Aligarh, India	Movile cave, Romania
n	19 ♀	1♂	♀	♀	♀
L	1131.0±122.0 (999.9-1384.6)	932	1151	960 950-1300)	1290
a	53.0±8.4 (42.2-75.0)	71.3	44.2	45.8 (37-64)	55
b	5.7±0.7 (4.7-7.5)	5.1	4.4	4.6 (3.9-4.8)	5.1
c	9.0±1.1 (7.4-10.7)	8.7	7.2	6.5 (6.5-9.4)	9.3
c'	11.9±2.2 (9.0-16.0)	8.3	9.9	7 (7-11.5)	-
V%	45.3±7.0 (42.0-47.3)	-	53.8	48 (45-50)	4.9
LRW	7.5±1.0 (6.0-8.5)	5.4	-	-	-
Nerve ring ABE	72.0±4.5 (64.2-77.4)	69.8	-	-	-
Basal Bulb L	23.0±1.5 (20.0-25.5)	21.5			

Table 6.4 (continued): Morphometrical data of *Chronogaster* sp. 1 collected from the Seekoeivlei Nature Reserve, Memel and known species. All measurements in µm and in format mean±stdev (range) for current morphometrics

SPECIES	*Chronogaster* sp. 1		*Chrongaster laxus* Sultana 2011	*Chronogaster neotypica* Tahseen 1994	*Chronogaster troglodytes* Poinar 1994
LOCATION	SKVNR Memel, South Africa		Pichola lake, Rajasthan	Sewage slurry, Aligarh, India	Movile cave, Romania
Basal Bulb W	14.0±1.4 (12.0-16.0)	15.0			
Pharyngeal L	199.0±10.0 (184.6-211.5)	181.5	-	207 (157-242)	-
Body width: **Neck base**	18.5±2.5 (15.4-23.1)	13.1	-	-	-
Mid-body	21.5±2.0 (18.0-25.0)	13.8	-	-	(21-27)
Anus	8.0±4.0 (8.0-12.5)	13.1	16	-	-
Ph. Bulb ABE	167.0±11.5 (154.0-183.0)	169.8	-	-	-
Tail length	126.0±12.5 (104.0-137.0)	107.6	159	105 (102-140)	141
Spicule		13.2	-	-	
S1-S2		11.3	-	-	-
S2-S3		20.8	-	-	-
S3-S4		18.8	-	-	-
S4-S5		11.3	-	-	-
S5-S6		13.2	-	-	-

Table 6.4 (continued): Morphometrical data of *Chronogaster* sp. 1 collected from the Seekoeivlei Nature Reserve, Memel and known species. All measurements in µm and in format mean±stdev (range) for current morphometrics

SPECIES	*Chronogaster* sp. 1		*Chrongaster laxus* Sultana 2011	*Chronogaster neotypica* Tahseen 1994	*Chronogaster troglodytes* Poinar 1994
LOCATION	SKVNR Memel, South Africa		Pichola lake, Rajasthan	Sewage slurry, Aligarh, India	Movile cave, Romania
S6-S7		13.2	-	-	-
S7-S8		13.2	-	-	-
S8-S9		11.3	-	-	-
S9-S10		11.3	-	-	-
S10-S11		15.1	-	-	-
S11-S12		15.1	-	-	-
S12-S13		13.2	-	-	-
S13-S14		9.4	-	-	-
S14 – S15		11.3	-	-	-

