Cosmocercidae (Nematoda) of selected species of amphibians of South Africa

FF Harnoster

orcid.org/0000-0001-9750-1845

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Supervisor: Dr R Svitin

Co-supervisor: Prof LH du Preez

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Declaration

I, Fransinah Florence Harnoster, declare that this dissertation is my own unaided work, except where otherwise acknowledged in the text. This dissertation is being submitted for the degree of Masters of Science in Environmental Sciences to the North-West University, Potchefstroom Campus. It has not been submitted for any degree or examination in any other university.

Fransinah Florence Harnoster

19 March 2021

Dedication

This Masters dissertation is dedicated to two of the dearest people, Dr Roman Svitin and Mrs Anneke Lincoln Schoeman (PhD candidate), for their support and encouragement throughout my post-graduate studies. I cannot be more grateful.

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Abstract

Recent studies revealed that parasites outweigh their host species in terms of species richness. This suggests parasitism as the most common lifestyle. In present times, parasites have an even higher species richness and abundance mainly due to host species dispersal, invasion and human activities. Of these, members of the family Cosmocercidae are found globally, most commonly parasitising Anura. These nematodes were found to be one of the most abundant groups of parasites with a wide host range which includes freshwater fish, amphibians, reptiles, with some recorded in birds and mammals. Species of Cosmocercidae are distinguished mainly by the differences in male genital morphology. Numerous inaccurate descriptions, redescriptions and the synonymisation of found species, followed by the lack of male found specimens and molecular data, caused taxonomic uncertainties in this group. Despite the numerous studies and the development of advanced methods and techniques, fauna of cosmocercid nematodes in South Africa is still represented by four species of *Aplectana*, Railiet and Henry, 1916 and one species of *Cosmocerca* Diesing, 1861. As a result, the most common species found in many amphibians (and reptiles) throughout South Africa is *Cosmocerca ornata* (Dujardin, 1845), initially described from the European green (=edible) frog and European common frog in France. This seems unlikely due to the geographical distance and difference in host species. In the present study helminthological material was collected from 193 frog specimens and the nematodes were studied under light microscopes. The morphological and morphometrical data illustrate significant differences between found specimens in comparison to the known species. Morphological differences are also supported by the molecular data of partial 18S and ITS-28S nuclear genes. Species were morphologically different in the number and/or arrangement of papillae from the known species with some resembling *Aplectana capensis* Baker, 1981 and *A. macintoshii* Stewart, 1914. These characteristics are used as a common distinguishing feature in species differentiation of cosmocercin nematodes. The number of host species was significantly extended from the known records. Thus, we believe that cosmocercin fauna of South African amphibians are more diverse than previously documented.

Key words: Taxonomy, Amphibians, Anura, Nematoda, Cosmocercidae, *Cosmocerca*, *Aplectana*

Opsomming

Onlangse studies het getoon dat parasiete in die algemeen 'n hoër spesierykheid het as hul gasheerspesies. Dit bevestig parasitisme as die mees algemene lewenstyl op aarde. In onlangse tye het parasiete selfs hoër spesierykheid en -veelheid, hoofsaaklik as gevolg van die verspreiding van gasheerspesies, indringing en menslike aktiwiteite. Hiervan word lede van die familie Cosmocercidae, wat meestal Anura parasiteer, wêreldwyd aangetref. Daar is gevind dat hierdie nematode een van die volopste groepe parasiete is met 'n wye verskeidenheid van gashere insluitend varswatervisse, amfibieërs, reptiele, voëls en soogdiere. Spesies van Cosmocercidae word veral onderskei deur die verskille in die morfologie van die manlike geslagsorgane. Talle onakkurate beskrywings, herbeskrywings en sinonimisering van die opgetekende spesies, tesame met ‘n gebrek aan molekulêre data, het gelei tot baie onduidelikheid in die taksonomie van die groep. Ten spyte van talle studies en die ontwikkeling van gevorderde metodes en tegnieke word die fauna van Afrika-amfibiese Cosmocercidae nematode in Suid-Afrika steeds beperk tot vier spesies van *Aplectana* Railiet en Henry, 1916 en een spesie van *Cosmocerca* Diesing, 1861. As gevolg hiervan is *Cosmocerca ornata* (Dujardin, 1845) die mees algemene spesie in amfibieërs (en reptiele) regoor Suid-Afrika, alhoewel dit oorspronklik van die Europese (= eetbare) groenkikker en die Europese gewonekikker in Frankryk beskryf is. Dit is onwaarskynlik as gevolg van die geografiese afstand en verskil in gasheerspesies. Helmintologiese materiaal is versamel uit 193 padda-eksemplare en die nematode is onder ligmikroskope bestudeer. Die morfologiese en morfometriese data illustreer beduidende verskille tussen gevonde eksemplare in vergelyking met die voorheen bekende spesies. Morfologiese verskille word ook ondersteun deur die molekulêre data van gedeeltelike 18S- en ITS-28S-kerngene. Spesies was morfologies onderskeidbaar van die bekende spesies in die aantal en/ of rangskikking van papille, waarvan sommige soos *Aplectana capensis* Baker, 1981 en *A. macintoshii* Stewart, 1914 gelyk het. Hierdie eienskappe word gebruik as 'n algemene kenmerk van spesiedifferensiasie van Cosmocercinae nematode. Gasheerspesies was meer uiteenlopend as wat voorheen gerapporteer is. Dus glo ons dat die Cosmocercinae fauna van Suid-Afrikaanse amfibieërs meer uiteenlopend is as wat voorheen gedokumenteer is.

Sleutelwoorde: Taksonomie, Amfibieë, Anura, Nematoda, Cosmocercidae, *Cosmocerca*, *Aplectana*

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Chapter 1

Introduction

Cosmocerca sp. 1. Image Ms Florence Harnoster

1.1 Nematoda

Nematodes, commonly known as roundworms, have a global distribution (Baker, 1987; Hickman et al. 2008; Bursey et al. 2015; Sou et al. 2018), follow a free-living or parasitic lifestyle and can have a combination of both lifestyles during their life cycle. Nematodes can inhabit marine, freshwater and soil biotypes, as well as parasitize plants, fungi, invertebrates and vertebrate animals (Baker, 1987; Anderson, 2000a; Zhang et al. 2020). Parasitic nematodes can infect their hosts in different ways: orally, percutaneously (through the skin) and via intermediate or paratenic hosts. Some nematodes are known to be autoinfectants (able to infect the same host) (Anderson, 2000a). Some free-living nematodes feed on bacteria, yeast or algae, some prey on rotifers, small annelids and other small nematodes, whilst others live in faecal matter feeding on host tissue (Traunspurger, 2000).

A number of marine, soil and plant nematodes are of agricultural or veterinary importance and their associations, species, feeding habits, life cycles, reproduction and biochemistry, are generally well documented. In contrast, to the parasitic nematodes (Traunspurger, 2000).

The most commonly known freshwater nematodes are about 0,5 – 2 mm long with few exceeding 3 mm (Poulin and Morand, 2000; Traunspurger, 2000), having a cylindrical and elongated body with the mouth opening located centrally on the tip of the anterior extremity. Most species have a buccal cavity between the mouth and oesophagus (Traunspurger, 2000; Poulin and Jorge, 2019). The buccal cavity may differ in shape depending on its feeding habits and is used as a taxonomically important characteristic. Freshwater nematodes are covered in sensory receptors (sensilla). The oesophagus is elongated connecting the buccal cavity to the intestine. It may remain cylindrical or have muscular expansions which are called bulbs (Traunspurger, 2000). Females may be monodelphic or didelphic (having one or two sets of genital organs) and the number and structure of the ovaries may be used as major taxonomic distinguishing characteristics (Traunspurger, 2000).

For monodelphic groups, the vulva may be close to the anus. In didelphic groups it is situated at mid-body level, which is also the situation in parasitic groups (Bursey et al. 2012; Majdi and Traunspurger, 2015). Males may also be monorchic or diorchic (having one or two testes). Typically they have a pair of cuticularized spicules with a guiding structure called the gubernaculum (Baker, 1916; Baker, 1980a; Baker, 1980b; Bursey et al. 2012). These structures facilitate copulation and sperm transfer, and are of taxonomic importance for differentiation of species (Traunspurger, 2000).

The most common terrestrial nematode, *Caenorhabditis elegans* Maupas, 1900, reaches one to two millimeters in length as adults (Félix and Braendle, 2010). These nematodes are easily cultured in the laboratory, where they are maintained on the surface of nutrient agar and fed on a mutant strain of *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919 (Félix and Braendle, 2010). As a result, they are commonly used as research models (Félix and Braendle, 2010; Sommer and Ogawa, 2011; Cook, 2013). There are four larval stages (L1-L4) which are of importance for comparative and evolutionary studies (Félix and Braendle, 2010; Sommer and Ogawa, 2011). One of the stages, namely L2, can develop into dauer larvae (Stasiuk et al. 2012). The dauer larva is a facultatively arrested and stress resistant survival stage, which develops during stressful environmental changes such as starvation, increased temperatures and overcrowding (Viney et al. 2005). Dauer larvae are found to be crucial to the survival of species (Sommer and Ogawa, 2011). Phenotypic plasticity, an organism's capacity to undergo phenotypical changes in response to environmental conditions, has been proposed to explain the development of dauer larvae (Sommer and Ogawa, 2011). The dauer stage retains the second stage larval cuticle, which is shed when molting to third stage larvae under normal circumstances (Stasiuk et al. 2012; Cook, 2013). This characteristic is of particular interest, since a similar layer is observed in parasitic nematodes where infective stage larvae are enveloped in a modified second stage cuticle or layer (Viney et al. 2005; Stasiuk et al. 2012). However, the link between the phenomenon in free-living and parasitic nematodes remains largely understudied (Cook, 2013). Regardless, it is clear that nematodes as a group display remarkable ecological and evolutionary flexibility (Cook, 2013).

1.2 Taxonomy

The science of taxonomy is an arrangement system produced to formulate naming and grouping of species (Hickman et al. 2008; Dantas-Torres, 2018). Taxonomy groups species to their most recent and common ancestor in accordance with their common characteristics (Michener, 1963). Studies have shown that Linnaean taxonomy, based on morphology, clearly pre-dates that of evolutionary taxonomy as the latter uses modern techniques (Baker, 1984; Hickman, et al. 2008).

The use of the theory of evolution changed taxonomy from simple classification to systematisation, thus two different approaches now determine classification by their most appropriate characteristics. The earlier approach being whether the organisms contained a defining feature and, the latter, whether the specimen rejects or confirms descendant hypothesis from the most recent common ancestor (Michener, 1963). Since Linnaean taxonomy dates back more than 250 years, many taxonomists described species in ignorance of the others' work thus leading to the confusion in species nomenclature, causing numerous inaccuracies in different groups of animals (Baker, 1984; Godfray, 2002; Cristofolini, 2019).

Of these, the Phylum Nematoda is documented with much criticism as it is one of the largest groups in number of species (second only to the Phylum Arthropoda) with one of the lowest levels of phylogenetic studies (Wu et al. 2002; Bursey et al. 2011; Sou et al. 2014). In traditional systematics Nematoda is divided into two Classes namely Adenophorea and Secernentea (Anderson, 2000a). Most freshwater nematodes belong to the Adenophorea that are typically characterised by the presence of setae (locomotive structures found on free-living nematodes), adhesive glands and conspicuous amphids (sensory organs on anterior extremity). Terrestrial, parasitic and pathogenic nematodes are mainly in the Secernentea. These nematodes have neither setae nor adhesive glands and amphids are greatly reduced in size (Baker, 1984; Traunspurger, 2000).

The group Secernentea hosts about 33% of all vertebrate parasites (Baker, 1984; Traunspurger, 2000; Poulin, 2014; Poulin and Jorge, 2019). Currently there are more than 2 000 described genera of parasitic nematodes, and new ones are being discovered every year due to the increasing focus on systematic parasitology (Anderson, 1984; Baker, 1984; Anderson, 2000b; Poulin and Jorge, 2019).

While conducting full parasite accounts, we came across a variety of nematodes. From these the candidate was involved in the publication of two papers with the following references:

Kuzmin, Y., Svitin, R., Harnoster, F and Du Preez, L. (2020). Description and molecular characterisation of a new nematode species parasitic in the lungs of *Strongylopus grayii* (Smith) (Anura: Pyxicephalidae) in South Africa. *Systematic Parasitology*, 97, 369–378.

Svitin, R., Kuzmin, Y., Harnoster, F and Du Preez, L. (2020). New records of A*mphibiophilus* spp. (Nematoda: Amphibiophilidae) parasitic in *Strongylopus grayii* (Smith) and *Amietia delalandii* (Duméril & Bibron) (Amphibia: Anura: Pyxicephalidae) in South Africa, with a description of *Amphibiophilus bialatus* n. sp. *Systematic Parasitology,* 97, 713–725. doi.org/10.1007/s11230-020-09947-z.

However, we realized that for the purpose of this dissertation we needed to focus on one taxonomic grouping, where the study would make a big contribution. As the most represented among the amphibians, we selected the Family Cosmocercidae.

Among parasitic nematodes the Family Cosmocercidae is not an exception and the taxonomic position, as well as taxonomic structure of this group, has been changed several times by different authors.

1.3 Phylogenetic studies

The number of phylogenetic studies of nematodes have increased in recent years though their position is still one of the most debatable issues in the animal kingdom (Wu et al. 2002; Blaxter, 2011; Chen et al. 2018; De Silva et al. 2018). Traditionally, systematics placed nematodes as one of the primitive metazoans, although, the molecular evidence suggests that this group should be highly derived (Wu et al. 2002; Blaxter, 2011; Chen et al. 2018; De Silva et al. 2018). Aguinaldo et al. (1997) studied phylogenetic relationships of different animals based on the 18S rRNA gene marker and have suggested that the division based on segmentation and the presence of a coelom is rather flawed. In taxonomy, nematodes, which are pseudocoelomates, and platyhelminthes which are acoelomates, are believed to be intermediate taxa between diplobasts and triplobasts (Traunspurger, 2000). Although this concept has been generally accepted, the molecular phylogeny indicates no linkage and there are no transitional forms between the taxa. To date, nematodes are mostly still classified based on morphological characteristics by many authors (Blaxter, 2011; Chen et al. 2018; De Silva et al. 2018). All major phylogenetic classification of nematodes by Maggenti, Adamson, Anderson, Malakhov and Lorenzen (Maggenti, 1981a, 1981b; Maggenti et al. 1987; Aguinaldo et al. 1997; Traunspurger, 2000), however, have divided the phylum into two Classes: Adenophora and Secernentea. The classification systems by Anderson and Maggenti, suggest that both classes are monophyletic while the other three suggest paraphyletic Adenophora which gave rise to the monophyletic Secernentea (Aguinaldo et al., 1997; Maggenti, 1981a; Maggenti, 1981b; Traunspurger, 2000). This inconsistency in the group remained unclear until the introduction of molecular investigations.

Molecular phylogenetic studies of nematodes started in the early 1980s and gave rise to the five major clades: Clade I includes Mononchida, Mermithida, Dorylaimida and Trichocephalida; Clade II includes Triplanchid and Enoplida; Clade III - Ascaridida, Rhigonematida, Spiruirida and Oxyrida; Clade IV - Tylenchida, Aphelenchida and partial Rhabditida; Clade V includes Diplogasterida and partial Rhabditida and Strongylida (Anderson, 2000a; Manktelow, 2010; Traunspurger, 2000). All the groups include parasitic and free-living taxa. This was a great advancement for nematode phylogeny, yet further testing is needed to support this with independent molecular evidence (Maggenti, 1981a, 1981b; Maggenti et al. 1987; Aguinaldo et al. 1997; Traunspurger, 2000).

Phylogenetic studies of cosmocercin nematodes are limited to a single study, published in early 2021 (Chen et al. 2021). In their work authors provided phylogenetic trees based on Maximum Likelihood (ML) and Bayesian Inference (BI) analyses of partial 28S rDNA and 18S rRNA sequences. Both analyses confirmed distinctness of the clade consisting of *Aplectana*, *Cosmocerca* and *Cosmocercoides* species among other cosmocercin nematodes (Chen et al. 2021).

1.4 Cosmocercinae

Knowledge of the development, transmission and classification of the family Cosmocercidae has increased in recent years (Anderson, 2000b; Saad et al. 2009). Parasitising the digestive tract of amphibians, reptiles and also some snails (Baker, 1987), they are transmitted orally or through the skin in most cases in amphibians (Saad et al. 2009). There are three subfamilies: Austraplectaninae, parasitising amphibians in Australia, Maxvachoniae, parasitising amphibians in Australo-Papuan Region (Bursey et al. 2011), and Cosmocercinae, reported from amphibians and rarely reptilians, worldwide. Of these, only Cosmocercinae are known from the African continent.

This group of nematodes is characterised by having an oesophagus divided into three parts (a short pharynx, cylindrical corpus and the oesophageal bulb). Males have paired spicules and numerous caudal papillae and females have an equatorial vulva, two uteri, thin shelled eggs and a short tail (Bursey et al. 2012). There are currently 11 accepted genera within Cosmocercinae (Bursey et al. 2012).

- *Aplectana* Railliet and Henry, 1916

The genus *Aplectana* was erected for the assignment of species *Aplectana acuminata* (=*Ascaris acuminate*) Shrank, 1788 as a result of the pre-occupied genus *Aplecta* Railliet and Henry, 1916 (Baker, 1980). Species of the genus are identified by the lack of rosettes or plectanes on the tail of males, and by the females having both ovaries and uteri anterior to the vulva (Baker, 1980; Bursey et al. 2012). There are currently 52 species assigned to the genus, of which six are known from the Afrotropical region (Bursey et al. 2018). Of those six, two have been described in South Africa (Baker, 1981a).

Type species: *Aplectana accuminata* (Schrank, 1788) Railliet and Henry 1916

- *Cosmocerca* Diesing, 1861

Representatives of the genus *Cosmocerca* are characterised by their small, stout bodies. These nematodes display sexual dimorphism, with males not more than half the length of the females and having numerous papillae and plectanes in the tail region (Baker, 1980; Bursey and Goldberg, 2004; Bursey et al. 2012). There are currently 31 species assigned to the genus, with only one species reported from the Afrotropical region (Bursey et al. 2015; Sou et al. 2018) - *Cosmocerca ornata* (Dujardin, 1845). This species is the most commonly found, occurring globally and has been recorded throughout the Afrotropical realm (including South Africa) although none of the records of the species found were supported by images, detailed descriptions or molecular data (Baker, 1981a; Halajian et al. 2013)

Type species: *Cosmocerca trispinosa* Railliet and Henry, 1916

- *Cosmocercella* Steiner, 1924

As per the revision of the genus by Baker and Adamson (1977), the genus is distinguished from others by the possession of the precloacal, vesiculated, rosette papillae and prominent longitudinal striation. Some species of the genus have digitiform rosette papillae. The number and location of the rosette, caudal, vesiculated papillae are significantly varied within the genus and play the main role in the species differentiation within this genus (Baker and Adamson, 1977; Bursey et al. 2012; Bursey et al. 2014). There are currently 10 assigned species in this group with no species recorded from the Afrotropical region.

Type species: *Cosmocercella haberi* Steiner, 1924

- *Cosmocercoides* Wilkie, 1930

Members of the genus *Cosmocercoides* are morphologically similar to ones of the genus *Cosmocerca*, however, they differ in the morphology of rosette papillae . Both males and females are found to have lateral alae, and the cuticle of the specimens is striated transversely without somatic papillae (Bursey et al. 2012; Bursey et al. 2015). There are currently 26 (Liu et al. 2020) assigned species, none of which are described from the Afrotropical region.

Type species: *Cosmocercoides pulcher* Wilkie, 1930

- *Nemhelix* Morand and Petter, 1986

The genus *Nemhelix* is characterised by having a rounded mouth opening without lips, the absence of somatic papillae, plectanes and rosettes. Characteristically it parasitizes molluscs. The only other species of the subfamily Cosmocercinae found to parasitise molluscs is *Cosmocercoides dukae* Holl, 1928. The type species is the only species of the genus and, thus, has not been recorded from the Afrotropical region but is limited to the molluscs of Europe (Morand and Petter, 1986)

Type species: *Nemhelix bakeri* Morand and Petter, 1986

- *Neocosmocercella* Baker and Vaucher, 1983

The genus was erected from *Cosmocercella* to accommodate the species *Neocosmocercella paraguayensis* Baker and Vaucher, 1983. The species was found to resemble species of *Cosmocercella* (Draghi et al. 2015), with both genera sharing the same characteristic vesiculated, rosette, caudal papillae. However, the type species *N. paraguayensis* appeared to be significantly different in that it has three bilobed lips and a hexagonal shaped mouth and has only vesiculated, caudal papillae (Draghi et al. 2015;dos Santos et al. 2017). There are currently three species assigned to the genus all of which are found in South America (Draghi et al. 2015; dos Santos et al. 2017, dos Santos et al. 2018).

Type specie: *Neocosmocercella paraguayensis*

- *Oxyascaris* Travassos, 1920

The genus closely resembles *Aplectana* with its absence of rosettes and is distinguished by more prominent sexual dimorphism, the length of females being over 15 mm long and the female reproduction system having various terminations (Baker and Vaucher, 1985; Bursey and Goldberg, 2007). There are currently only four species ascribed to this group from the Americas', none of which occur in the Afrotropical region.

Type species: *Oxyascaris oxyascaris* Travassos, 1920

- *Oxysomatium* Railliet and Henry, 1916

Oxysomatium are also characterised by the males lacking rosettes or plectanes, the presence of somatic papillae and lateral alae and females producing rather small eggs (Baker, 1980b). The genus closely resembles *Aplectana* and is distinguished by the location of the ovaries. The ovaries in *Oxysomatium* are connected to the uterus anteriorly but are posterior to the vulva, characterising the genus as amphidelphic (Baker, 1980c). There are three species assigned to this genus, occurring in Europe, Asia and the Americas, none of which are found in the Afrotropical region.

Type species: *Oxysomatium brevicaudatum* (Zeder, 1800) Railliet and Henry, 1916

- *Paradollfusnema* Baker, 1982

Paradollfusnema was erected to replace *Dollfusnema* Baker, 1982, which was pre-occupied by *Dollfusnema* Caballero, 1974 (Baker, 1981b; Bursey, 2002). The genus is described as having cephalic lips parted by cuticular interlabia, with the tails of males bearing simple caudal papillae with no rosettes nor plectanes, and didelphic females with both ovaries in an anterior position to the vulva. There are currently two species of this genus, both parasitising lizard hosts that were described from Brazil and Florida (USA) (Baker, 1981; Baker, 1987; Bursey, 2002) .

Type species: *Paradollfusnema amphisbaenia* (Baker, 1981) Baker, 1982

- *Paraplesiohedruris* Bursey, Goldberg et Kraus, 2012

Paraplesiohedruris was assigned to this genus based on the common characteristic of the anterior portion found in the subfamily Cosmocercinae (Bursey et al. 2012). The posterior end of the females possesses a sclerified hook, a feature commonly found in the genus *Hedruris* Nitzsch, 1821 (Bursey et al. 2012) and the authors have stated that further investigation is required to examine the hook feature. Other than the type species described from *Hylarana volkerjane* Günther, 2003 no other record of species has been documented in this genus.

Type species: *Paraplesiohedruris ranae* Bursey, Goldberg et Kraus, 2012

- *Raillietnema* Travassos, 1927

The genus was described to include species of Cosmocercinae that are found to have simple amphidelphic uteri with few, relatively large, ova (Walton, 1940). There are currently 24 species of *Raillietnema described*, 14 of which were described from the Afrotropical region in the Ethiopian realm (Walton, 1940; Bursey and Goldberg, 2006; Bursey et al. 2006). Species of the genus commonly parasitize lizards (Bursey et al. 2006). Bursey et al. (2006) tabulated species, showing that two species from Ethiopia parasitize frogs. There are no records of species from the genus in South Africa.

Type species: *Raillietnema simples* (Travassos, 1925) Travassos, 1927.

Four more genera namely *Paracosmocerca* Kung et Wu, 1945 *Neoxysomatium* Ballesteros-Mârquez, 1945 and *Neoraillietnema* Ballesteros Marquez, 1945 and *Neyraplectana* Ballesteros Marquez, 1945 were erected from *Cosmocerca*, *Oxysomatium*, *Raillietnema* and *Aplectana*, respectively. In subsequent studies, however, many authors considered proposed morphological differences as insufficient for the generic status (Baker, 1980b; Anderson, 2000a)

1.5 Life cycle of Cosmocercinae

There are very few studies conducted on the members of the subfamily Cosmocercinae (Anderson, 2000a) most of which dealt only with free-living stages (Ogren, 1953; Saad et al. 2009). Some studies have shown the life-cycle of these nematodes being direct, without intermediate hosts and infection of hosts occurring *per os* or rarely subcutaneously (Ogren, 1953; Anderson, 2000a; Saad et al. 2009; Bursey et al. 2015). Saad et al. (2009) studied the life-cycle of *C. ornata* (reported as *Paracosmocerca macronata* Kung and Wu, 1945) and showed that second stage nematode larvae penetrated the skin around the eyes of tadpoles within seven days after their first moult, although none were found in the gut. The life-cycle investigation by Kirillov, and Kirillova (2016) of *C. ornata*, showed that the species did not infect tadpoles, although they successfully infected frogs *per os*. In the light of available literature, it is clear that the life cycle of different species within *Cosmocercinae* may vary and only further studies can clarify the adaptations of different nematode genera to their hosts.

1.6 Cosmocercinae records in South Africa

In 1981, Baker published a comprehensive survey of cosmocercids in southern Africa. To date this remains the only publication of a survey conducted in this region which included species descriptions. In his study, Baker found five species, two of which were newly described. The species described were *Aplectana capensis* Baker, 1981 and *Aplectana degraaffi* Baker, 1981. He also recorded the occurrence of species *Aplectana chamaeleonis* Baylis, 1929, *Aplectana macintoshii* Stewart, 1914 and *C. ornata.* The host diversity from which they were recovered is as follows:

- *Aplectana capensis* recorded in species *Breviceps rosei* Power, 1926 and *B. montanus* Power, 1926.
- *Aplectana chamaeleonis* recorded in *Sclerophys capensis* (= *Bufo rangeri*) Tschudi, 1838, *Vandijkophrynus angusticeps* (= *Bufo angusticeps*) Smith, 1848, *Amietia delalandii* (= *Rana angolensis*) Dumeril and Bibron, 1841, *Ptychadena oxyrhynchus* Smith, 1849 and *Cacosternum capense* Hewitt, 1925.
- *Aplectana degraaffi* recorded in *Breviceps sylvestris* (= *B. sylvestris sylvestris*) FitzSimons, 1930.
- *Aplectana macintoshii* recorded in *Schismaderma carens* (= *Bufo carens*) Smith, 1848, *Sclerophrys gutturalis* (= *Bufo gutturalis*) Power, 1927, *Sclerophrys pusilla* (= *Bufo maculatus*) Mertens, 1937, *Sclerophrys garmani* (= *Bufo garmani*) Meek, 1897, *Phrynomantis bifasciatus* (= *Phrynomerus bifasciatus)* Smith, 1847 and *Breviceps adspersus* Peter, 1882.
- *Cosmocerca ornata* recorded in *B. rosei*, *S. capensis*, *S. gutturalis*, *S. pusillus*, *S. garmani*, *Mertensophryne anotis* (= *Bufo anotis*) Boulenger, 1907, *Ptychadena anchietae* Bocage, 1867, *Ptychadena porosissima* Steindachner, 1867, *Kassina senegalensis* Duméril and Bibron, 1841 and *Cacosternum namaquense* Werner, 1910.

1.7 Problem statement, Aims and Objectives

1.7.1Problem statement

The number of potential amphibian host species for Cosmocercinae found to occur in South Africa, differ from species found in Europe, Asia and the Americas. There are, however, also a substantial lack of cosmocercin nematode investigations on the Afrotropical region, most of which are narrowed to multiple records of *C. ornata* in different hosts. These are obscured by the numerous inaccurate descriptions, re-descriptions and synonymising of species. Considering the wide variety of host species, species of cosmocercinae from South Africa are highly unlikely to be limited to only five species with European *C. ornata* found from most of the studied frogs.

1.7.2 Aim

This study aims to focus on the biodiversity of cosmocercin nematodes in several species of amphibians from South Africa using advanced morphological and molecular methods to resolve the taxonomic status and phylogenetic position of the parasite species.

1.7.3 Objectives

Specific objectives of this study included:

- Study the relevant literature and compile a species list of known cosmocercin nematodes in South Africa.
- Collect and study Cosmocerinae from selected anuran hosts in South Africa.
- Conduct molecular and phylogenetic analyses of collected material to determine undescribed morphotypes.
- Study the morphology of collected nematodes and provide detailed species description.

Chapter 2

Materials and methods

Field laboratory. Image Prof Louis H du Preez

2.1 Study areas and sampling

For this study freshly collected material from localities (Fig. 1) not previously sampled during field experiments, as well as nematode specimens from the African Amphibian Conservation Research Group parasite collection, were used.

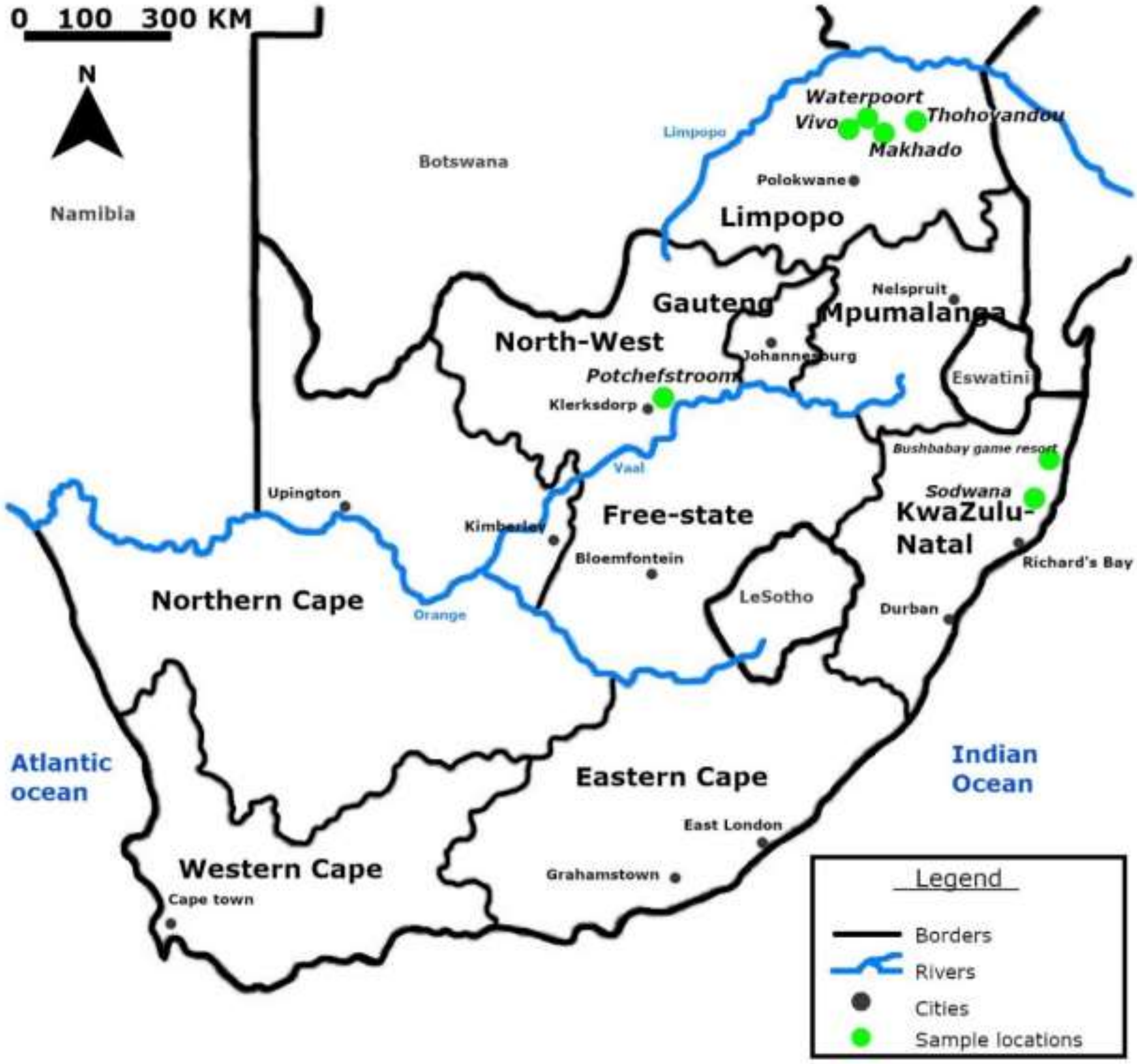

Figure1: Map of South Africa representing sampling localities

The collective analyses surveyed 16 localities and sampled 553 anuran specimens over the time period of four years (2017–2020). Of these, 193 were infected with cosmocercin nematodes. In total 1 586 nematode specimens were retrieved and 206 were studied under the light microscope. Only ten species were used in the description due to lack of male found specimens and the indistinguishable characters of females. All measurements in the text are given in micrometres, unless stated otherwise.

2.2 Field experiments

Figure 2: Preparations for sampling

To obtain fresh material, host specimens were captured at various sites at night with the aid of headlamps, disposable gloves and water-proof safety boots (Fig. 2). Captured anurans were placed in clear plastic bags, according to species and locality and field numbers were assigned on site. Not more than three frogs were placed in a bag with smaller and larger specimens in separate bags. Wet vegetation and about 50 ml water was placed in each bag and the bag was aerated and closed. Bags containing frogs were placed in a cool box. Collected specimens were identified using the field guide “Complete Guide to the Frogs of Southern Africa” (Du Preez and Carruthers, 2017) as well as other field guides that were relevant to the specific area.

For helminthological studies, amphibians were humanely euthanised with tricane ethyl-4-aminobenzoate (MS222). Then, in accordance with the standard operating procedure (SOP NWU-00492-16-A5), the spinal cord was cut to destroy the brain. Specimens were dissected at the field stations by opening a small cut in the abdomen in order to preserve carcases for the amphibian collection. The digestive system, lungs and tissue samples from the liver were carefully removed and placed separately in Petri dishes with 0.9 % saline where they were screened for parasites.

Nematodes were carefully collected with the tip of a bird feather or a soft paint brush, washed in saline, and fixed with hot 70% ethanol. They were then stored in cryogenic vials in 70% ethanol with their assigned field numbers to be later retrieved for further laboratory studies. The collected tissue samples were cut with sharp, sterile dissecting scissors or disposable insulin syringe needles and stored in cryogenic vials containing 70% molecular ethanol for molecular vouchering of the host specimens.

Anuran carcases were closed and fixed in 10% neutrally buffered formalin (NBF) and assigned a field number which was attached to the left thigh. The anurans were positioned on a tray in a form that imitates their natural posture in 10% NBF for 24 hours. Thereafter, at the campus laboratory, the formalin-fixed anurans were placed in a jar that is designed to wash the specimens with slow running tap water overnight. Specimens were accessed into the SAIAB (South African Institute of Aquatic Biodiversity) amphibian collection at the North-West University. Tissue samples were stored in a -80 ˚C freezer. The sample data is archived in the SAIAB Specify database.

2.3 Laboratory analyses

For morphological studies, freshly collected nematodes were placed in distilled water for about 20 minutes followed by 60 – 90 minutes in lactophenol. Preserved specimens were placed in distilled water for about two hours and cleared in lactophenol for 24 hours, then studied under light microscopes on temporary mounts. Apical sections, as well as tail fragments, were prepared manually using a thin razor blade. Photomicrographs and measurements were taken using Microscopic Axio imager Z2 (ZEISS Z2) and Nikon E800 compound microscopes for morphological analyses. Measurements are given as ranges followed by mean values in parentheses. All measurements are given in micrometres unless otherwise indicated.

Nematode measurements of both sexes were as follows; body length and width, oesophageal (pharynx, cylindrical corpus and bulb) length and width, distance of the nerve ring and excretory pore from anterior extreme, tail from the anus to posterior tip and in females, vulva from the anterior extreme. In males; gubernaculum length from the later or ventral view, spicules and the tail tip if present. The plectanes were studied lateraly and ventrally. For ventral studies, males were carefully rolled on temporary slides. The apical section was study by cutting a small section of the apical extremity and carefully rolled to *en face* view.