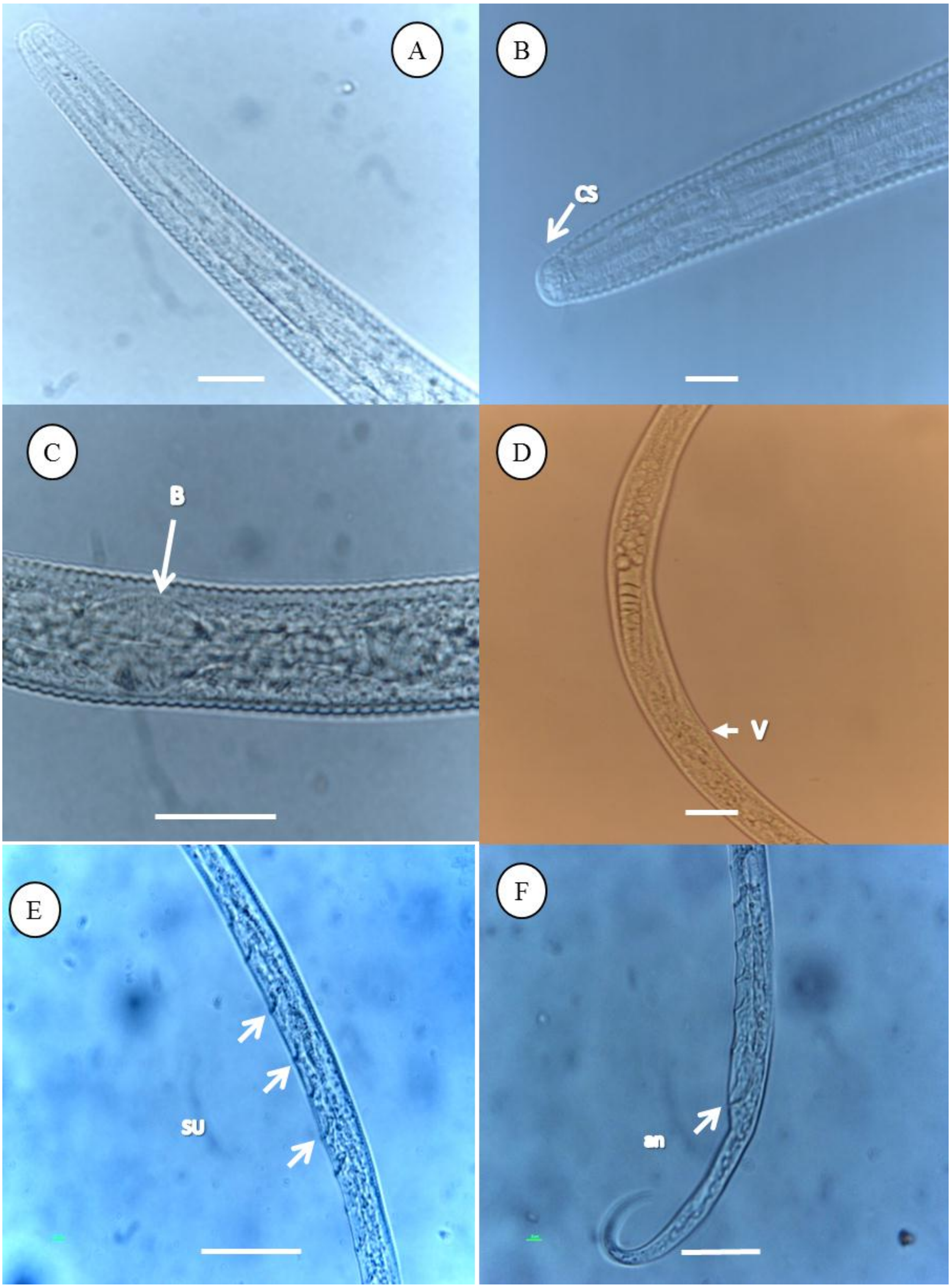


Figure 6.8: *Chronogaster* sp. 1 collected from the Seekoeivlei Nature Reserve, Memel (LM) **A**. Neck region. **B**. Anterior end at level of stoma showing cephalic setae. **C**. Basal bulb. **D**. Female reproductive system. **E**. Male supplements. **F.** Male posterior end, copulatory apparatus. [an - anus, B - bulb, CS - cephalic setae, V - vulva opening, SU - supplements] Scale bars: A, B = 10 µm; C, D, E, F = 20 µm

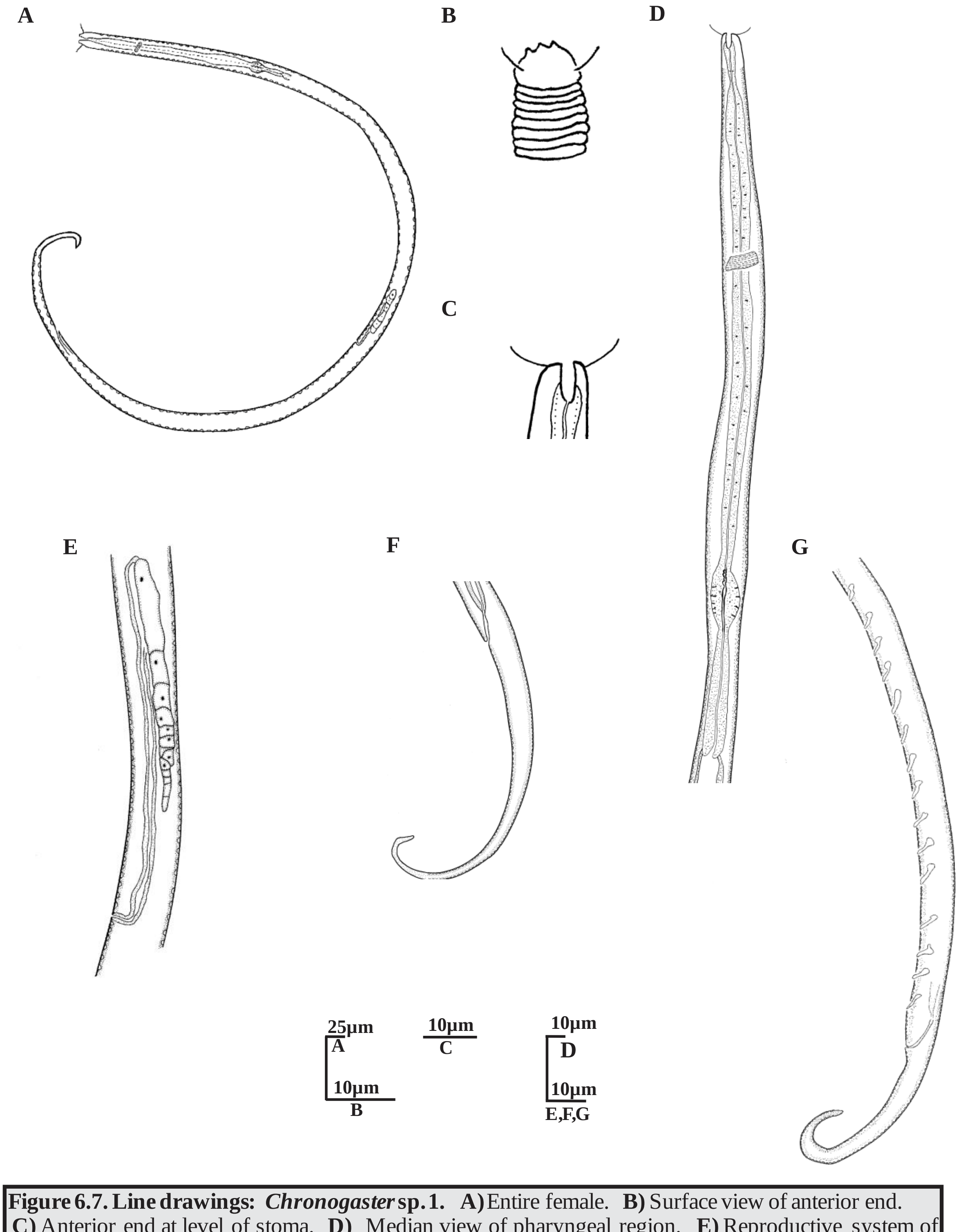


Figure 6.7. Line drawings: *Chronogaster* sp.1. A) Entire female. **B)** Surface view of anterior end. **C)** Anterior end at level of stoma. **D)** Median view of pharyngeal region. **E)** Reproductive system of mature female. **F)** Female tail showing rectal region. **G)** Male posterior end showing spicules and

Chapter 7

DISCUSSION

ECOLOGICAL DISCUSSION

NEMATODE GENERA: COMPOSITION, RICHNESS, DIVERSITY AND DOMINANCE

A range of biotic and biotic factors at the Seekoeivlei Nature Reserve (SKVNR) contributed to the nematode composition, richness, diversity and dominance at the different localities, during the different seasons. Abiotic aspects include the geology, hydrology, climate and physical parameters such as temperature, dissolved oxygen concentration, pH and salinity. Biotic factors include the ecosystem structure, which is the fauna and flora present at the reserve. The morphology and bionomics of the nematodes as well as other factors such as grazing, farming and the presence of invasive species all add to the type and magnitude of nematode species present.

Concerning the geology of the wetland, the flat floodplain which is surrounded by an undulating landscape allowed for the development of both lentic and lotic environments. According to Buffagni *et al.* (2009), the lentic-lotic character of an aquatic ecosystem is significant and can greatly impact its ecological status. The Mamba Pool and Bird Lookout were characterised as lentic environments, whilst the Hippo Pool was characterised as a lotic environment. Tobrilidae (*Brevitobrilus* sp. 1, *Eutobrilus* sp. 1), Leptolaimidae (*Leptolaimus* sp. 1), Monhysteridae (*Monhystera* sp. 1), Plectidae (*Plectus* sp. 1) and numerous species of the order Dorylaimida (*Aporcelaimellus* sp. 1, *Dorylaimus* sp. 1, *Mesodorylaimus* sp. 1, *Prodorylaimus* sp. 1, *Eudorylaimus* sp. 1, *Parastomachoglossa* sp. 1) were found in samples collected from the Hippo Pool and according to Traunspurger (2000), these families are amongst those associated with running water. The presence of caudal glands on the tip of the tail of tobrilid nematodes act as an adhesive to temporarily attach themselves to the substratum.

The SKVNR wetland is classified as being a valley bottom wetland (FS DTEEA IMP 2005) and the hydrology is composed of various water transfer mechanisms. The physical habitat of nematodes and other factors, such as the availability of food is affected by these wetland ecosystem dynamics. These dynamics include the periodic local flooding and drought, water pools remaining on wetland soil surface as a result of poor drainage, as well as seasonal fluctuations in the shallow wetland water table (Ettema *et al.* 2000). In aquatic environments, as well as the ever-changing soil environments, nematodes face the problem of water loss or water gain (Lee 1965; Tahseen 2012). The cuticle of nematodes is permeable to certain ions, non-electrolytes as well as water (Lee 1965).

When compared to Heyns (2002b) checklist on the free living nematodes recorded from freshwater habitats in Southern Africa, only ten (Aporcelaimidae, Cephalobidae, Dorylaimidae, Leptolaimidae, Monhysteridae, Rhabditidae, Aphelenchoidida Hoplolaimidae, Tobrilidae and Chronogasteridae) of the 36 families that are listed, was found at the SKVNR. This may be explained by the fact that Heyns (2002b) checklist is limited to those species which are known/assumed to be able to live and reproduce in aquatic or semi-aquatic habitats only and excludes those soil-living species which are frequently washed into water bodies through water transfer mechanisms. Members of the families Actinolaimidae (*Parastomachoglossa* sp. 1) , Aporcelaimidae (*Aporcelaimellus* sp. 1) and Qudsianematidae (*Allodorylaimus* sp. 1, *Eudorylaimus* sp. 1, *Labronema* sp. 1, *Microdorylaimus* sp. 1) which have been identified at the reserve are predominantly terrestrial, although species from limnic or semi-limnic habitats do occur (Andrássy 2009). Some nematodes end up in the benthos and may survive there for relatively long periods. However they cannot play a significant role in the aquatic ecosystem considering they do not feed or reproduce there (Heyns 2002b). The orders Rhabditida, Tylenchida and Dorylaimida are the most frequently encountered among the benthos in freshwater habitats (Heyns 2002a).

In terms of flora, the natural vegetation, previous land use and presence of alien plants contributed to the plant parasitic nematodes. Prior to proclamation of the area as a nature reserve, the land was used for grazing cattle. Plant parasitic nematodes such as *Aphelenchoides* and *Ditylenchus*, use their stylet to pierce the plant cell and thereafter extract the cell contents by means of suction (Lee 1965). Members of some genera such as *Tylenchorynchus* from the family Belonolaimidae are found associated with fodder crops and maize (Kleynhans *et al*. 1996). During the present study, members of the genus *Tylenchorynchus* were found to be the most abundant from all three collection sites, although a large number of juveniles were present in these samples. Species from this genus generally have a relatively fast reproduction rate and an average life-cycle of 31-38 days. This could explain their omnipresence. *Hirschmanniella* species were present at all three sites and according to Van den Berg *et al.* 2009, they are well adapted to an aquatic environment. They parasitise paddy rice and a number of aquatic plants. Criconematidae which is a cosmopolitan family has previously been found in natural veld in the Free State Province, South Africa. Interestingly, they were most abundant at the Hippo Pool during this study and this is likely due to the presence of a weeping willow tree (*Salix babylonica*). This tree is an invasive woody perennial and members of the family Criconematidae are plant parasitic with a preference to woody perennials. Besides the family Criconematidae being common around woody perennials, members of the genus *Helicotylenchus* from the family Hoplolaimidae have previously been found to occur around gum trees, *Eucalyptus* spp. (Kleynhans *et al*. 1996), which are also present at the reserve. Alien trees therefore contributed to the composition of nematodes in this grassland biome.