2.3.1 DNA extraction

Using sharp, sterile razor blades DNA was extracted from the middle fragments of male nematodes, preserving the morphologically important structures, the anterior and the posterior ends. The DNA was extracted using a PCR Bio express extraction kit (PRCBiosystems, UK) or a ZYMO Research DNA miniprep extraction kit (Zymo Research, USA) following the animal tissue protocols recommended by the manufacturers. The 18S rRNA, ITS-28S rDNA and COI gene markers were selected as the most representative for the species identification and phylogenetic analyses.

2.3.2 Amplification and sequencing

Polymerase chain reactions (PCR) were used to amplify approximately 1 500 bp fragments of the nuclear 18S rDNA gene, 2 000 bp fragments of the ITS-28S region and 1 000 of mitochondrial COI gene. The 18S regions were amplified using the primer pair F18Scf.1 (5′-ACC GCC CTA GTT CTG ACC GTA AA-3′) and F18ScR1 (5′-GGT TCA AGC CAC TGC GAT TAA AGC-3′) or NSF418 (5`-CTG GTT GAT CCT GCC AGT-3’) and NSR1787-18 (5`-CGA CGG GCG GTG TGT ACA-3`) for species the F18Scf.1 and F18ScR1 primer pair did not amplify (Lefoulon et al. 2015).

The ITS-28S region were amplified using the primer pair rift (5’-GCG GCT TAA TTT GAC TCA ACA CGG-3’) and 1500R (5’-GCT ATC CTG AGG GAA ACT TCG-3’). The COI gene region was amplified using the primer pair LCO1490 (5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’) and HCO 2198 (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’) (Folmer et al. 1994). PCR reactions were performed with volumes of 25 µL, using 12,5 µL OneTaq® 2X Master Mix with Standard, 1,25 µL (10 µM) of each of the primer sets, and at least 25 ng DNA. Unpurified PCR products were sent to a commercial sequencing company Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa), where sequences were obtained using BigDye® Terminator v3.1 Cycle Sequencing on an ABI3500XL sequencer. DNA products were sequenced in both directions using pairs of PCR primers and additional internal primers: 18S-1192R/20 (5`-CAG GTG AGT TTT CCC GTG TT-3`) and NSF573/19 (5`-CGC GGT AAT TCC AGC TCC A-3`) for 18S amplified with NSF418 and NSR1787-18 primers; ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’), 300R (5’-CAA CTT TCC CTC ACG GTA CTT G-3’), ITS5 (5’- GGA AGT AAA AGT CGT AAC AAG G-3’), and ECD2 (5’-CTT GGT CCG TGT TTC AAG ACG GG-3’) for ITS-28S region.

Table 1: Thermocycling primer profiles

Primer pairs	Thermocycle				
	Denaturation				Extension
F18Scf.1 F18ScR1	94°C	95°C	58°C	75°C	72°C
Time	2 min	30 secs	45 secs	90 secs	10 min
NSF418 NSR1787	94°C	94°C	59.5°C	72°C	72°C
Time	3 mins	45 secs	1 min	1 min	10 mins
Rift 1500R	94°C	95°C	54°C	75°C	72°C
Time	2 mins	45 secs	45 secs	3 mins	7 mins
Cycles	1x	40x			1x
LCO1490 HCO 2198	94°C	94°C	47°C	72°C	72°C
Time	2 mins	1 min	30 secs	2 mins	7 mins
Cycles	1x		35x		1x

Contiguous sequences were assembled and edited using Geneious Prime software (https://www.geneious.com).

2.3.3 Phylogenetic analysis

The resulting alignment was shortened to the shortest sequence of 676 bp for the partial 18S alignments and 1 204 bp long for the ITS-28S alignment and used for Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. The Bayesian analyses were run with the following nucleotide substitution model settings: lset nst = 6, rates = invgamma, ncat = 4, shape = estimate, inferrates = yes and basefreq = empirical, that correspond to a general time reversible model GTR+G+I. Further analysis was provided using the next parameters: mcmc ngen = 4 000 000 for 18S and 3 000 000 for ITS-28S; samplefreq = 100, printfreq = 100 and diagnfreq = 1 000. Trees were visualised using the FigTree (V. 1.4.3) software. Maximum Likelihood analysis was run using Mega (V. 8) software with nodal support estimated from 100 bootstraps. The pairwise analyses (p-distance and the number of differences) were performed using Mega (V. 7.0) software.

Chapter 3

Results

Kassina senegalensis. Image Dr Yury Kuzmin

3.1 Species descriptions

Most of the species observed were identified to one of the two genera namely *Aplectana* and *Cosmocerca*. The two genera have similar apical end morphology with four papillae and two amphids arranged around a triangular mouth (Fig. 3A). Lateral alae are present in members of both genera but are not characteristics used to distinguish genera (Fig. 3B). Details of morphology of the digestive tract and female genital systems are also very similar in both genera and the principal differences are found in the morphology of the posterior end of males.

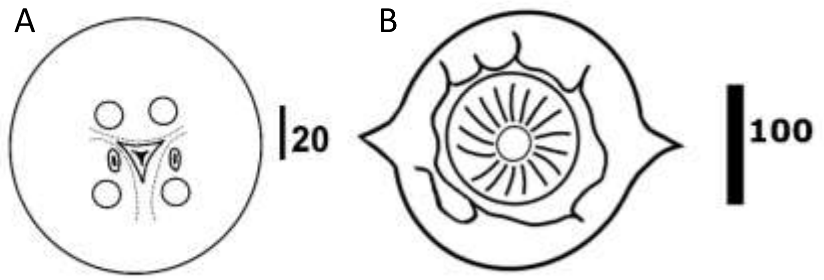

Figure 3: A, general apical section of the genera *Cosmocerca* and *Aplectana*. B, general lateral alae pattern found on members of *Cosmocerca* and *Aplectana.*

***Aplectana* sp. 1**

General. Small, slim nematodes. Males smaller than females. Triangular mouth opening with three lips. Lips surrounded by four papillae and two amphids. Males lacking rosettes or plectanes; females having both ovaries and uteri anterior to the vulva. Oesophagus divided into three parts: short pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to nerve ring and terminating at level of cloaca.

Male. Measurements based on one male. Body 2.8 mm long, 303 wide at mid-body level. Lateral alae beginning at 127 from apex. Oesophageal pharynx 26 long and 34 wide; corpus 301 long and 32 wide; oesophageal bulb 71 long and 80 wide. Nerve ring at 69 and excretory pore at 368 from apex (Fig. 4B). Tail 199 long. Gubernaculum triangular, visible without dissection of tail (Fig. 4C) 54 long. Spicules (Fig. 4C) equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 254 long, right one 264 long. Numerous pre-cloacal unpaired papillae present. Paired papillae (Fig. 4C) found at cloacal region: two pairs anterior to the cloaca; two pairs at level of cloaca; one unpaired papillae on anterior lip of cloaca; one pair posterior to cloaca. Numerous somatic papillae found at post-cloacal and tail region (Fig. 5D).

Female. Measurements based on three females. Body 4.6–5.2 (4.9) mm long, 309–338 (325) wide at mid-body level. Lateral alae 108–124 (115) from apex. Oesophageal pharynx 41–50 (45) long and 50–55 (53) wide; corpus 421–442 (429) long and 53–58 (56) wide; oesophageal bulb

118–131 (126) long and 128–139 (132) wide. Nerve ring at 200–230 (219) and excretory pore at 482–530 (502) from apex (Fig. 4A, 5A). Vulva (Fig 5C) at 2.4–2.6 (2.4) mm from anterior end of body, small, transversely slit, lips poorly developed, located around mid-body level (about 50% of body length). Tail (Fig. 4D) 505–513 (509) long, with elongated end, slightly rounded bluntly on tip (Fig. 5E).

Taxonomic summary.

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Aplectana* Railliet and Henry, 1916.

Species: *Aplectana* sp. 1.

Host: *Amietia delalandii* (Amphibia: Anura: Pyxicephalidae).

Locality: Mooi river, Potchefstroom, North-West Province, South Africa: Coordinates: S 26˚41'44" E 27˚06'13".

Site in host: Large intestine.

Infection parameters: Intensity: 1– 50 (16.3); Prevalence: 100%; Mean abundance: 16.3.

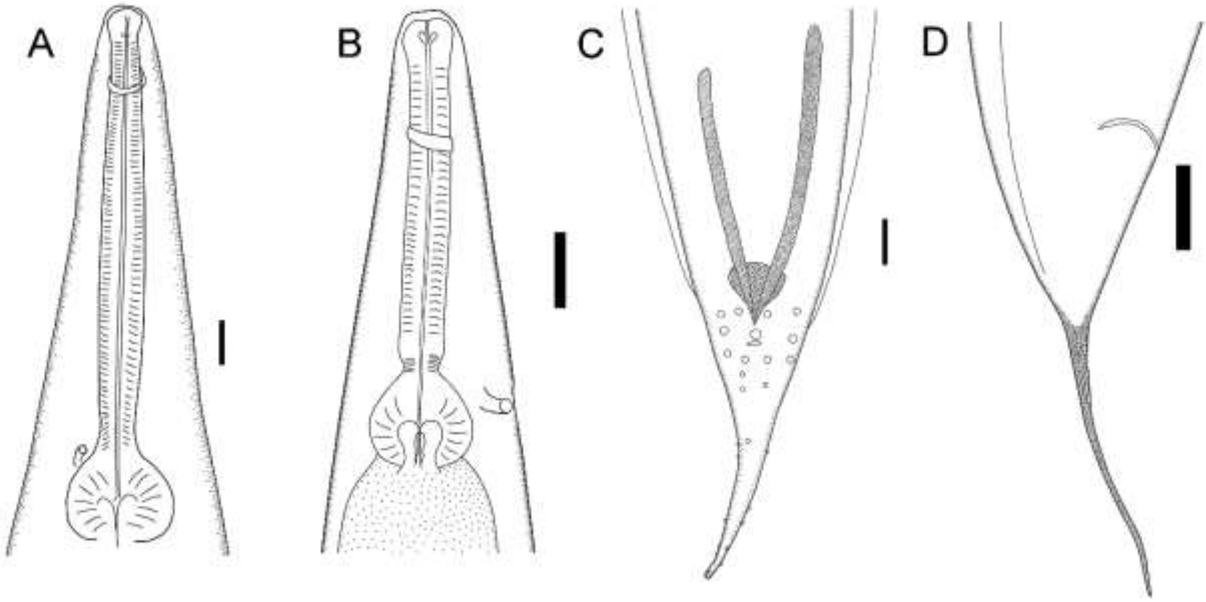

Figure 4. *Aplectana* sp. 1, line drawings. A – anterior end of body, female ventral view; B – anterior end of body, male, lateral view; C – posterior end of body, male, ventral view; D – posterior end of body, female, ventro-lateral view. Scale bars: 100µm.

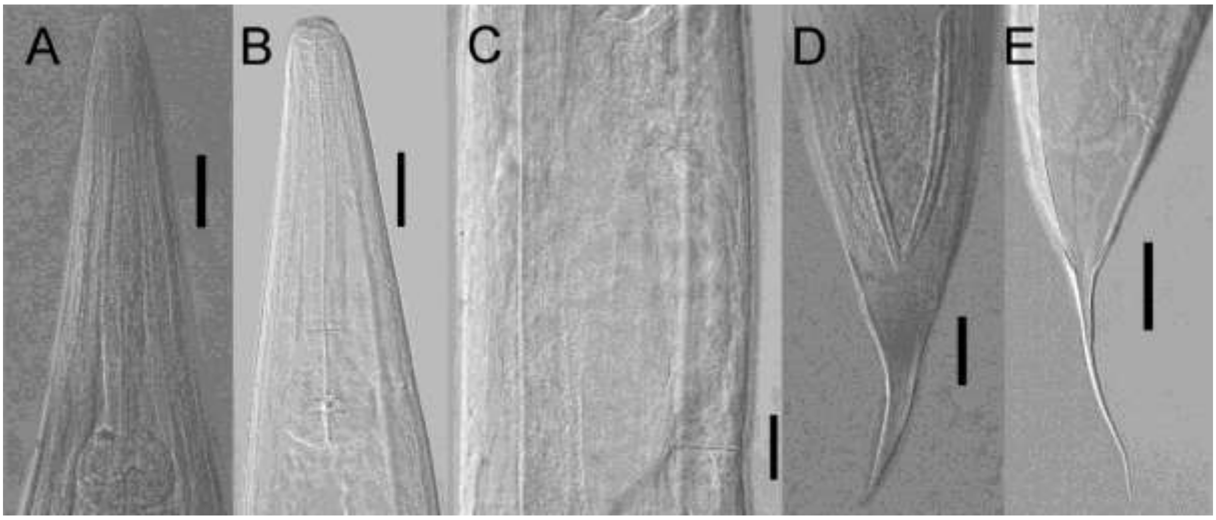

Figure 5. *Aplectana* sp. 1, photomicrographs. A – anterior end of body, female, ventral view; B – anterior end of body, male, lateral view; C – fragment of body at vulva region, lateral view; D – posterior end of body, male, ventral view; E – posterior end of body, female, lateral view. Scale bars: 100µm.

Remarks.

The species belongs to the genus *Aplectana* due to lacking rosettes or plectanes on posterior end of males and the uteri and ovaries are anterior to the vulva in females. *Aplectana* sp. 1 closely resembled *A. macintoshii* in having numerous unpaired papillae at the pre- and post-cloacal regions. However, *Aplectana sp.* 1 differed from *A. macintoshii* in having five paired papillae at the cloacal region, which is not found in *A. macintoshii*. *Aplectana* sp. 1. differed from *A. degraaffi*, *A. capensis* and *A. chamaeleonis* in having numerous unpaired papillae in the pre- and post-cloacal regions as well as having a small unpaired papillae anterior to the cloacal lip, whereas in

A. degraaffi, *A. capensis* and *A. chamaeleonis* the unpaired papillae found anterior to the lip is comparatively much larger in size. Based on the morphological and molecular differences, we believe that *Aplectana* sp. 1 is representing a new species. Unfortunately, only two male specimens were found, of which one was used for DNA extraction. After obtaining more specimens, the description will be amended and submitted for publication.

Aplectana sp. 2

General. Small, slim nematodes. Males smaller than females. Triangular mouth opening with three lips. Lips surrounded by four papillae and two amphids. Males lacking rosettes or plectanes, females having both ovaries and uteri anterior to the vulva. Oesophagus divided into three parts: short pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to the nerve ring and terminating at cloacal level.

Female. Measurements based on 15 females. Body 2.4–4.5 (3.6) mm long, 129–324 (231) wide at mid-body level. Lateral alae 114–241 (175) from apex. Oesophageal pharynx 23–62 (44) long and 41–76 (55) wide; corpus 372–500 (428) long and 40–69 (54) wide; oesophageal bulb 95–136 (117) long and 91–157 (128) wide. Nerve ring at 151–258 (190) and excretory pore at 339–499 (425) from apex (Fig. 6A, 7A). Vulva (Fig. 7B) at 1.2–2.3 (1.9) mm from anterior end of body, small, transversely slit, lips poorly developed. Vulva situated at around mid-body level, spanning about 53% of body length. Tail (Fig. 6C) 291–510 (401) long, with an elongated end, slightly rounded bluntly on tip.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925

Subfamily: Cosmocercinae Railliet, 1916

Genus: *Aplectana* Railliet and Henry, 1916

Species: *Aplectana* sp. 2

Host: *Schismaderma carens* (Amphibia: Anura: Bufonidae).

Locality: Vivo, Limpopo Province, South Africa: Coordinates: S 23˚14’40” E 29˚26’54”

Site in host: Large intestine.

Infection parameters: Intensity: 14– 72 (33); Prevalence: 66.7% (2 of 3 were infected); Mean abundance: 22

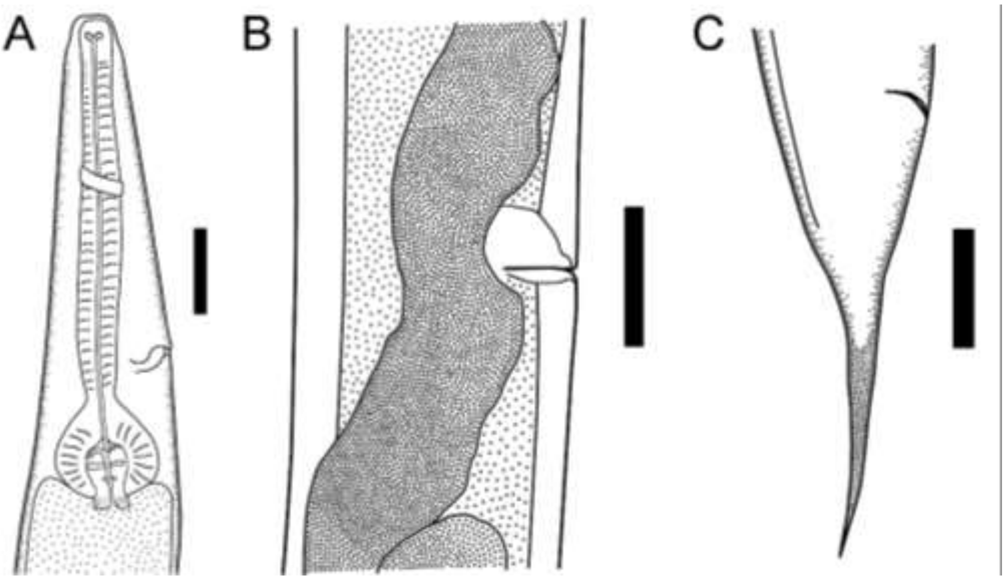

Figure 6. *Aplectana* sp. 2, line drawings. A – anterior end of body, female lateral view; B – fragment of body at vulva region, lateral view; C – posterior end of body, female, ventro-lateral view. Scale bars: 100µm.