Whilst free-living nematodes generally have a straight-forward lifestyle, it is known that they do inhabit temporary environments such as moss tussocks, the surface layer of arable land and dung. The third-stage larvae are found to be the dispersal stage in the life cycle of many dung-inhabiting species (Lee 1965). It is suspected that fauna present at the reserve both directly and indirectly had an impact on nematode composition. Yeates *et al.* (2003) listed nematode taxa found under cattle faecal pats. The list included some genera found at the SKVNR: *Tylenchus* (Tylenchidae), *Tylenchorynchus* (Belonolaimidae), *Aphelenchoides* (Aphelenchoididae), *Cephalobus* (Cephalobidae), *Plectus* (Plectidae), *Tobrilus* (Tobrilidae), *Eudorylaimus* (Dorylaimidae) and *Aporcelaimus* (Aporcelaimidae). During the present study a number of cattle were seen grazing in the vicinity of the Mamba Pool and this could be one of the reasons why these families were found at the reserve.

Nematodes are often microscopic and many have resistant life stages. This allows them to take advantage of efficient passive distribution mechanisms such as via wind, flowing water and biological agents. It is known that migratory birds generally move toward freshwater sources (Abebe *et al.* 2008). In a study by Abebe & Coomans (1996), it was hypothesised that the presence of certain freshwater nematodes on Galapagos Islands was a result of passive and occasional transport by birds. It was thus assumed that they are able to transport resistant stages of nematodes across long distances. The SKVNR supports a large number of local and migratory birds (FS DTEEA IMP 2005). It is expected that they may have contributed to the composition of nematodes found during this study.

According to Tahseen (2012), nematode species richness does not change in hypoxic-anoxic conditions although their trophic structure and species composition bestow significant changes. Species richness (S) was highest at the Bird Lookout Point during the summer collection. The Shannon-wiener diversity (H’) and Hills diversity (Hills N_1) was also highest here, during the summer period. This area had the highest level of activity with regards to birds and mammals in and around the site and could be a likely reason for the higher nematode diversity at this site. Both the Shannon-wiener diversity (H’) and Hills diversity

(Hills N_1) was lowest at the Mamba Pool during the preliminary survey. The majority of samples from this site were composed of plant feeders. Simpsons index was highest at this site (D = 0.69) and it was dominated by the family Belonolaimidae, possibly implying a competition for resources and the resulting low species richness and diversity.

Global observations do indicate that temperature is singularly the most important factor that fluctuates seasonally and in turn affects denizens of limnetic habitats through its multifaceted effect e.g. by directly affecting the biotic component and by mixing (DWAF, 1996). According to Antofica (2010), the life-cycles of nematodes depend strongly on temperatures. At low temperatures, the life-cycle of nematodes can range from 13-20 months. At temperatures ranging from 18-33°C, the life-cycle can take place over 2-30 days, which is characteristic for most free-living nematodes.

Physical data obtained during the summer and winter survey indicates the average temperature as 23.17°C during the summer survey and 15.08°C during the winter survey. During the winter seasons, Simpsons Index was highest at the Hippo Pool (D = 0.34) although similar to results obtained from the Mamba Pool and Bird Lookout (D = 0.29). The dominant family found overall during the winter survey was Tobrilidae. In a paper by Ocaña (1991), it was noted that members of the order Enoplida (to which Tobrilidae belongs) were found in a range of temperatures between 10-and-45°C. However, their presence peaked between 14-16°C. Schiemer and Duncan (1974) stated that in highly organic mud, the genus *Tobrilus* is most abundant well below the redox discontinuity layers. They seem to prefer definite depth and substratum at 1m layers. They inhabit the surface and mud layers in appreciable numbers only during winter, under ice and in deoxygenated water (Schiemer & Duncan, 1974). The average dissolved oxygen was calculated at 58.47% during the winter survey (Table 5.4). It is stated that the family Tobrilidae is the only real freshwater family known. It is said to have adapted to freshwater habitats during the oceanic regression. As a result, Tobrilidae is considered a relatively euryhaline group since they are found in salinity ranges from pure freshwater to brackish water. The average salinity during the winter season was calculated as 0.11 µS/cm (Table 5.4). This may possibly explain why the family Tobrilidae were only found during the winter survey when temperatures were lower.

Members of the family Monhysteridae was found at all three sites during all three surveys, although a relatively higher number was found during winter. Like the family Tobrilidae, Monhysteridae is also common in habitats where oxygen is absent or limited (Andrássy

2005) although the reason for this is still unknown. *Chronogaster* spp. are distributed worldwide and predominantly found in aquatic habitats. Andrássy (2005) stated that thefamily Chronogasteridae prefer warm zones where many species occur in tropical and subtropical zones. However, during this study, the highest numbers of *Chronogaster* specimens were found during the winter collection and at the Bird Lookout point. According to Poinar & Sarbu (1994), it does seem that a certain amount of genetic plasticity occurs within the genus *Chronogaster*, in terms of habitat selection and adaptation.

TROPHIC STRUCTURE AND C-P VALUES

With the exception of the Bird Lookout during the winter survey and Mamba Pool during the summer survey, it was found that all localities were dominated by plant feeders. This may be attributed to the fact the nature reserve is a preserved grassland area with dense vegetation around collection sites.

Bacterivores which were second only to plant feeders, were present at all three sites during the winter and summer collection with the highest being at the Mamba Pool during the summer collection. Bio-films are vital contributors to microbial production and nematodes play a considerable role in the consumption of this material (Traunspurger 2000). In addition to this, bird guano, faecal pats from mammals as well as the presence of peat which consists of decayed plant materials, are all rich in organic matter and present at the reserve. These are all likely to have acted as a food source for bacteria and contributed to the presence of bacterivorous nematodes.

General opportunists (c-p 1) and enrichment opportunists (c-p 2 and c-p 3) only develop under food-rich conditions (Bongers 1999) and respond to the presence of decomposing organic material. The overall dominance of c-p 2 and c-p 3 class nematodes may therefore be explained by the high food source injection.

Whilst bacteria feed on easily decomposable material, fungi are known to live off material which is relatively larger to breakdown, including wood and bark (Melendrez 2003). Overall, the three sites were dominated by grasses except for woody trees present at the Hippo Pool. This could likely explain the presence of fungi and as a result fungivorous nematodes being present in higher numbers at this site in comparison to the two other sites.

Omnivores, which were represented by the families Aporcelaimidae, Dorylaimidae, Qudsianematidae and Thornenematidae, were most prominent at the Mamba Pool during the summer season. According to Ristau & Traunspurger (2011) an increase of omnivores may be expected to occur under enriched conditions. This is likely the reason for omnivores dominating this site. Omnivorous nematodes can feed on a selection of different foods such as algae, protozoan cysts, fungi as well as other nematodes (Lee 1965). Predators which are represented by mainly the family Tobrilidae, but also the family Actinolaimidae, were most prominent at the Bird Lookout Point during the winter season. As depicted in the soil food web in Figure 2.3., predators are in the fourth trophic level and associated with arthropods. Futhermore, birds and other animals, including mammals, feed on arthropods which may allow for facilitation of the predaceous nematodes to the fifth trophic level in the food web. The Bird Lookout point had a large variety of bird species and mammals were present here in comparison to the other two sites.

Starting at a local (core sample) scale of interaction, the lack of species specific knowledge does make it harder to evaluate the degree of niche specialisation. Species with similar buccal and oesophageal morphology probably have the same diets although perhaps, with different preferences. It could be possible that they feed on different microsites due to their different body sizes (Ettema *et al.* 2000).

Nematode feeding type diversity offers an additional option for ecological indication. Pollution induces changes of feeding type composition. This reflects the varying sensitivities of different taxa which partly overlap with the information of other indices (e.g. MI). Pollution also influences the quality or quantity of food sources and the changes in the composition of nematode feeding types has secondary food web effects (Hoss *et al.* 2006).

ENVIRONMENTAL DISTURBANCE INDICES

According to McCarthy *et al.* (2010), investigations have indicated that even though parts of the floodplain wetlands in the Klip River valley have remained relatively pristine, there are wetlands within the reserve that have been seriously degraded. It is clear that the highest MI value was at the Hippo Pool during the preliminary collection, followed by the Bird Lookout point during the winter collection indicating pristine conditions during these spatial-temporal periods.

A massive stress factor for aquatic ecosystems is anthropogenic pollution. Pollution affects living organisms and as result ecosystem functions, such as nutrient cycling, are strongly influenced (Hoss *et al.* 2006). The MI values were the lowest during all three surveys at the

Mamba Pool, indicating nutrient-rich conditions. The Mamba Pool is situated close to the nearby town of Memel. Sanitation and low-cost housing sections of Memel are poor and low quality water from this source discharges into tributaries of the Klip River at the upstream end of wetlands (McCarthy *et al*. 2010). Sewage enters the wetland at this site and this is likely the reason for the disturbed conditions at the Mamba Pool, as it is located at the upstream end of the wetland.

The MI2-5 is identical to the MI, but excludes general opportunists (c-p 1). Results of the MI2-5 showed a virtually similar pattern to the MI overall, however, the c-p 1 class was represented by members of a single family (Panagrolaimidae) which were present in relatively low number. Removing the c-p 1 values from the equation had a narrow impact on the result and this is likely the reason for the similar pattern between MI and MI2-5.

The Plant-Parasitic Index (PPI) is comparable to the MI, but computed only for plant-feeding nematodes. PPI is lower under nutrient poor conditions. It is with the underlying principle that their large quantity is a result of the vigour of their host plants which in turn is determined by system enrichment (Ferris 2013b). The PPI value was lowest at the Bird Lookout during the winter survey which indicates nutrient poor conditions, which could be attributed to plants losing vitality over winter and decreased animal activity, resulting in less enrichment. The PPI value was highest at the Hippo Pool during the preliminary survey. Host plants and ecosystem type varied at these locations.

TAXONOMIC DISCUSSION

According to Zullini (2006) the family Tobrilidae is the only real freshwater family known. De Ley *et al.* (2006) in their new scheme of classification placed Tobrilina as a suborder of Triplonchida. Within Tobrilidae, they considered nine genera under the subfamily Tobrilinae including *Tobrilus* and *Eutobrilus*. Four genera were placed under Neotobrilinae including *Brevitobrilus*.