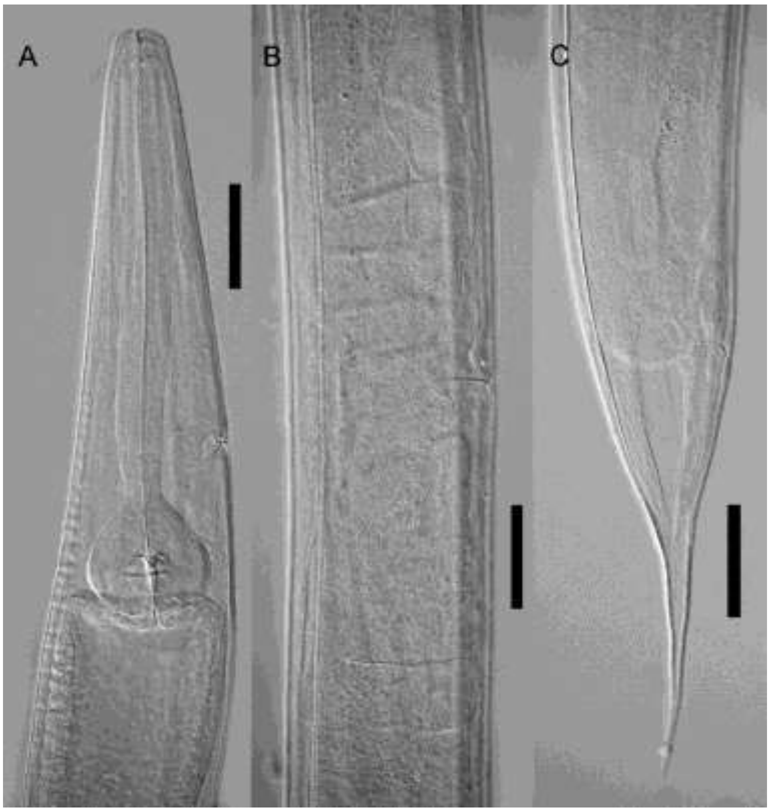

Figure 7. *Aplectana* sp. 2, photomicrographs A – anterior end of body, lateral ventral view; B – fragment of body at vulva region, lateral view; C – posterior end of body, female, ventro-lateral view. Scale bars: 100µm.

Remarks.

Based on molecular data and morphology of females having both uteri and ovaries anterior to the vulva, the species was assigned to the genus *Aplectana*. Unfortunately, there were no male specimens found in the sample for morphological comparison, although molecular data clearly shows its distinctness from other *Aplectana* spp. found in present study.

Aplectana cf. _capensis_ sp. 1

General. Small, slim nematodes. Males smaller than females. Triangular mouth opening with three lips. Lips surrounded by four papillae and two amphids. Males lacking rosettes or plectanes, females having both ovaries and uteri anterior to the vulva. Oesophagus divided into three parts: short pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to the nerve ring and terminating at cloacal level.

Male. Measurement based on one male. Body 2.9 mm long, 262 wide at mid-body level. Lateral alae beginning at 155 from apex. Oesophageal pharynx 28 long and 30 wide; corpus 345 long and 30 wide; oesophageal bulb 81 long and 67 wide. Nerve ring at 96 and excretory pore at 401 from apex (Fig. 8B, 9B). Tail 240 long. Gubernaculum triangular, visible without dissection of tail (Fig. 8C) 44 long. Spicules (Fig. 8C) equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 221 long, right one 275 long. Somatic papillae and cloaca papillae numerous (Fig. 8C) indistinguishable from each other; one large unpaired papillae observed anterior to cloaca.

Female. Measurements based on four females. Body 5.8–6.9 (6.5) mm long, 436–552 (479) wide at mid-body level. Lateral alae 186–206 (198) from apex. Oesophageal pharynx 43–57 (48) long and 54–68 (61) wide; corpus 511–543 (523) long and 63–80 (70) wide; oesophageal bulb 134–155 (144) long and 157–197 (168) wide. Nerve ring at 177–287 (238) and excretory pore at 571–600 (588) from apex (Fig. 8A). Vulva (Fig. 9C) at 3.3–3.9 (3.5) mm from anterior end of body, small, transversely slit, lips poorly developed. Vulva located at around mid-body level (about 54% of body length). Tail (Fig. 8D) 517–648 (585) long, with elongated end, slightly rounded bluntly on the tip (Fig. 9D).

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Aplectana* Railliet and Henry, 1916.

Species: *Aplectana* cf. *capensis* sp. 1.

Host: *Sclerophrys garmani* (Amphibia: Anura: Bufonidae).

Locality: Potchefstroom, North-West Province, South Africa. Coordinates: S 26˚45'50" E 27˚03'56".

Site in host: Large intestine.

Infection parameters: Intensity: 21– 39 (30); Prevalence: 66.7% (2 of 3 were infected); Mean abundance: 20

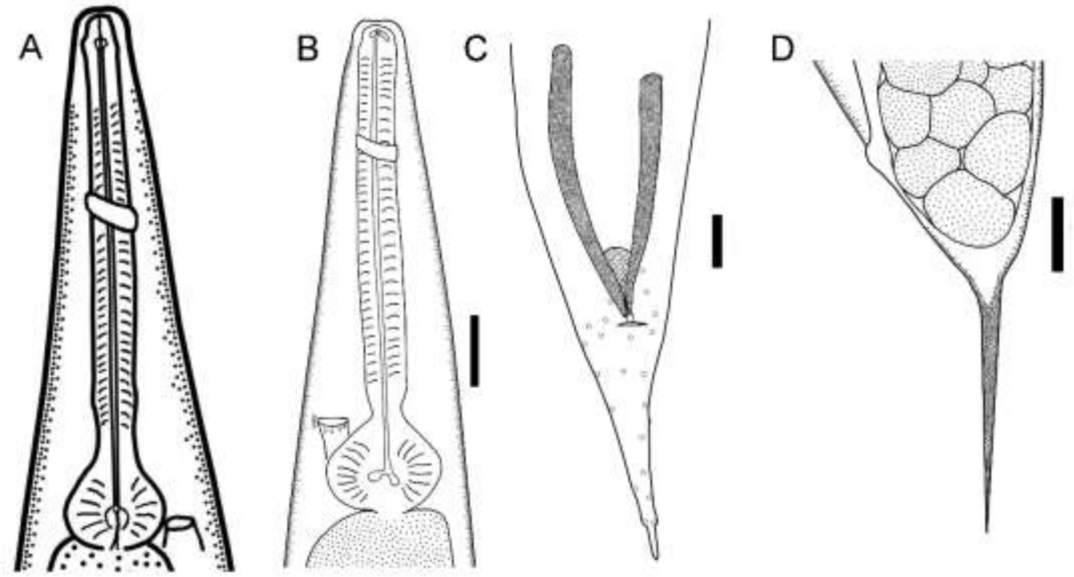

Figure 8. *Aplectana* cf. *capensis* sp. 1, line drawings. A – anterior end of body, female ventro-lateral view; B – anterior end of body, male, ventro-lateral view; C – posterior end of body, male, ventral view; D – posterior end of body, female, lateral view. Scale bars: 100µm.

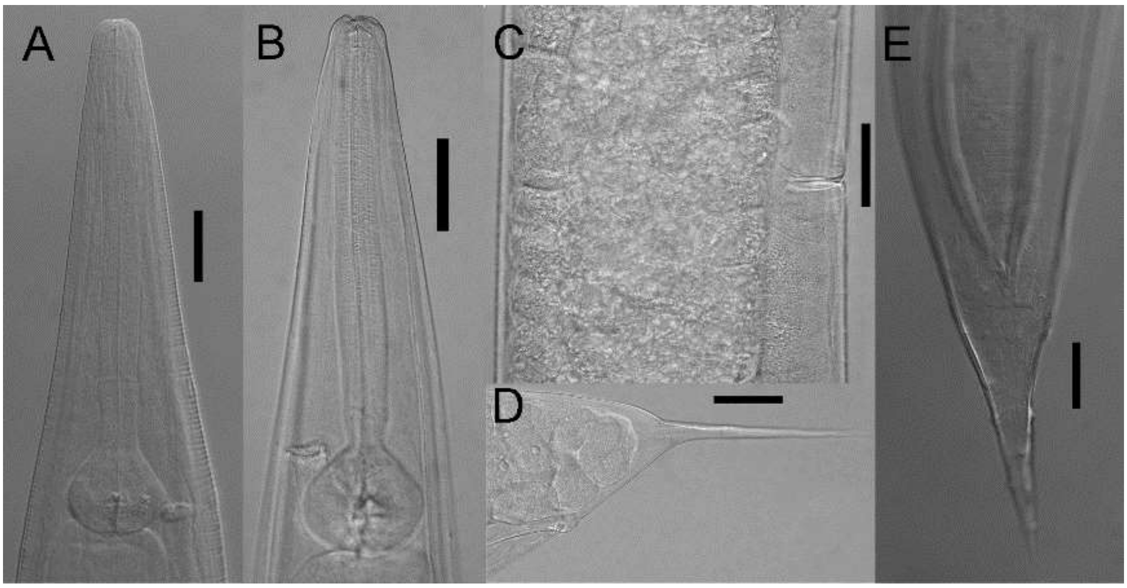

Figure 9. *Aplectana* cf. *capensis* sp. 1, photomicrographs. A – anterior end of body, female, ventro-lateral view; B – anterior end of body, male, ventro-lateral view; C – fragment of body at vulva region, lateral view; D – posterior end of body, female, lateral view; E – posterior end of body, male, ventral view. Scale bars: 100µm.

Remarks.

Aplectana cf. *capensis* sp. 1 resembles *A. capensis* in having a sclerotized bulb cap at the capitulum of the spicules, numerous somatic and caudal papillae and one large unpaired papillae anterior to the cloaca. However, males of *A.* cf. *capensis* sp. 1 possess a short tail tip which is not found in *A. capensis*. Moreover, the species is clearly differed from *Aplectana* cf. *capensis* sp. 2 (please see below) in 18S and ITS-28S sequences. Due to absence of sequences of *A. capensis* from its type host (*Breviceps rosei*) and type locality (Cape Town) it is not possible to either assign present specimens to *A. capensis* or to describe as a new species.

***Aplectana* cf. *capensis* sp. 2**

General. Small, slim nematodes. Males smaller than females. Triangular mouth opening with three lips. Lips surrounded by four papillae and two amphids. Males lacking rosettes or plectanes, with females having both ovaries and uteri anterior to the vulva. Oesophagus divided into three parts: short pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to the nerve ring and terminating at cloacal level.

Male. Measurements based on three males. Body 2.2–2.4 (2.3) mm long, 160–201 (180) wide at mid-body level. Lateral alae beginning at 85–129 (112) from apex. Oesophageal pharynx 20–34 (29) long and 26–28 (27) wide; corpus 264–322 (291) long and 26–35 (29) wide; oesophageal bulb 26–35 (29) long and 68–79 (73) wide. Nerve ring at 94–145 (122) and excretory pore at 319–348 (333) from apex (Fig. 10B). Tail (Fig. 10C) 160–224 (188) long. Gubernaculum triangular, visible without dissection of tail (Fig. 10C) 40–70 (51) long. Spicules (Fig. 10C) equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 186–249 (209) long, right one 171–262 (204) long (Fig. 11D). Somatic papillae and cloaca papillae numerous (Fig. 10C) indistinguishable from each other; one large unpaired papillae observed anterior to cloaca.

Female. Measurements based on 11 females. Body (Fig. 10A) 3.5–7.1 (5.7) mm long, 188–420 (328) wide at mid-body level. Lateral alae 135–235 (168) from apex. Oesophageal pharynx 34–52 (43) long and 39–60 (54) wide; corpus 360–461 (414) long and 48–68 (57) wide; oesophageal bulb 110–134 (123) long and 105–149 (126) wide. Nerve ring at 150–237 (196) and excretory pore at 411–523 (472) from apex. Vulva (Fig. 11C) at 1.9–3.8 (2.9) mm from anterior end of body, small, transversely slit, lips poorly developed. Vulva located around mid-body level (about 51% of body length). Tail (Fig. 10D) 324–478 (425) long, with elongated end, slightly rounded bluntly on tip.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Aplectana* Railliet and Henry, 1916.

Species: *Aplectana* cf. *capensis* sp. 2.

Host: *Amietia delalandii* (Amphibia: Anura: Pyxicephalida).

Other Hosts: *S. garmani*, *S. gutturalis* and *Ptychadena anchietae.*

Locality: Makhado, Limpopo Province, South Africa: Coordinates: S 26˚45'50" E 27˚03'56".

Site in host: Large intestine.

Infection parameters: Intensity: 1– 29 (5.3); Prevalence: 20% (8 of 40 were infected); Abundance: 1.4.

Infection parameters for all hosts: Intensity: 1– 79 (11.8); Prevalence: 36.5% (23 of 63 were infected); Mean abundance: 4.5.

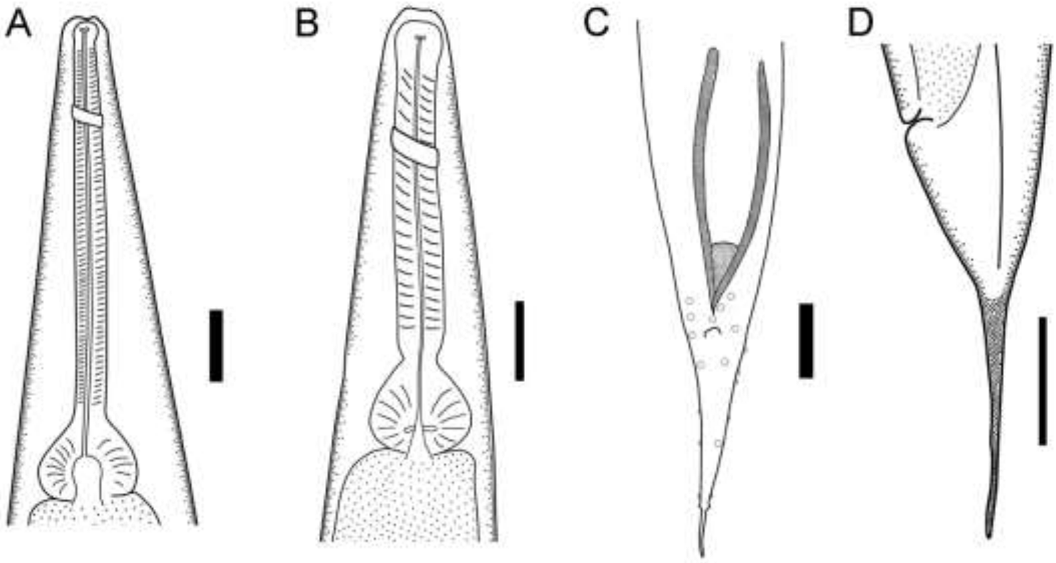

Figure 10. *Aplectana* cf. *capensis* sp. 2, line drawings. A – anterior end of body, female ventral view; B – anterior end of body, male, ventral view; C – posterior end of body, male, ventral view; D – posterior end of body, female, lateral view. Scale bars: 100µm.

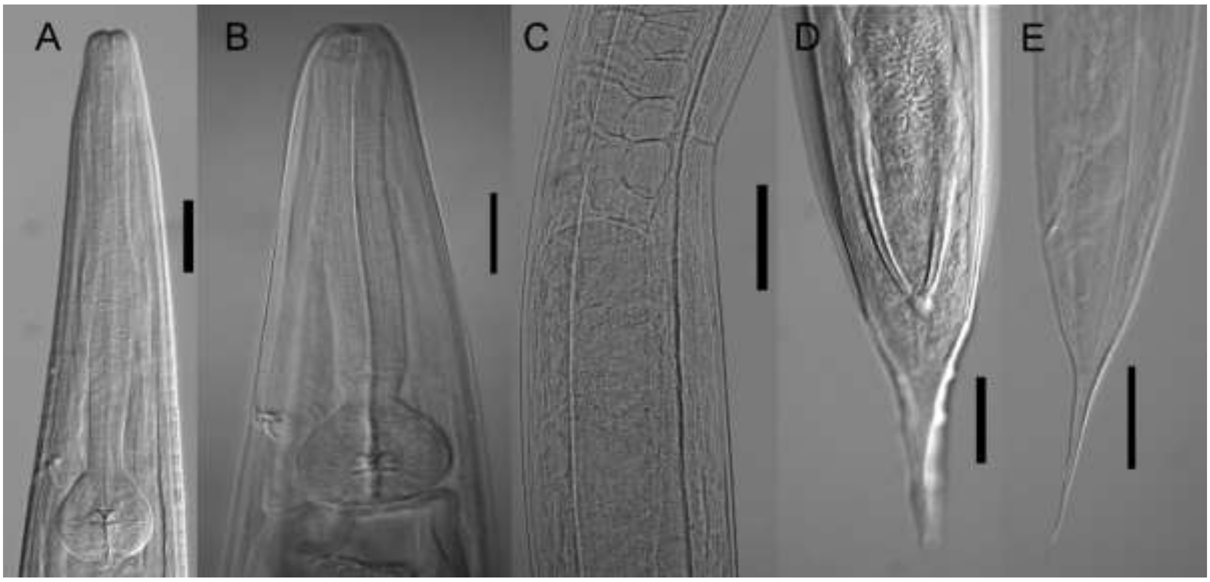

Figure 11. *Aplectana* cf. *capensis* sp. 2, photomicrographs. A – anterior end of body, female, ventro-lateral view; B – anterior end of body, male, lateral view; C – fragment of body at vulva region, lateral view; D – posterior end of body, male, ventral view; E – posterior end of body, female, lateral view. Scale bars: 100µm.

Remarks.

Aplectana cf. *capensis* sp. 2 resembles *A. capensis* in having a sclerotized bulb cap at the capitulum of the spicules, numerous somatic and caudal papillae and one large unpaired papillae anterior to the cloaca. However, *A.* cf. *capensis* sp. 2 differed from *A. capensis* due to the presence of papillae at the posterior end of the tail whereas *A. capensis* has no papillae at the posterior tail end. Similar to previous species, based on molecular and morphological data, the species cannot be assigned to *A. capensis* nor described as a new species.

***Aplectana* cf. *macintoshii* sp. 1**

General. Small, slim nematodes. Males smaller than females. Triangular mouth opening with three lips. Lips surrounded by four papillae and two amphids. Males lacking rosettes or plectanes, females having both ovaries and uteri anterior to the vulva. Oesophagus divided into three parts: short pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to the nerve ring and terminating at cloacal level.

Male. Measurements based on six specimens. Body 1.2–1.7 (1.6) mm long, 106–169 (143) wide at mid-body level. Lateral alae beginning at 47–143 (96) from apex. Oesophageal pharynx 21–30 (25) long and 20–28 (23) wide; corpus 239–289 (270) long and 26–32 (29) wide; oesophageal bulb 51–70 (65) long and 60–85 (76) wide. Nerve ring at 84–124 (102) and excretory pore at 221–303 (261) from apex. Tail (Fig. 13E) 145–296 (207) long (Fig. 13B). Gubernaculum oval with narrowing end, only visible after dissection of tail (Fig. 12D) 33–81 (50) long. Spicules (Fig. 12E) equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 166–236 (204) long, right one 187–224 (203) long. Numerous unpaired papillae present at posterior end.

Female. Measurements based on nine nematodes. Body (Fig. 12A) 2.6–3.8 (3.2) mm long, 197–289 (234) wide at mid-body level. Lateral alae 45–124 (97) from apex. Oesophageal pharynx 27–37 (32) long and 32–47 (40) wide; corpus 333–406 (364) long and 38–52 (46) wide; oesophageal bulb 90–110 (102) long and 102–125 (118) wide. Nerve ring at 104–209 (163) and excretory pore at 318–407 (357) from apex. Vulva (Fig. 12B) at 1.5–2.5 (1.8) mm from anterior end of body, small, transversely slit, lips poorly developed. Vulva located at around mid-body level (about 57% of body length). Tail (Fig. 12C) 278–389 (330) long, with an elongated end slightly rounded bluntly on tip.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Aplectana* Railliet and Henry, 1916.

Species: *Aplectana* cf. *macintoshii* sp. 1.

Host: *Sclerophrys gutturalis* (Amphibia: Anura: Bufonidae).

Locality: Bushbaby game reserve, Kwa-Zulu Natal Province, South Africa: Coordinates: S 28˚04’56” E 32˚17’32”.

Site in host: Large intestine.

Infection parameters: Intensity: 1– 24 (14); Prevalence: 100%; Mean abundance: 8.

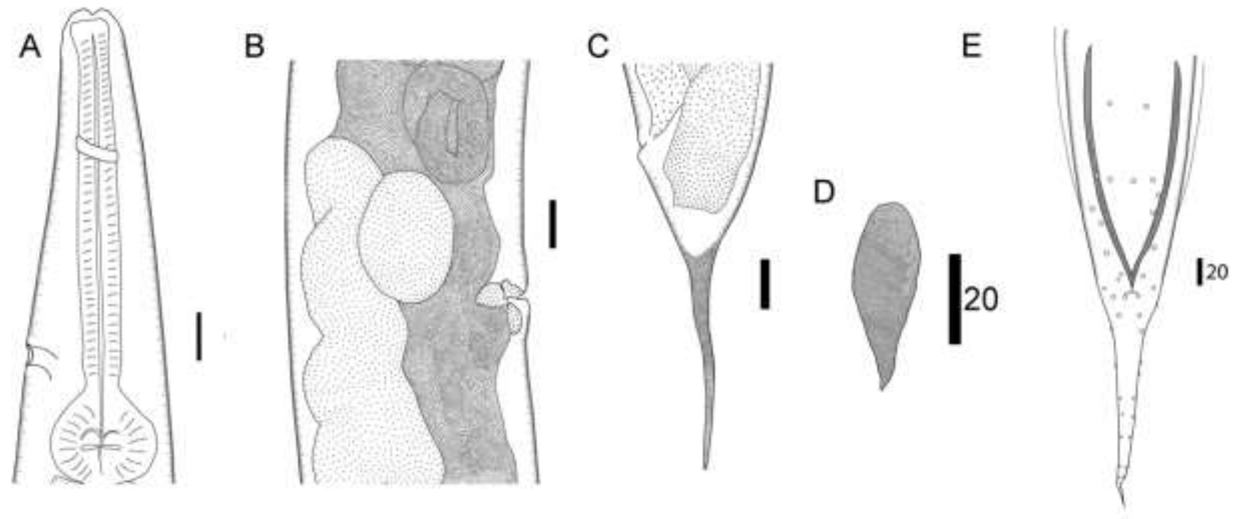

Figure 12. *Aplectana* cf. *macintoshii* sp. 1, line drawings. A – anterior end of body, female lateral view; B – fragment of body, female, lateral view; C – posterior end of body, female, lateral view; D – gubernaculum, ventral view; E – posterior end of body, male, ventral view. Scale bars: A–C, E – 100µm, D - 20µm.

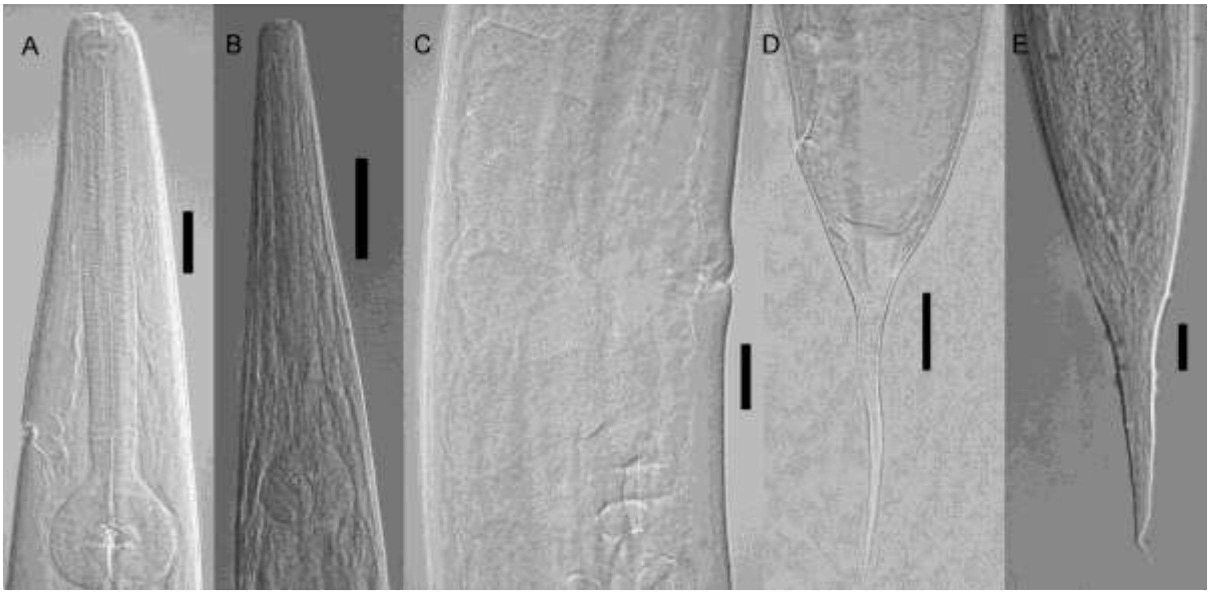

Figure 13. *Aplectana* cf. *macintoshii* sp. 1, photomicrographs. A – anterior end of body, male, lateral view; B – anterior end of body, female, lateral view; C – fragment of body at vulva region, lateral view; D – posterior end of body, female, lateral view; E – posterior end of body, male, ventral view. Scale bars: 100µm.

Remarks.

Aplectana cf. *macintoshii* sp. 1 closely resembles the species *A. macintoshii* in having numerous somatic papillae. The cloacal papillae are numerous and unpaired, one unpaired papilla found anterior to the cloaca indistinguishable from somatic papillae. Gubernaculum weakly sclerotized. The only slight difference found, was a short tail tip, not found in the description of *A. macintoshii*. Unfortunately, due to absence of the molecular data of *A. macintoshii* from the type host

(*Hoplobatrachus tigerinus* (Daudin, 1803)) and type locality (India) we can neither assign our specimens to that species, nor describe them as a new species.

***Aplectana* cf. *macintoshii* sp. 2**

General. Small, slim nematodes. Males smaller than females. Triangular mouth opening with three lips. Lips surrounded by four papillae and two amphids. Males lacking rosettes or plectanes, females having both ovaries and uteri anterior to the vulva. Oesophagus divided into three parts: short pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to the nerve ring and terminating at cloacal level.

Male. Measurements based on two males. Body 1.7 (1.6) mm long, 189 (146) wide at mid-body level. Lateral alae beginning at 95 (163) from apex. Oesophageal pharynx 29 (22) long and 28 (27) wide; corpus 284 (281) long and 31 (27) wide; oesophageal bulb 62 (56) long and 63 (62) wide. Nerve ring at 115 (129) and excretory pore at 325 (299) from apex (Fig. 14A). Tail (Fig. 14E) 187 (228) long. Gubernaculum triangular, visible without dissection of tail (Fig. 14E) 45 (43) long. Spicules equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 183 (170) long, right one 196 (186) long.

Female. Measurements based on 11 females. Body 2.1–6.0 (4.1) mm long, 156–396 (291) wide at mid-body level. Lateral alae 75–132 (105) from apex. Oesophageal pharynx 33–50 (42) long and 38–56 (50) wide; corpus 335–465 (391) long and 39–58 (50) wide; oesophageal bulb 82–131 (107) long and 94–139 (117) wide. Nerve ring at 140–230 (189) and excretory pore at 308–543 (433) from apex (Fig. 14B). Vulva (Fig. 15C) at 1.0–2.9 (2.1) mm from anterior end of body, small, transversely slit, lips poorly developed. Vulva located at around mid-body level (about 50% of body length). Tail (Fig. 14D) 309–593 (442) long, slightly rounded bluntly with an elongated end.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Aplectana* Railliet and Henry, 1916.

Species: *Aplectana* cf*. macintoshii* sp. 2.

Host: *Ptychadena mossambica* Peters, 1854 (Amphibia: Anura: Ptychadenidae).

Locality: Sodwana, Kwa-Zulu Natal Province, South Africa: Coordinates: S 27˚29’19” E 32˚39’50”.

Site in host: Large intestine.

Infection parameters: Intensity: 5 – 23 (11); Prevalence: 80% (4 of 5 were infected); Mean abundance: 8.8.

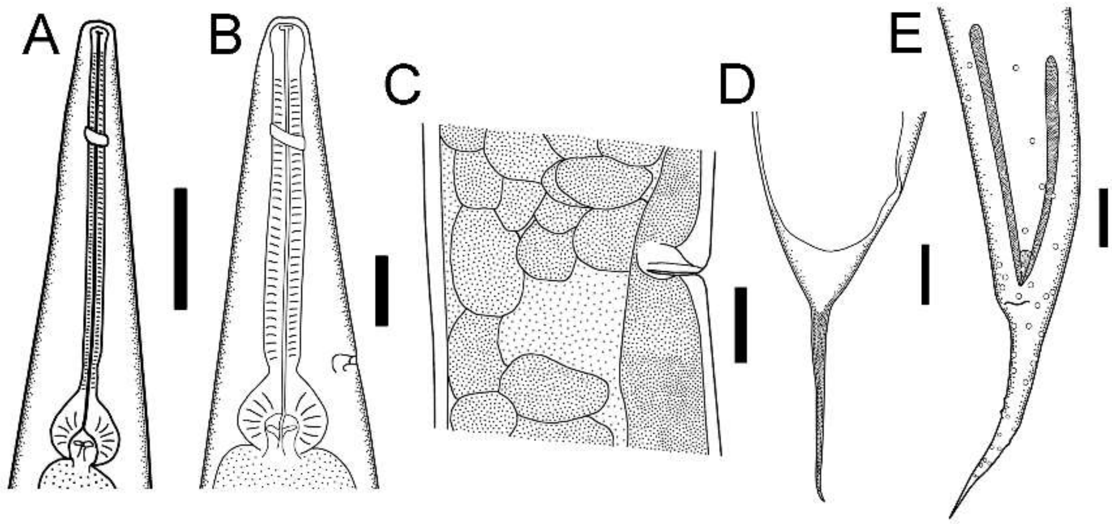

Figure 14. *Aplectana* cf. *macintoshii* sp. 2, line drawings. A – anterior end of body, male ventral view; B – anterior end of body, female, ventral view; C – fragment of body at vulva region, female, lateral view; D – posterior end of body, female, lateral view; E – posterior end of body, male, ventral view. Scale bars: 100µm.

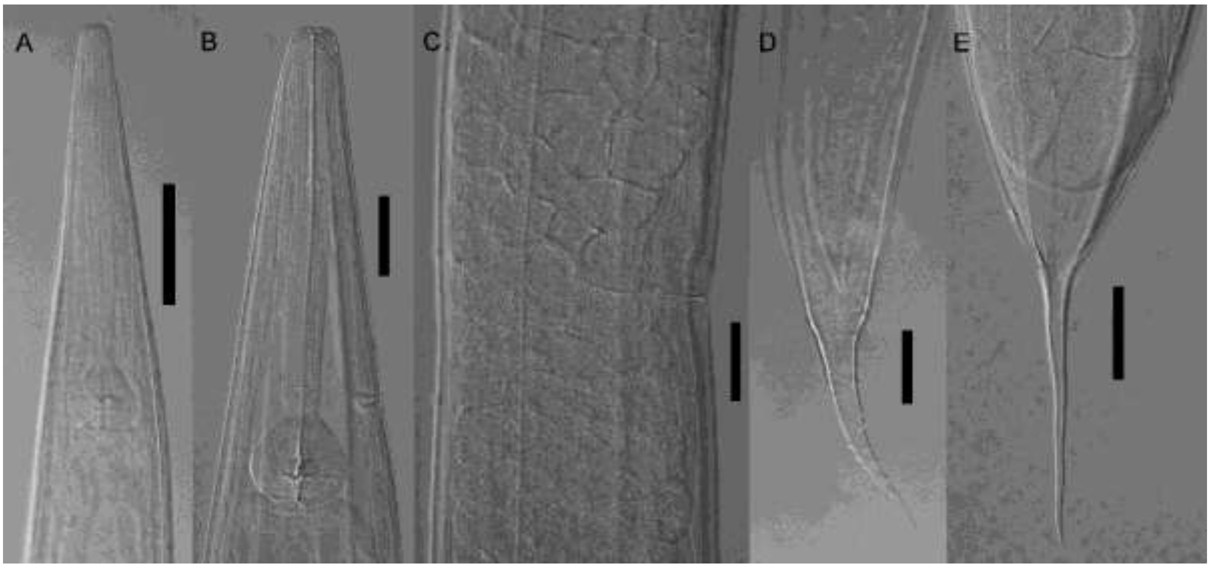

Figure 15. *Aplectana* cf. *macintoshii* sp. 2, photomicrographs. A – anterior end of body, male, ventral view; B – anterior end of body, female, lateral view; C – fragment of body at vulva region, lateral view; D – posterior end of body, male, ventral view; E – posterior end of body, female, lateral view. Scale bars: 100µm.

Remarks.

Aplectana cf. *macintoshii* sp. 2 also closely resembles the species *A. macintoshii* in having numerous somatic papillae. The cloacal papillae are numerous and unpaired, one unpaired papilla found anterior to the cloaca indistinguishable from somatic papillae. Gubernaculum weakly sclerotized. As with the previous *Aplectana* cf. *macintoshii* sp. 1, the present specimens are morphologically almost indistinguishable from *A. macintoshi*, however, molecular data clearly indicates that the two species are different and neither of them can be assigned to *A. macintoshii*.

***Cosmocerca* sp. 1**

General. Body small, stout, attenuated anteriorly. Mouth triangular with three lips, dorsal lip bearing two prominent cephalic papillae, two ventro-lateral lips each bearing one cephalic papilla and an amphid, with eight body papillae surrounding the cephalus. Three lips opening into oesophagus. Oesophagus divided into three parts: pharynx, cylindrical corpus and oesophageal bulb. Nerve ring encircling oesophagus at level of its mid-length. Excretory pore anterior to beginning of oesophageal bulb. Lateral alae beginning anterior to level of nerve ring and terminating anterior to cloaca. Tail rounded, evenly narrowing with short process on tip; straight in females and curved ventrally in males.

Male. Measurements of five specimens. Body (Fig. 16B) 1.0–2.7 (1.6) mm long, 72–198 (93) wide at mid-body level. Lateral alae beginning at 29–37 (33.5) from apex. Oesophageal pharynx 11–31 (19) long and 13–21 (15) wide; corpus 198–379 (251) long and 20–36 (24) wide; oesophageal bulb 43–81 (56) long and 42–77 (51) wide. Nerve ring at 104–159 (111) and excretory pore at 220–387 (288) from apex. Tail 121–146 (132) long, bearing short process at tip 11–22 (18) long. Gubernaculum (Fig. 16D) 60–89 (73) long, V-shaped, with well sclerotised edges, bearing hook-like structures on its margins. Spicules equal in shape, evenly narrowing with sharpened tips, poorly sclerotised, observed only when dissected. Left spicule 61–98 (77) long, right one 61–102 (80) long. Fourteen pairs of post-cloacal papillae (Fig. 16F) and five pairs of plectanes (Fig. 16C, G) observed in tail region.

Female. Measurements based on 30 specimens. Body (Fig. 16H) 2–6 (3.7) mm long, 74–323 (225) wide at mid-body level. Lateral alae (Fig. 16E) 42–188 (84) from apex. Oesophageal pharynx 17–42 (27) long and 20–41 (31) wide; corpus 240–433 (296) long and 27–47 (34) wide; oesophageal bulb 70–120 (82) long and 62–128 (78) wide. Nerve ring at 117–214 (171) and excretory pore at 206–539 (371) from apex. Vulva at 0.6–2.9 (1.8) mm from anterior end of body, small, transversely slit, lips poorly developed, located around mid-body level (about 46% of body length). Tail 138–408 (289) long, slightly rounded bluntly with an elongated end.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Cosmocerca* Diesing, 1861.

Species: *Cosmocerca* sp. 1.

Host: Boettger's dainty frog *Cacosternum boettgeri* (Amphibia: Anura: Microhylidae).

Locality: Daly farm, Potchefstroom, North-West Province, South Africa: Coordinates: S 26˚39'1.2" E 27˚03'45".

Site in host: Large intestine.

Infection parameters: Intensity: 1– 24 (7.9); Prevalence: 27% (16 of 59 were infected); Mean abundance: 2.2.