***Tobrilus* sp. 1**

Initially, the genus *Tobrilus* housed a large number of species. Its taxonomy has been studied and revised by several workers including: Andrássy (1964), Schiemer (1971), Loof & Riemann (1976) and Joubert & Heyns (1979). In 1981, Tsalolikhin subdivided *Tobrilus* into 13 different genera on the basis of similarities primarily in body size, stoma, lip sensilla, supplements and spicules. This categorization removed much heterogeneity from the earlier known genus *Tobrilus*. Species belonging to *Tobrilus* can be found in freshwater habitats and rarely occur in brackish water or in wet soil (Zullini 2006). From the available literature for this study, it can be deduced that many species are still incorrectly being placed in the genus *Tobrilus* when they clearly do not belong there. This has led to the conclusion that the genus *Tobrilus* has become the labyrinth destination point for many of the species within the family Tobrilidae. As a result, *Tobrilus* sp. 1 was identified as such, since there is still some confusion as to whether the known species listed actually belong to this genus.

***Eutobrilus* sp. 1**

Species belonging to the genus *Eutobrilus* are generally found in freshwater habitats, but rarely in brackish water or moist soil. Species from the present study were found in all three localities during the winter months. There is a wide inconsistency in opinions concerning this genus and it has been subjected to several revisions. The most current was in 2009, Gagarin reviewed and emended the genus and provided a brief description and identification key to 15 species.

As a member of the family Tobrilidae, *Eutobrilus* is a true freshwater genus. Species belonging to this genus are predators and feed on small invertebrates as well as diatoms (Andrássy 2007).

***Brevitobrilus* sp. 1.**

The genus *Brevitobrilus* represents smaller species of about 1.5 mm (0.9 - 2.4 mm). Diagnostic characteristics such as the size of stoma, distance between two teeth and the position of amphids do not seem to be very reliable features for species differentiation. This is because these characteristics generally appear to be similar for species which have similar body lengths. Similar findings were also reported by Tahseen *et al*. (2009).

This genus is mostly found in freshwater but may also be found in brackish water or wet soil (Zullini 2006). During this study, *Brevitobrilus* sp. 1. was found in both lentic (Mamba Pool and Bird Lookout Point) and lotic habitats (Hippo Pool) at the reserve. A taxonomic key formulated by Tahseen *et a*l. (2009) was used to identify the species. The key indicated the nematode at hand to be *B. sardus*. However further research on *B. sardus* indicated too many differences and thus the species from the present study was identified as *Brevitobrilus sp. 1.*

***Chronogaster* sp. 1.**

The genus *Chronogaster* is morphologically a very distinct genus. It has a cosmopolitan distribution, although more abundant in warmer tropical regions. Key diagnostic characteristics include the presence of longitudinal and serrate valvular apparatus in the oesophagus bulb and the absence of caudal glands and a spinneret. *Chronogaster* species are usually suited in freshwater bodies, but also in various types of terrestrial habitats. During the present study *Chronogaster* sp. 1 were found from all three localities (which included both lotic and lentic environments) that were sampled at the Seekoeivlei Nature Reserve, Memel. The main difference of *Chronogaster* sp. 1 to the known South African species as listed by Heyns (2002b) is that two of the species, (*C. africana* and *C. andrassyi*) have either a mucron or more than one mucron on the tip of the tail, the other species *C. glandifera* has lateral bodies within its body. Therefore, based on all the other differences between *Chronogaster* sp. 1 and the known species, it was decided to preliminary name it as *Chronogaster* sp. 1.

FUTURE WORK

A high level of similarities exists between species within each of the genera at hand. In order to avoid incorrect identification, it would be best to do molecular work in addition to the morphology-based studies. Considering the limited literature available for these families, especially in South Africa, it would be valuable to try and describe these species.

During the present study, it also became apparent that a taxonomic revision for each of the genera *Tobrilus*, *Eutobrilus* and *Brevitobrilus* is needed.

Many descriptions of the genus *Chronogaster* do not include a description of male specimens, as males are extremely rare. It was therefore exciting to find a male specimen of *Chronogaster* from soil samples collected at the Seekoeivlei Nature Reserve. Male specimens were also found for the genus *Brevitobrilus.* Thus, it would be interesting to carry out more fieldwork in an attempt to find additional males.

The study should form a foundation for future ecological work at the SKVNR, with data being used as reference material. Collections should also be carried out over a longer time period in order to have a more complete view on the taxonomical as well as the ecological status of the wetland.