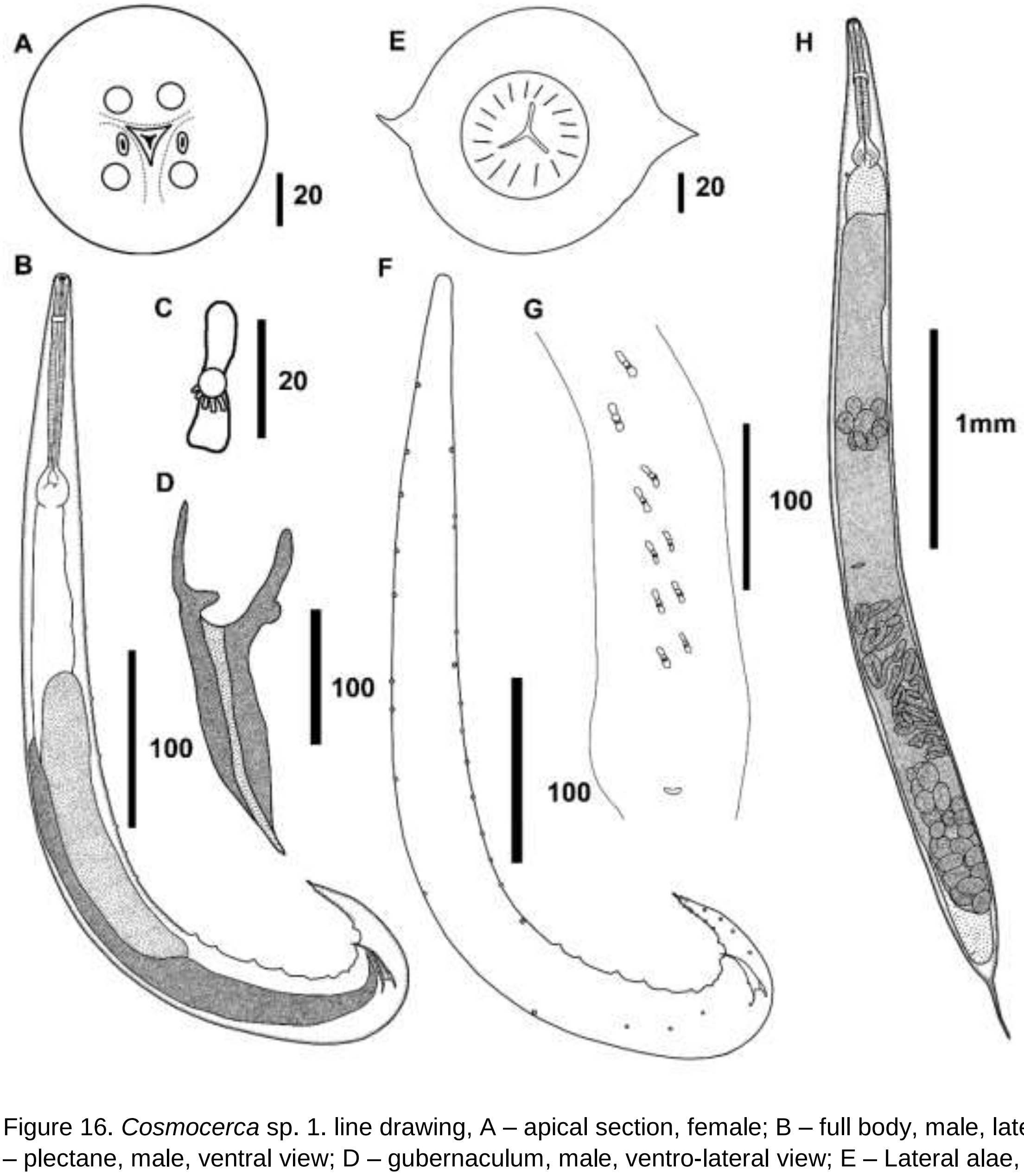

Figure 16. *Cosmocerca* sp. 1. line drawing, A – apical section, female; B – full body, male, lateral view; C – plectane, male, ventral view; D – gubernaculum, male, ventro-lateral view; E – Lateral alae, female; F somatic papillae, male, lateral view; G – fragment of posterior end, male, ventral view; H. full body, female, lateral view.

Remarks.

This species belongs to the genus *Cosmocerca* due to the presence of plectanes, numerous papillae along the body and sexual dimorphism with males being half the size of females (Baker, 1980).

Hitherto, 30 species of the genus *Cosmocerca* are considered valid of which *C. ornata* is the only species recorded in Southern Africa. *Cosmocerca* sp. 1 evidently differed from *C. ornata* in the shape of the gubernaculum, having prominent hook-like structures on its margins in contrast to the simple Y shaped gubernaculum in *C. ornata*. Moreover, mature females of *Cosmocerca* sp. 1 have excretory pore located posterior to the level of oesophageal bulb whereas it is described as being anterior to the level of oesophageal bulb in *C. ornata*.

Cosmocerca. sp. 1 (Fig. 19 A-C) differs from *Cosmocerca* sp. 2 (Fig. 19 D-F) in the shape of the gubernaculum: *Cosmocerca* sp. 1 (Fig. 16 D) has V-shaped gubernaculum with hook-like structures on margins while *Cosmocerca* sp. 2 (Fig. 17 E) has Y-shaped gubernaculum without hook-like structures. *Cosmocerca* sp. 1 differed from *Cosmocerca* sp. 2 by having 14 pairs of post-cloacal papillae whereas *Cosmocerca* sp. 2 has 13 pairs. Additionally, mature females of *Cosmocerca* sp. 1 have an excretory pore posterior to the level of the oesophageal bulb whereas it is depicted as being anterior to the level of the oesophageal bulb in *Cosmocerca* sp. 2.

Cosmocerca sp. 1 differs from *Cosmocerca* sp. 3 (Fig. 18 E) in the shape of the gubernaculum: *Cosmocerca* sp. 1 (Fig. 16 D) has a V-shaped gubernaculum with hook-like structures on margins whereas *Cosmocerca* sp. 3 (Fig. 18 E) has a club-shaped gubernaculum without hook-like structures. Additionally, *Cosmocerca* sp. 1 possesses somatic papillae located only on the lateral sides of the body while somatic papillae in *Cosmocerca* sp. 3 are found evenly spread on entire body, including dorsal and ventral sides. Moreover, plectanes *of Cosmocerca* sp. 1 are clearly visible in lateral and ventral views whilst plectanes of *Cosmocerca* sp. 3 having almost transparent edges and almost inconspicuous in the ventral view. Mature females of *Cosmocerca* sp. 1 have excretory pore located posterior to the level of the oesophageal bulb whereas it is depicted anterior to the level of the oesophageal bulb in *Cosmocerca* sp.3.

The description of this new species is currently under review with *Parasitology Research*.

***Cosmocerca* sp. 2**

General. Body small, stout, attenuated anteriorly. Mouth triangular with three lips, dorsal lip bearing two prominent cephalic papillae, two ventro-lateral lips each bearing one cephalic papilla and an amphid, with eight body papillae surrounding the cephalus. Three lips opening into oesophagus. Oesophagus divided into three parts: pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to nerve ring and terminating anterior to cloaca. Nerve ring encircles oesophagus at mid-length. Excretory pore anterior to oesophageal bulb. Tail rounded with straight, elongated end in females and curved ventrally in males, evenly narrowing with a short process on the tip.

Male. Measurements based on four specimens. Body (Fig. 17A) 1.77–2.13 (1.97) mm long, 177–203 (190) wide at mid-body level. Lateral alae beginning at 122–216 (182) from apex. Oesophageal pharynx 12–20 (15) long and 16–22 (19) wide; corpus 267–326 (299) long and 27–42 (34) wide; and oesophageal bulb 61–76 (67) long and 70–84 (78) wide. Nerve ring at 91–239 (155) and excretory pore at 328–369 (354) from apex. Tail bluntly rounded, 130–162 (149) long, bearing a short process at the tip 9–15 (12) long. Gubernaculum (Fig. 17E) Y-shaped with well sclerotised edges, 100–108 (102) long. Spicules equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 87–96 (92) long, right one 92–107 (97) long. Fifteen post-cloacal papillae (Fig. 17B) located in tail region. Five pairs of plectanes (Fig. 17F, G) located anterior to cloaca, each bearing five to six tubercles directed posteriorly. In total about 146 papillae observed on the entire body.

Female. Measurements based on 14 specimens. Body (Fig. 17H) 2.24–4.24 (3.31) mm long, 154–332 (250) wide at mid-body level. Lateral alae (Fig. 17D) 138–293 (212) from apex. Oesophageal pharynx 25–35 (30) long and 38–67 (57) wide; cylindrical corpus 368–469 (410) long and 35–68 (51) wide and oesophageal bulb 81–124 (103) long and 86–146 (125) wide. Nerve ring at 110–184 (155) and excretory pore at 352–477 (410) from apex. Vulva at 1.22–2.07 mm from anterior end of body (about 55 % of body length), small, transversely slit with poorly developed lips. Tail 308–455 (371) long, rounded, with long point.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Cosmocerca* Diesing, 1861.

Species: *Cosmocerca* sp. 2.

Host: Senegal running frog *Kassina senegalensis* Dumeril and Bibron, 1841 (Amphibia: Anura: Hyperoliidae).

Localities: Daly farm, Potchefstroom, North-West Province, South Africa, Coordinates: S 26˚39'1.2" E 27˚03'45".

Louis Trichardt, Limpopo Province, South Africa, Coordinates: N 23˚06'17" E 29˚54'32", Makhado, Limpopo Province, South Africa, Coordinates: S 23˚06'36" E 29˚54'30".

Site in host: Large intestine.

Infection parameters: Intensity: 4 – 33 (13.9); Prevalence: 100%; Mean abundance: 13.9

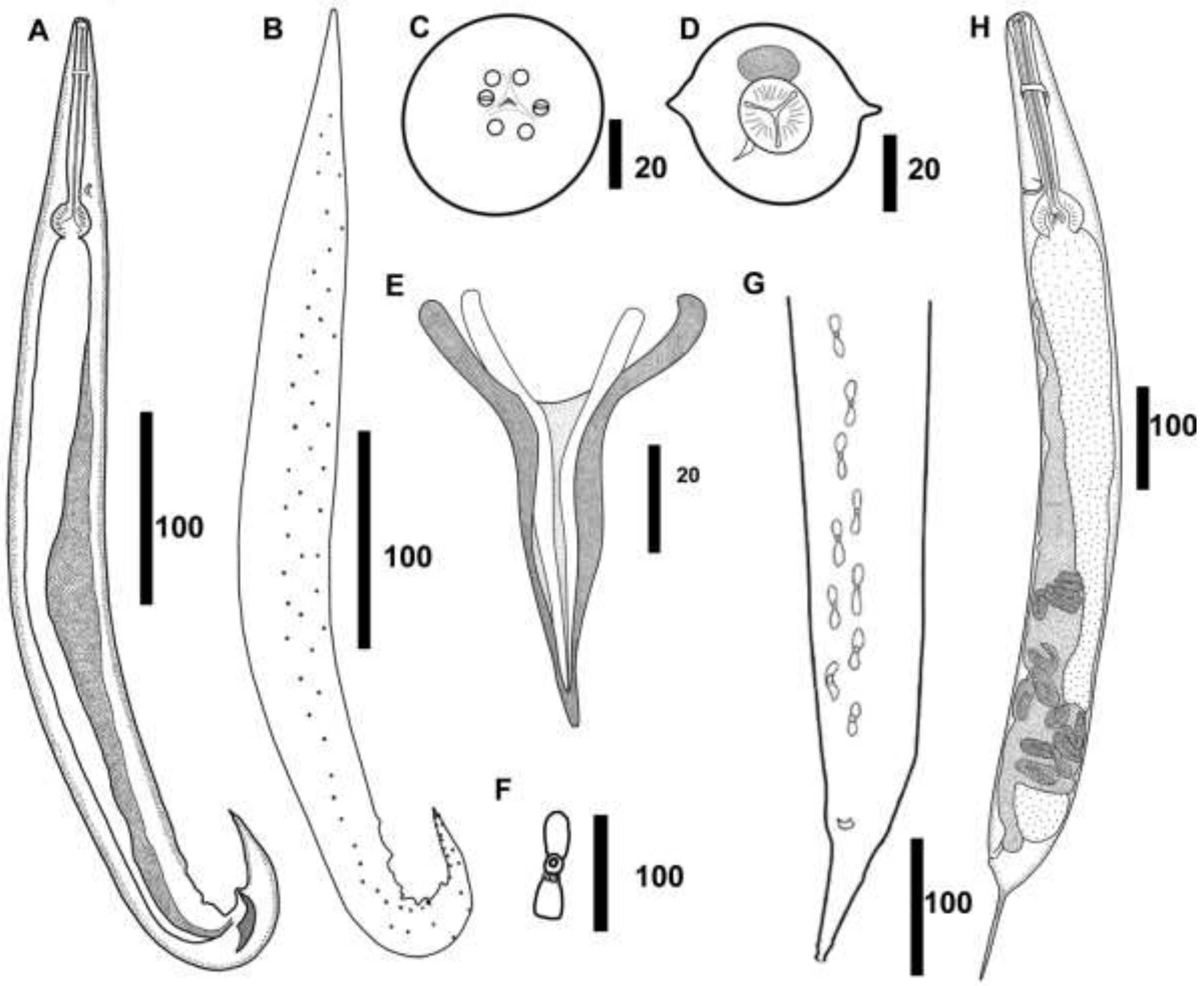

Figure 17. *Cosmocerca* sp. 2. line drawing, A – full body, male, lateral view; B – somatic papillae, male, lateral view; C – apical section, female; D – lateral alae, female; E – gubernaculum, male, ventral view; F – plectane, male, ventral view G – fragment of posterior end, male, ventral view; H. full body, female, lateral view.

Remarks.

The new species belong to the genus *Cosmocerca* due to the presence of plectanes, numerous papillae along the body and displays sexual dimorphism with males half the size of females (Baker, 1980).

Cosmocerca sp. 2 differs from *C. ornata* in having 13 pairs of post-cloacal papillae whereas the maximum number reported on *C. ornata* is 14 pairs. The shape of the gubernaculum in *Cosmocerca* sp. 2 is different to that of *Cosmocerca* sp. 1: *Cosmocerca* sp. 2 (Fig. 19 D-F) having a Y-shaped gubernaculum while *Cosmocerca* sp. 1 (Fig. 16 D) has a V-shaped gubernaculum with hook-like structures on the margins. *Cosmocerca* sp. 2 has 13 pairs of post-cloacal papillae whereas *Cosmocerca* sp. 1 has 14 pairs. Adult females of *Cosmocerca* sp. 2 have excretory pore located anterior to the level of the oesophageal bulb whereas it is found posterior to the level of the oesophageal bulb in *Cosmocerca* sp. 1*. Cosmocerca* sp. 2 (Fig. 17 E) differs from *Cosmocerca* sp. 3 by the presence of a V-shaped gubernaculum whereas *Cosmocerca* sp. 3 (Fig. 18 E) has a club-shaped gubernaculum. The plectanes of *Cosmocerca* sp. 2 are clearly visible in the lateral and ventral view whilst plectanes of *Cosmocerca* sp. 3 have almost transparent edges and are inconspicuous in the ventral view.

The description of this new species is currently under review with *Parasitology Research*.

***Cosmocerca* sp. 3**

General. Body small, stout, attenuated anteriorly. Mouth triangular with three lips, dorsal lip bearing two prominent cephalic papillae, two ventro-lateral lips each bearing two cephalic papillae. Three lips opening into the oesophagus. Oesophagus is divided into three parts: pharynx, cylindrical corpus and oesophageal bulb. Lateral alae beginning anterior to the nerve ring and terminating anterior to cloaca. Nerve ring at mid-length of the corpus. Excretory pore anterior to oesophageal bulb. Tail straight in females and curved ventrally in males, evenly narrowing with short process on tip.

Male. Measurements based on four specimens. Body (Fig. 18A) 1.50–2.29 (1.72) mm long, 122–172 (142) wide at mid-body level. Lateral alae at 81–160 (115) from apex. Oesophagus divided into three parts: pharynx 16–22 (20) long and 15–21 (18) wide; corpus 317–368 (339) long and 24–31 (28) wide; oesophageal bulb 67–76 (71) long and 70–88 (78) wide. Nerve ring at 101–163 (124) and excretory pore at 268–344 (313) from apex. Tail bluntly rounded 134–166 (152) long, bearing short process at tip 13–23 (19) long. Gubernaculum (Fig. 18E) club-shaped, having a narrowed small bulb at anterior part and well sclerotised edges, 98–110 (105) long. Spicules equal in shape, evenly narrowing with sharpened tips, observed without dissection of tail. Left spicule 38–95 (71) long, right one 41–103 (75) long. Fourteen post-cloacal papillae (Fig. 18D) in tail region. Five pairs of plectanes (Fig. 18F, G) almost transparent with indistinguishable margins when observed in ventral view, easily visible in lateral view, each bearing from five to six trabeculae. In total about 108 papillae observed on entire body.

Female. Measurements based on 30 specimens. Body (Fig. 18H) 2.8–3.9 (3.2) mm long, 230–420 (302) wide at mid-body level. Lateral alae (Fig. 18C) at 116–367 (291) from apex. Oesophagus divided into three parts: pharynx 23–47 (33) long and 32–58 (44) wide; cylindrical corpus 355–776 (417) long and 36–59 (49) wide and oesophageal bulb 97–129 (113) long and 118–165 (133) wide. Nerve ring 118–244 (174) and excretory pore 362–454 (403) from apex. Vulva small slit, lips poorly developed, at mid body level 1.3–1.9 (1.6) mm from apex. Tail 131–486 (408) long with short point.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925.

Subfamily: Cosmocercinae Railliet, 1916.

Genus: *Cosmocerca* Diesing, 1861.

Species: *Cosmocerca* sp. 3.

Host: *Phrynomantis bifasciatus* Smith, 1847 (Amphibia: Anura: Microhylidae).

Locality: Makhado, Limpopo Province, South Africa; Coordinates: S 23˚06'36" E 29˚54'30".

Site in host: Large intestine.

Infection parameters: Intensity: 4-136 (29.88); Prevalence: 89.45% (17 of 19 were infected); Mean abundance: 26.7.

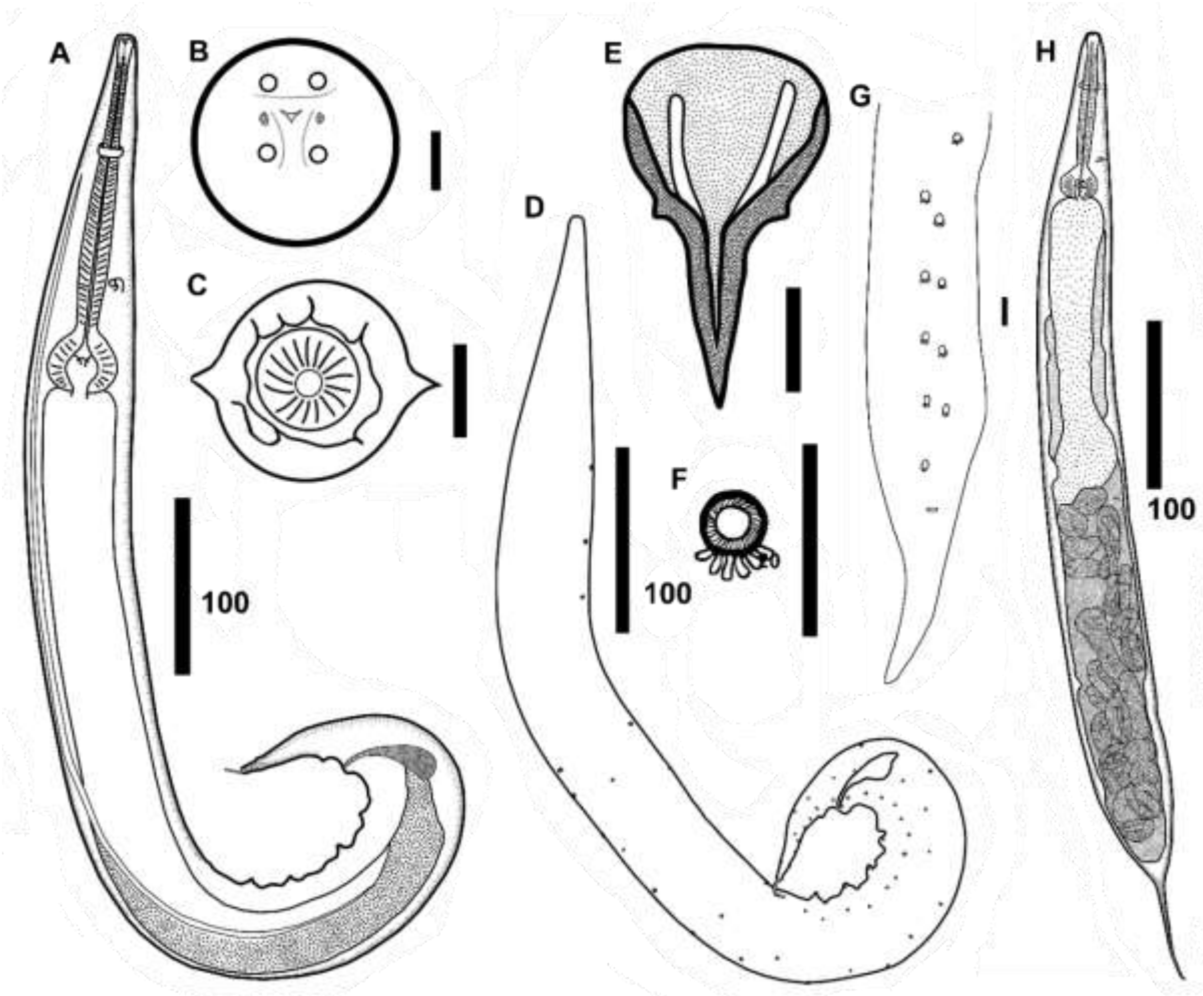

Figure 18. *Cosmocerca* sp. 3. line drawing, A – full body, male, lateral view; B – apical section, female; C – lateral alae, female; D – somatic papillae, male, lateral view; E – gubernaculum, male, ventral view; F – plectane, male, ventral view G – posterior end, male, ventral view; H. full body, female, lateral view.

Remarks.

The new species belong to the genus *Cosmocerca* due to the presence of plectanes, numerous papillae along the body and sexual dimorphism with males being half the size of females (Baker, 1980).

Cosmocerca sp. 3 differs from *C. ornata* in the shape of the gubernaculum which is club-shaped in *Cosmocerca* sp. 3 while the gubernaculum of *C. ornata* is Y-shaped. Moreover, the plectanes of *Cosmocerca* sp. 3 are almost transparent when observed from the lateral view and almost inconspicuous in the ventral view in contrast to prominent plectanes in *Cosmocerca ornata*.

Cosmocerca sp. 3 differed from *Cosmocerca* sp. 1 in the shape of the gubernaculum: *Cosmocerca* sp. 3 (Fig. 18E) has a club-shaped gubernaculum while *Cosmocerca*. sp. 1 (Fig. 16D) has a V-shaped gubernaculum with hook-like structures on the margins. Additionally, *Cosmocerca* sp. 3 has somatic papillae spread evenly on the entire body while papillae of *Cosmocerca* sp. 1 are located only on the lateral parts of the body. The plectanes of *Cosmocerca* sp. 3 are found to be almost transparent in the lateral view and almost inconspicuous in the ventral view while *Cosmocerca* sp. 1 were clearly visible. Mature females of *Cosmocerca* sp. 3 have an excretory pore anterior to the level of the oesophageal bulb whereas in Cosmcerca sp. 1 it is posterior to the level of the oesophageal bulb.

Cosmocerca sp. 3 differed from *Cosmocerca* sp. 2 in possession of a club-shaped gubernaculum (Fig. 18E) whereas *Cosmocerca* sp. 2 (Fig. 17E) have V-shaped gubernaculum. The plectanes of *Cosmocerca* sp. 3 are almost transparent in the lateral view and almost inconspicuous in the ventral view while plectanes of *Cosmocerca* sp.2 are prominent and clearly visible.

The description of this new species is currently under review with *Parasitology Research*.

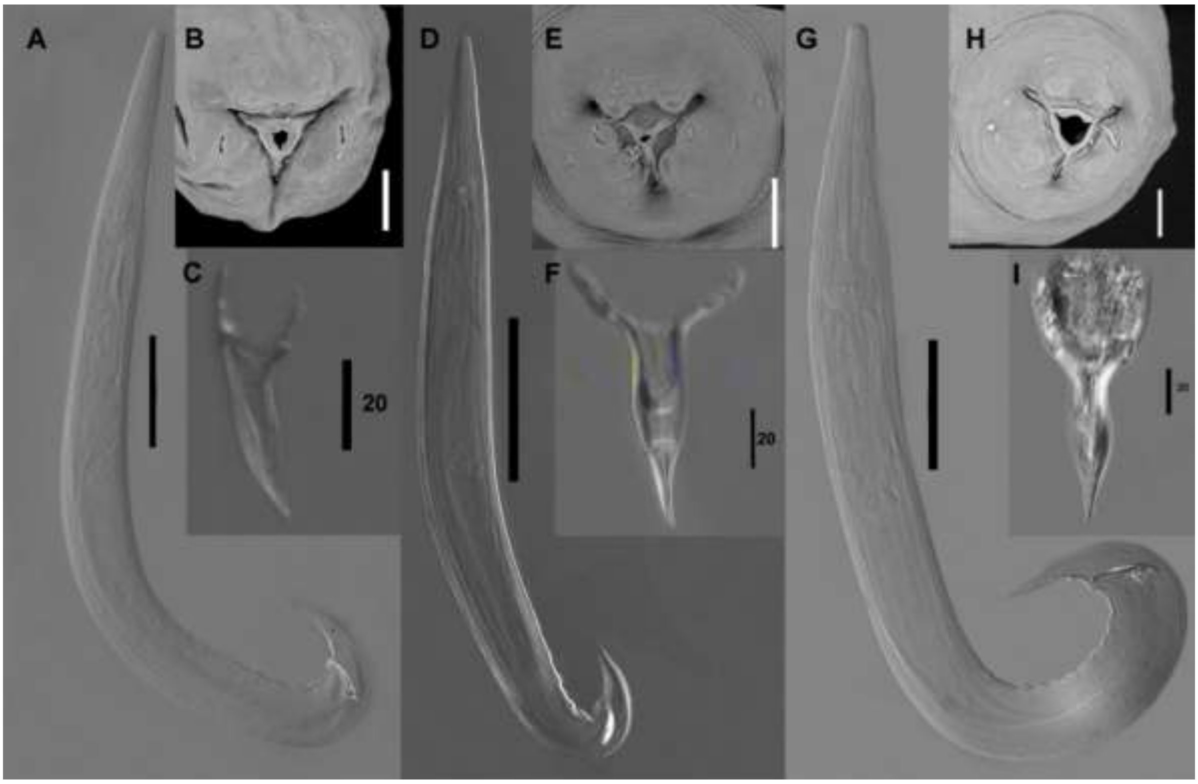

Figure 19. Photomicrographs, A–C. *Cosmocerca* sp.1. A – Full body male, B – *en face* view female SEM, C – Gubernaculum. D–F. *Cosmocerca* sp. 2. D – Full body male, E – *en face* view female SEM. F – Gubernaculum, G–I. *Cosmocerca* sp. 3. G – Full body male, H – *en face* view female SEM, I. Gubernaculum.

Cosmocercidae gen. sp. 1

General. Oesophagus divided into three parts: short pharynx, cylindrical corpus and wide posterior bulb. Excretory pore anterior to oesophageal bulb. Vulva at mid-body level.

Female. Measurements based on three females. Body 2.3–2.9 (2.7) mm long, 210–254 (227) wide at mid-body level. Lateral alae 98–104 (101) from apex. Oesophageal pharynx 28–35 (32) long and 15–26 (20) wide; corpus 303–321 (312) long and 21–24 (23) wide (Fig. 20B); oesophageal bulb 82–87 (84) long and 69–82 (74) wide. Nerve ring at 97–123 (111) and excretory pore at 201–303 (242) from apex. Tail 199–239 (214) long, slightly rounded bluntly with an elongated end.

Taxonomic summary

Family: Cosmocercidae Travassos, 1925

Subfamily: Cosmocercinae Railliet, 1916

Host: *Chiromantis xerampelina* (Amphibia: Anura: Rhacophoridae).

Locality: Makhado, Limpopo Province, South Africa: Coordinates: S 23˚05’23” E 29˚54’30”

Site in host: Large intestine.

Infection parameters: Intensity: 5; Prevalence: 25% (1 of 4 was infected); Mean abundance: 1.25.

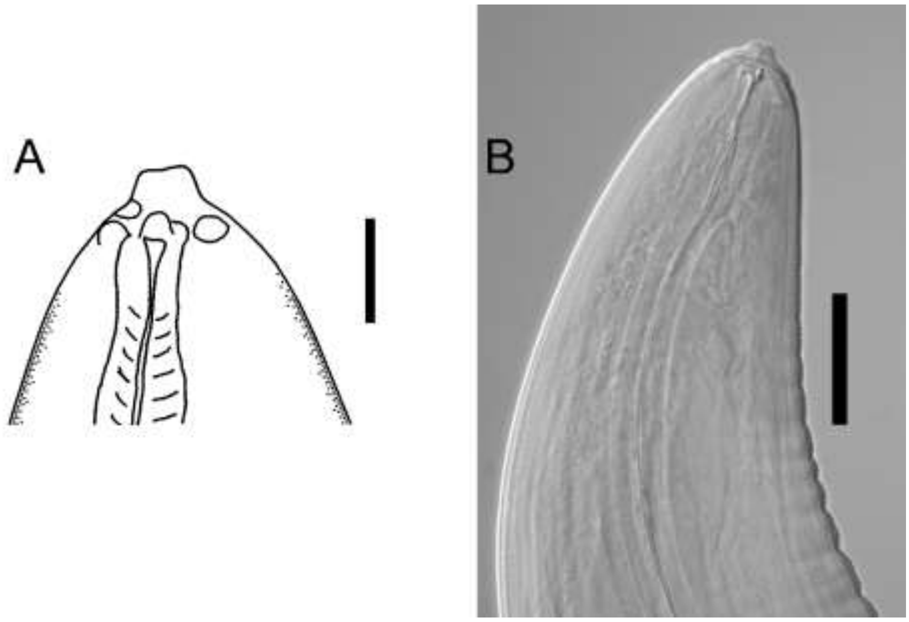

Figure 20. Cosmocercidae gen sp. A – line drawings, anterior end of body, female ventral view; B – photomicrograph, anterior end, female, lateral view. Scale bars: 20µm.

Remarks.

Only young females were found with no eggs in their uteri. The vulva was not observed as the specimens were compromised through the fixing and thus the vulva was not visible under microscopic analysis. The anterior end and tail are similar to that found in the genus *Cosmocercoides*, with females less than three millimetres in length while adult females of the genus can be, on average, 10 mm. The BLAST search of the ITS--28S region placed it closely to the genus *Cosmocercoides*. However, the phylogenetic analysis (Fig. 19) showed its distant relation to available sequences of *Cosmocercoides*, *Cosmocerca* and *Aplectana*. It is, therefore, not possible to assign found specimens to any of existing genera of cosmocercids.

In the present study three more species of *Aplectana* (*Aplectana* sp. 3, 4 and 5) and two of *Cosmocerca* (*Cosmocerca* sp.4 and 5) were found. Morphological description could not be completed due to the specimens all being juvenile. However, molecular analyses of the partial 18S sequence suggest that these specimens were different from all the other species in the present study.

3.2 Free-living stages.

The free-living stages of two specimens from *Aplectana* and *Cosmocerca* were obtained from frogs *Breviceps adspersus* Peters, 1882 and *Tomopterna adiastola* Boulenger, 1907, respectively. Larvae cultivated within several days on their hosts' faeces and studied under the light microscope.

First stage larvae (Fig. 21C–D) were immediately released when collected females were placed in a water sample from the host's locality. These larvae were thin and elongated with a blunt anterior end and a transparent cuticularised sheath. Their oesophagi were without striations, the oesophageal bulb was not visible as the body consisted of many small granules that increased gradually until no other structural characteristics were distinguishable. This stage was highly mobile.

Within three days the first stage larva began to moult into a second stage larva. This stage became sessile within a day after moulting. After adding more freshwater from the host location, the second stage larva moulted into infective third stage larva (Fig. 21 A–B). The third stage larva had an elongated oesophagus, rapidly decreasing granules, a now visible oesophageal bulb (although the intestine area was covered with granules) and a small genital primordium present at the mid-body region.

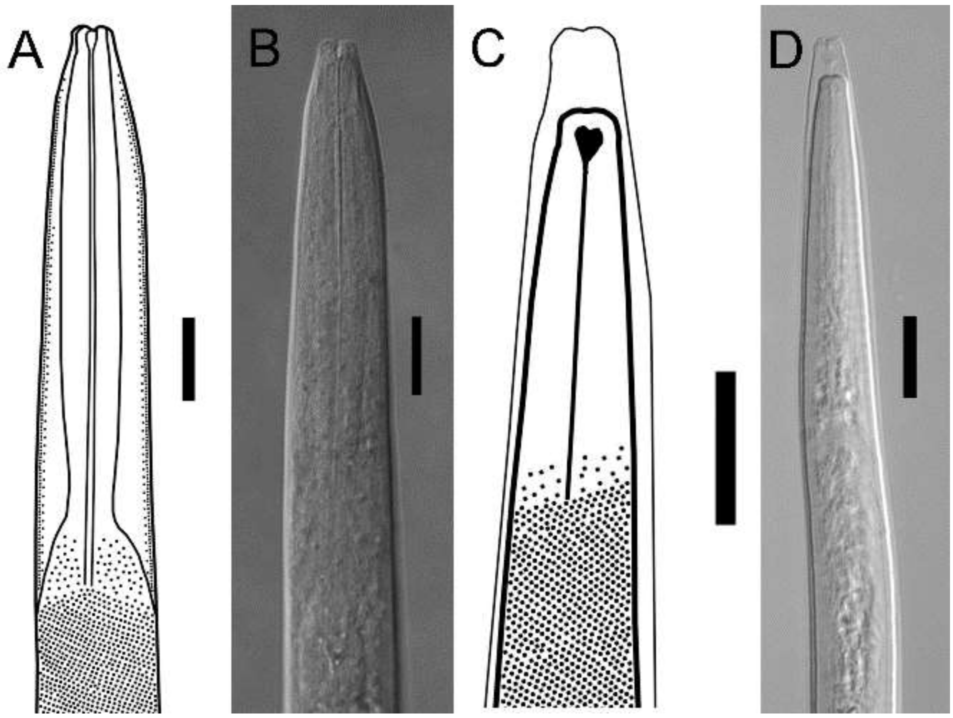

Figure 21. *Aplectana* sp. Juvenile, A – line drawings, anterior end of body, stage 3 larva; B – photomicrograph, anterior end of body, stage 2 larva; C – line drawing, anterior end of body, stage 1 larva; D – photomicrograph, anterior end of body, stage 1 larva. Scale bars: 20µm.

3.3 Molecular analyses

Molecular analyses were conducted on specimens collected from 20 anurans from different localities. Sequencing of the partial 18S, ITS-28S and COI genes were attempted. The COI gene successfully amplified for only two species and was not used in further analyses. Of the 20 samples analysed, 17 samples were successfully sequenced for the partial 18S alignment and nine samples for the ITS-28S region (Table2).

Table 2: List of species analysed for morphological and/or molecular studies.

Species	Morphology studies	Molecular studies 18rRNA/	ITS-28S
Aplectana sp. 1	+	+	+
Aplectana sp. 2	+	+	-
Aplectana sp. 3	-	+	-
Aplectana sp. 4	-	+	-
Aplectana sp. 5	-	+	-
A. cf. *capensis* sp. 1	+	+	+
A. cf. *capensis* sp. 2	+	+	+
A. cf. *macintoshii* sp. 1	+	+	+
A. cf. *macintoshii* sp. 2	+	+	+
Cosmocerca sp. 1	+	-	-
Cosmocerca sp. 2	+	+	+
Cosmocerca sp. 3	+	-	+
Cosmocerca sp. 4	-	+	-
Cosmocerca sp. 5	-	+	-
Cosmocercidae gen sp.	+	+	+

In addition to those obtained in the present study, several sequences were downloaded from GenBank. These included the following species: *Cosmocercoides pulcher*, *Cosmocercoides dukae*, *Cosmocercoides qingtianensis* Chen et al. 2018, *Cosmocercoides tonkinensis* Tran et al. 2015, *Neoraillietnema bakeri*, *Raillietnema* sp., *Cosmocerca simile* Chen et al. 2020 and as an outgroup *Insulanema longispiculum* Malysheva et al. 2012 for 18S. For the ITS-28S the species were: *Co. tonkinensis* and *Co. pulcher* and as an outgroup *Meteterakis occidentalis* Sata, 2018.