Chapter 8

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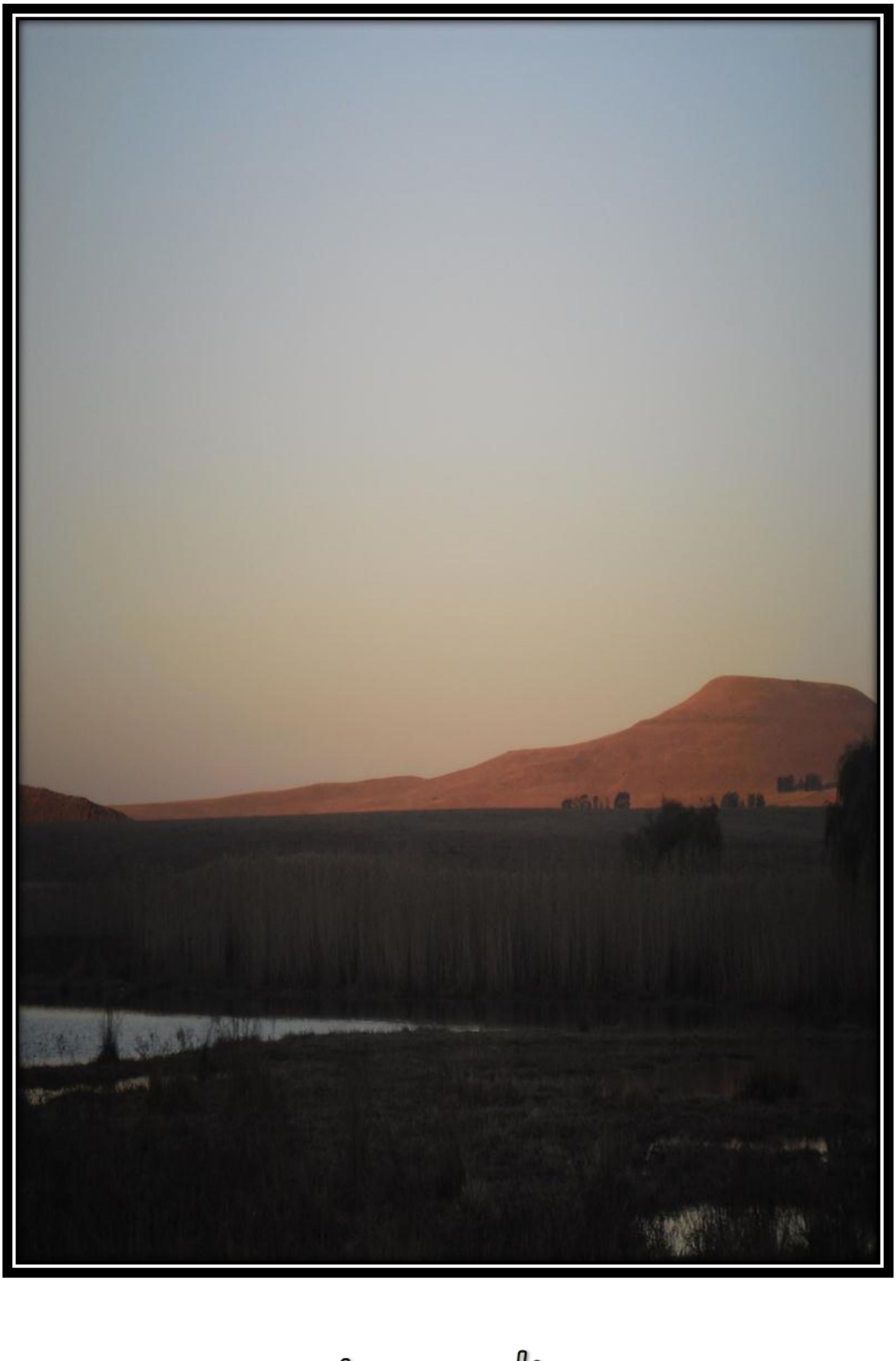

Appendix

The department of economic development, tourism and environmental affairs

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The department of economic development, tourism, and environmental affairs
FREE STATE PROVINCE

98 Zastron Street
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Permitee Details

Ayesha Mobara

Westdene Bloemfontein
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This Permit is issued in terms of the Biodiversity Act 10 of 2004 (Threatened or Protected Species Regulations) and In Terms of Nature Conservation Ordinance no 8 of 1969, permission is hereby granted to the holder of this permit to;

General Permit

To collect nematodes for research purposes at Seekoeivlei Wetland.

Permitee's Signature	**Approved** on behalf of the MEC department of economic development, tourism and environmental affairs .	
Expiry Date 2012-09-30 Return Permit After Expiry Date	Permit Number 01/11281 D Nel	Date Issued 2011-09-14

Permit obtained from the Free State Department of Economic Development, Tourism and Environmental Affairs for collection of nematodes at the Seekoeivlei Wetland. Personal details omitted.

Abstract
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Opsomming

The Seekoeivlei Nature Reserve wetland is a designated Ramsar site, situated on the north-eastern boundary of the Free State Province. Field trips were made on three occasions between October 2011 and May 2012. A total of 43 genera belonging to 20 families and 7 orders were identified from the three localities. Of the three sites sampled, Bird Lookout point showed the highest species richness (S = 16) and species diversity in terms of the Shannon-Wiener diversity index (H' = 2.18) and Hills diversity index (Hills N1 = 8.78) during the summer survey. It was found that all localities were dominated by plant parasitic nematodes with the exception of Bird Lookout during the winter survey and Mamba Pool during the summer survey, which was dominated by predators and omnivores, respectively. The Maturity Index (MI) value was highest at the Hippo Pool during the preliminary collection (MI = 14.22) and lowest at the Mamba Pool on all three collections. Key genera found at the site included *Tobrilus* Andrássy, 1959, *Eutobrilus* Tsalolikhin, 1981; *Brevitobrilus* Andrássy, 1959 and *Chronogaster* Cobb, 1913. *Tobrilus* sp. 1 is characterised by a vulva at mid body (49%), the tail long and cylindrical (216-295 µm) with a spinneret present. *Eutobrilus* sp. 1 is characterised by labial sensillae papilli form, cephalic setae just over one third labial region diameter, vulva placement at midbody, tail elongate tapering, 6.5 anal body widths long. *Brevitobrilus* sp. 1 is characterised by body length (1019-1792 µm female; 1123-1526 µm male), labial sensillae papilliform, cephalic setae one third of labial region diameter, vulva at 46% body length, tail five anal body widths long and spinneret present. Males characterised by six supplements with largest distance between S5 and S6, spicule 21 µm, tail three anal body widths long and subterminal setae present. *Chronogaster* sp. 1. is recognised by female habitus being straight to completely ventrally curved and male habitus being ventrally curved, J-shaped, lip region not annulated, cephalic setae as long or longer than lip region diameter, cylindroid pharynx with denticulated valve in basal bulb, female reproductive system monodelphic-prodelphic, vulva at (45.3%) body lengths from anterior end, male tail conoid, spicule slender, with fifteen dissimilar supplements. Descriptions, measurements and illustrations including light microscope micrographs are provided for the four species.

Keywords: *Brevitobrilus* sp. 1, *Chronogaster* sp. 1, *Eutobrilus* sp. 1, *Tobrilus* sp. 1, ecology, freshwater, Memel, morphology, nematode, taxonomy

Die Seekoei-Natuurreservaat Vleiland is ŉ Ramsargebied wat aan die noord-oostelike grens van die Vrystaat geleë is. Veldwerkbesoeke is op drie geleenthede tussen Oktober 2011 en Mei 2012 na hierdie gebied onderneem. ŉ Totaal van 43 genera in 20 families en sewe ordes wat voorkom, is vanaf drie spesifieke areas geïdentifiseer. Een van hierdie areas, die Voëluitkykpunt het die grootste spesieverskeidenheid (S = 16) asook spesiediversiteit in terme van die Shannon-Weiner diversiteitsindeks (H' = 2.18) opgelewer en gedurende die somer was die Hill's diversiteitsindeks (Hills N1 = 8.78) die grootste. Die algemene bevinding was dat die gebied deur plant-parasitiese nematode oorheers word, behalwe die Voëluitkykpunt in die winter en die Mambapoel in die somer, wat onderskeidelik deur predatore en omnivore oorheers was. Gedurende die aanvanklikeondersoek, was die volwassenheidindeks (VI) die hoogste by die Seekoeipoel (VI = 14.22) en ten tye van al drie besoeke die laagste by die Mambapoel. Sleutelgenera wat hier aangetref was, sluit *Tobrilus* Andrássy, 1959, *Eutobrilus* Tsalolikhin, 1981, *Brevitobrilus* Andrássy, 1959 en *Chronogaster* Cobb, 1913 in. *Tobrilus* sp. 1 word gekenmerk deur ŉ vulvaplasing by die middellyf, 'n stertlengte van 216-295 µm en 'n silindriese spineret. *Eutobrilus* sp. 1 word gekenmerk deur labiale sensiliums wat papillivormig is, sefaliese setas wat een derde van die labiale-area deursnee is, asook 'n vulvaplasing by die middellyf, 'n verlengde vernouende stert wat 6.5 anale liggaamsbreedtes lank is. *Brevitobrilus* sp. 1 word gekenmerk deur 'n liggaamslengte van 1019-1792 µm by die wyfie en 1123-1526 µm by die mannetjie, asook labiale sensiliums wat papillivormig is, sefaliese setas een derde van die labiale areadeursnit, vulva 46% van die liggaamslengte, die stert vyf anale liggaamsbreedtes lank, asook 'n spineret wat teenwoordig is. Mannetjies word gekenmerk deur ses byvoegsels met die grootste afstand tussen S5 en S6, spikulums 21 µm lank, stert drie anale liggaamsbreedtes lank en subterminale setas wat teenwoordig is. *Chronogaste*r sp. 1 is uitkenbaar aan die habitus wat reguit of ventraal gebuig is vir wyfies terwyl dié van die mannetjies ventraal gebuig en J-vormig is, die liparea is nie verwyd nie, sefaliese setas is lank, of langer as die liparea-omtrek, silindervormige farinks bevat 'n getande klep in die basale knop, wyfie se voortplantingstelsel is monodelfies-prodelfies, vulva (45.3%) van liggaamslengte, manlike stert konies verleng, spikulums skraal asook vyftien verskillende byvoegsels. Beskrywings, afmetings en illustrasies insluitende ligmikroskoopmikrograwe van die vier spesies word voorsien.

Sleutelwoorde: *Brevitobrilus* sp. 1, *Chronogaster* sp. 1, *Eutobrilus* sp. 1, *Tobrilus* sp. 1 ekologie, varswater, Memel, morfologie, nematode, taksonomie

Acknowledgements

The ALMIGHTY

…for blessing me with the opportunity to get this far in my studies and for giving me the strength and ability to complete it.

Dr. Candice Jansen van Rensburg

…for her input, patience and guidance as my study leader and allowing me the opportunity to carry out the project.

Prof. Linda Basson

… for her continual willingness to guide, support and counsel me.

Prof. Jo van As & Prof. Liesl van As

…for their constant advice, help and support throughout my studies.

Dr. Sonja Brink & Isabel Human

…for ongoing motivation, concern and advice.

Dr. Mariette Marais & Dr. Antoinette Swart from the ARC-Biosystematics Unit

…for willingness to help and provide literature and provision of data from SAPPNS data base.

Dr. Nacelle Collins & the Free State Department of Economic Development, Tourism and Environmental Affairs

in providing me with research information and helping me obtain a permit.

The Seekoeivlei Nature Reserve Administration: Erla, Morne and December

…for their enthusiastic willingness to assist me in setting up fieldwork opportunities and research assistance.

The Department of Zoology and Entomology, University of the Free State, Bloemfontein

…for use of their facilities and support received throughout my studies.

Prof. Neil Heideman

…for his support and encouragement throughout my studies.

The National Research Foundation

…for financial support during this project.

Zubeida & Ebraheim Mobara & Aysa Mobara

… *My beloved parents and grandmother for their unending love, support, patience, prayers and guidance through each and every step of my study career.*

Zaynab Mobara

…my dear sister for being there for me, for her understanding, love and encouragement and helping me whenever she could.

Ruaan Schlebusch, Deidre West, Josef Möller, Anneke Vermeulen & **Carl Pohl**

…my colleagues and friends for assisting me in research, fieldwork, obtaining data and encouraging me when I needed it.

To everybody else not mentioned here who was involved in making this project possible and supporting me throughout.