According to the divergence of the partial 18S alignment (Table: 3) specimens were assigned to the genera which depicted low divergence values and similar morphological characters.

Table 3: Genetic divergence based on 676 nucleotides between species of Cosmocercinae based on the partial 18S rDNA gene sequences. Presented number of nucleotides (above the diagonal) and percent (Below the diagonal).

	1.	2	3.	4.	5.	6.	7.	8.	9.	10	11.	12.	13.	14.	15.	16.	17
1.*Aplectana* sp. 1	-	0	0	0	0	0	0	0	0	0	0	1	1	1	3	2	21
2.*Aplectana* sp. 2	0	-	0	0	0	0	0	0	0	0	0	1	1	1	3	2	21
3.*Aplectana* sp. 3	0	0	-	0	0	0	0	0	0	0	0	1	1	1	3	2	21
4.*Aplectana* sp. 4	0	0	0	-	0	0	0	0	0	0	0	1	1	1	3	2	21
5.*Aplectana* sp. 5	0	0	0	0	-	0	0	0	0	0	0	1	1	1	3	2	21
6.*A.* cf. *capensis* sp. 1	0	0	0	0	0	-	0	0	0	0	0	1	1	1	3	2	21
7.*A.* cf. *capensis* sp. 2	0	0	0	0	0	0	-	0	0	0	0	1	1	1	3	2	21
8.*A.* cf. *capensis* sp. 2	0	0	0	0	0	0	0	-	0	0	0	1	1	1	3	2	21
9.*A.* cf. *capensis* sp. 2	0	0	0	0	0	0	0	0	-	0	0	1	1	1	3	2	21
10.*A.* cf. *macintoshii* sp. 1	0	0	0	0	0	0	0	0	0	-	0	1	1	1	3	2	21
11.*A.* cf. *macintoshii* sp. 2	0	0	0	0	0	0	0	0	0	0	-	1	1	1	3	2	21
12.*Cosmocerca* sp. 2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	-	0	0	2	3	21
13.*Cosmocerca* sp. 2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	-	0	2	3	21
14.*Cosmocerca* sp. 2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0	-	2	3	21
15.*Cosmocerca* sp. 4	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	-	5	21
16.*Cosmocerca* sp. 5	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.01	-	21
17.Cosmocercidae gen sp.	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	-

The tree based on the partial 18S alignment is comprised of 17 newly obtained sequences and six species retrieved from GenBank (Fig. 22). On the tree, most of the species formed only polytomy without any supported clades. This is most likely due to the high level of conservatism of the 18S rDNA gene (Table 3).

The tree based on ITS-28S sequences is comprised of nine species obtained in the present study plus two species retrieved from GenBank (Fig. 23). The resultant tree consisted of three main branches. One formed by species of *Aplectana* and *Cosmocerca* appeared polyphyletic in the low-supported clade. Another branch formed by two well-supported clades: one comprised of two species of *Cosmocercoides* and another of two species of *Aplectana*. The third branch formed by one species of Cosmocercidae gen. sp. appeared basal lineage to the other analysed genera. The nucleotide differences in ITS-28S sequences between different species (Table. 4) is notably bigger than in 18S (Table. 3) and played a basic role in distinguishing species for which morphological comparison was not possible. Difference between several species comprised more than 50 % which is normally represented in the difference between different families or superfamilies. Nevertheless, the BLAST search placed all of the species close to the genera with which they are identified. The situation is most likely explained by different regions of ITS-28S sequenced and thus, not properly aligned.

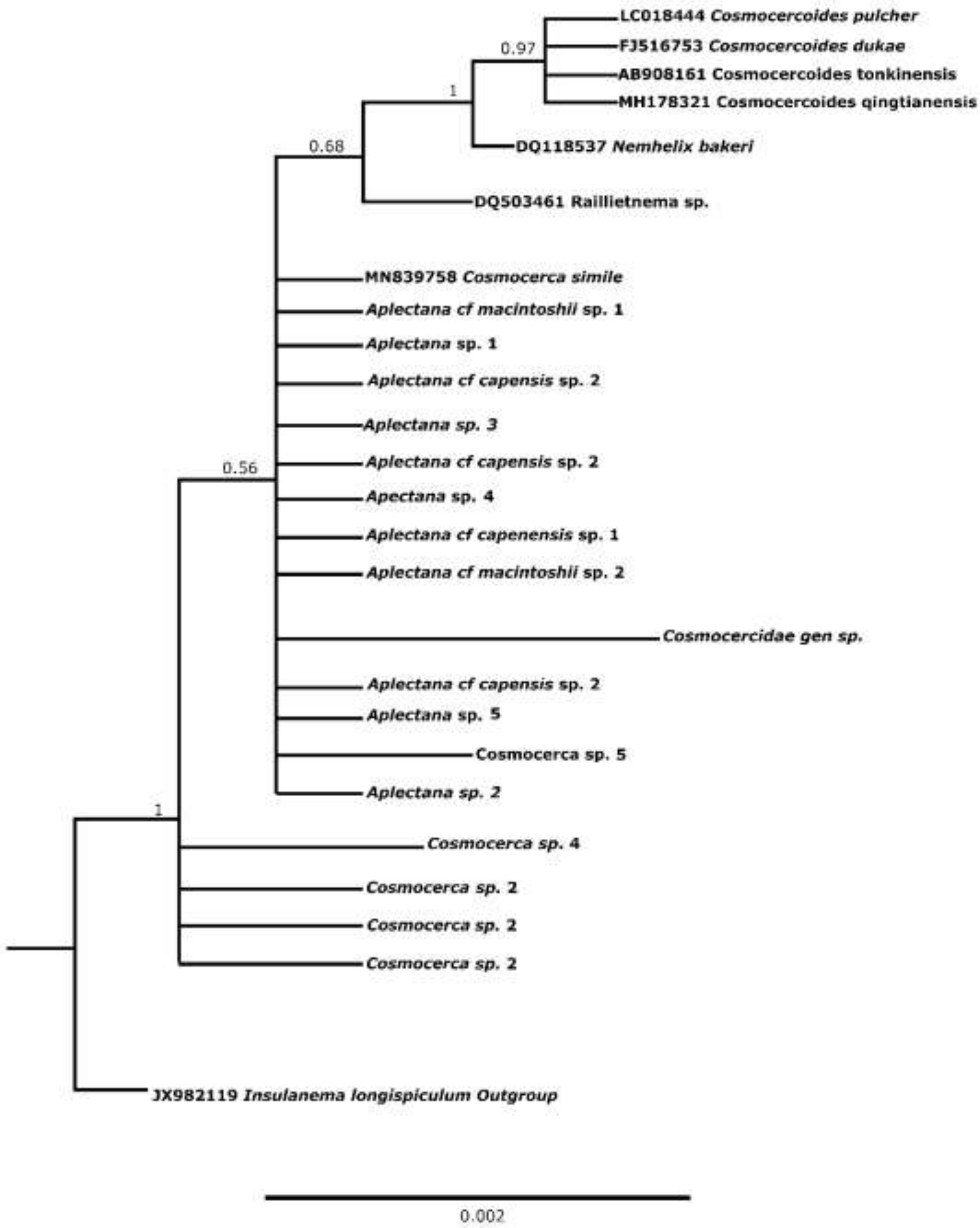

Figure 22. Phylogenetic tree of Cosmocercinae nematodes based on 676 nucleotides of partial 18S rDNA gene. Nodal support presented for Bayesian Inference.

Table 4: Genetic divergence based on 1 204 nucleotides between species of Cosmocercinae based on partial ITS-28S. Presented number of nucleotides (above diagonal) and percent (below diagonal).

	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**
1. *Aplectana* sp. 2	-	6	4	3	737	735	4	367	11	6	70
2. *A.* cf. *capensis* sp 1	0.04	-	6	5	735	734	2	368	12	6	73
3. *A.* cf. *capensis* sp. 2	0.03	0.05	-	0	735	735	4	366	12	6	68
4. *A.* cf. *capensis* sp. 2	0.02	0.04	0	-	736	735	3	366	11	5	67
5. *A.* cf. *macintoshii* sp. 1	64	64	64	64	-	3	735	739	737	736	730
6. *A.* cf. *macintoshii* sp. 1	64	64	64	64	0.03	-	734	738	736	735	728
7. *A* cf. *macintoshi* sp. 2	0.03	0.02	0.03	0.02	637	635	-	367	10	4	71
8. *Cosmocerca* sp. 2	0.09	0.10	0.12	0.09	64	64	0.08	-	365	367	362
9. *Cosmocerca* sp. 3	0.05	0.05	0.05	0.04	64	64	0.03	0.10	-	12	71
10. *Cosmocercidae* gen sp.	31	31	31	31	64	64	31	31	31	-	71
11. *Cosmocerca* cf. *ornata*	5.78	6.09	5.67	5,66	6.34	6.31	5.79	5.67	5.79	3.04	-

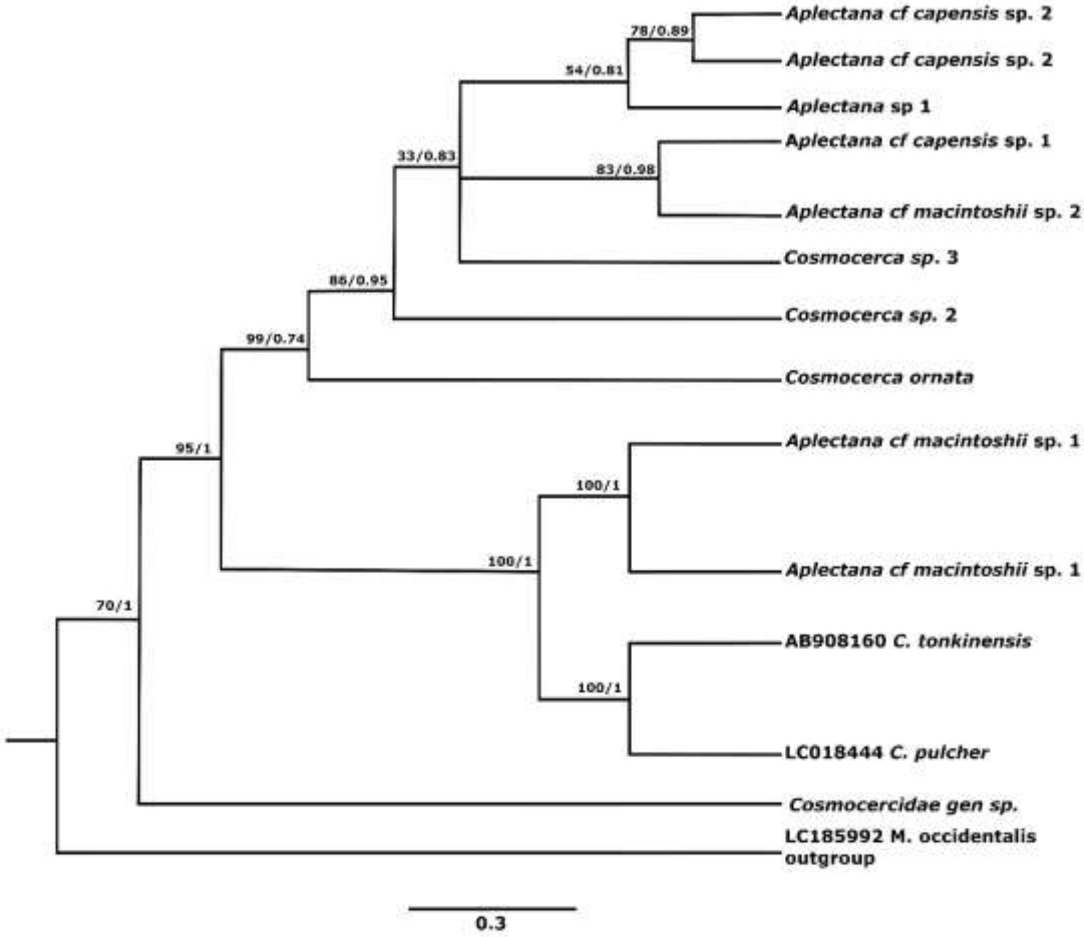

Figure 23. Phylogenetic tree of Cosmocercinae nematodes based on 1 204 nucleotides long alignment of ITS-28S region. Nodal support presented for Bayesian Inference / Maximum likelihood.

The recent study published by Chen et al. (2021) represented phylogenetic analyses among several species of *Aplectana* based on partial 18S and ITS-28S sequences. Unfortunately, the sequences are not yet released by GenBank and could not be included in the present study (Chen et al. 2021).

3.4 Host species record for this study.

In total, 18 different frog species were screened for the presence of cosmocercin nematodes. Of these, 14 listed below were infected with one or more species of nematodes:

- *Amietia delalandii* recorded *Aplectana* sp. 1 and *A.* cf. *capensis* sp. 2
- *Schismaderma carens* recorded *Aplectana* sp. 2 and *Aplectana* sp. 3
- *Tomopterma adiastola* recorded *Aplectana sp. 4* and *A.* cf. *macintoshii* sp. 1
- *Pyxicephalus edulis* Peters, 1854 recorded *Aplectana* sp. 5
- *Sclerophys garmani* recorded *A.* cf. *capensis* sp. 1 and *A.* cf. *capensis* sp. 2
- *Sclerophys gutturalis* recorded *A.* cf. *capensis* sp. 2 *and A.* cf. *macintoshii* sp. 1
- *Ptychadena anchietae* recorded *A.* cf. *macintoshii* sp. 1
- *Ptychadena mossambica* recorded *A.* cf. *macintoshii sp.2*
- *Cacosternum boettgeri* recorded *Cosmocerca* sp. 1
- *Kassina senegalensis* recorded *Cosmocerca* sp. 2
- *Phrynomantis bifasciatus* recorded *Cosmocerca* sp. 3
- *Breviceps adspesus* recorded *Cosmocerca* sp. 4
- *Poyntonophrynus fenoulheti* Hewitt and Methuen, 1912 recorded *Cosmocerca* sp. 5
- *Chiromantis xerampelina* recorded Cosmocercidae gen sp.

Additionally, four more species, namely *Breviceps mossambicus* Peters, 1854, *Leptopelis natalensis* Smith, 1849, *Afrixalus spinifrons* Cope, 1862 and *Hyperolius marmoratus* (Rapp, 1842) were found uninfected with Cosmocercinae.

The species of *Aplectana* were found in more than one host and often more than one species was found parasitising the same host species. At the same time, species of *Cosmocerca* appeared to be strictly host specific. Only one species of *Cosmocerca* was found in a host species from different localities and all the hosts analysed had only their specific species of *Cosmocerca*.

Chapter 4

Discussion and conclusion

Cosmocerca sp. 2. Image
Ms Florence Harnoster

4.1 Discussion

The list of cosmocercin species occurring in the Afrotropical region was compiled by Bursey et al. 2006, 2015, 2018) and Sou et al. (2018). Based on these there are currently only 16 species of Cosmocercinae described from the Afrotropical region and five species occur in South Africa (Baker, 1981a; Bursey et al. 2006; Bursey et al. 2018). This indicates that there is a big gap in cosmocercin nematode investigation in the southern African region. Unfortunately, of the species studied in South Africa, there are only two described in detail with images available for comparative study (Baker, 1981a). Although species of *Raillietnema* commonly occur on the African continent, none of these were found in South Africa during this study.

There are two species of the genus *Aplectana* described in South Africa (Baker, 1981a). In this present study *Aplectana* sp. 1 differed from all the species found in South Africa. The character of having numerous unpaired papillae on the anterior and posterior parts of the tail is generally considered as significant to the species differentiation and thus *Aplectana* sp. 1 is most likely representing a new species. The lack of male found specimens in *Aplectana* sp. 2, is one of the main reasons behind species identification obscurities. This species was placed as a different species of *Aplectana* based on the phylogenetic analysis (Fig. 22 and 23), placing it as different from all the species where male specimens were found. However, the lack of phylogenetic studies and the availability of sequences for the verification of samples lacking male found specimens endorses further investigation. Species identified as *A*. cf. *capensis* sp. 1 and *A*. cf. *capensis* sp. 2 and *A*. cf. *macintoshii* sp.1 as well as *A*. cf. *macintoshii* sp. 2 are morphologically very close to the previously known species, although molecular data indicated their distinctness. Unfortunately, it is impossible to say which of the species, if any, belongs to the previously described ones without data from type hosts and type locations.

Aplectana degraafi was previously found only in host *B. sylvestris,* thus, due to the lack of finding the host this study was unsuccessful in finding the species. *Aplectana capensis* was found only in endemic species of frogs occurring in the Western Cape Province. Species resembling *A. capensis* may have been found in the species *S. gutturalis*, as this host species has become invasive in the areas where species of *B. montanus* and *B. rosei* occur. The interaction between species of frogs was not observed in the study and can only remain a hypothesis.

Species of *A. chamaeleonis* were also not found during this study. These species had been found in the southern areas of KwaZulu-Natal Province, whereas only the north-eastern areas were sampled.

The study found no species of *C. ornata* occurring in the regions sampled. All three species of *Cosmocerca* (sp. 1, 2 and 3) were found only in their type hosts and in spite of presence of *C. boetgeri* and *K. senegalensis* at the same site, only their specific species were collected. Similarly, only *Cosmocerca* sp. 3. was found in *P. bifasciatus* and no other cosmocercids were recorded from this host. Therefore, we suppose that the host specificity of *Cosmocerca* is rather high and the presence of *C. ornata* throughout different African frogs is doubtful.

Phylogenetic studies of cosmocercidae are limited to five studies and four unpublished (Blaxter et al. 2005; Smythe et al. 2006; Ross and Gowen, 2010; Tran et al. 2015; Balezun et al. 2020; Chen et al. 2020; Maity et al. 2020). The overall lack of studies of African cosmocercin nematodes and the molecular data thereof, obscures the evaluation of species from the African continent. The only phylogenetic study on Cosmocercidae nematodes was recently published by Chen et al. (2021). In that study the authors included 18S and 28S sequences of a very few species of *Aplectana*, *Cosmocerca*, *Cosmocercoides*, *Cruzia*, *Falcaustra*, *Megalobatrachonema*, *Orientatracis* and *Rondoria* from different areas, although southern Africa was excluded. Phylogenetic studies in this present study also based on 18S and 28S sequences showed somewhat controversial results which might be due to rather short fragments used in analyses and the small number of species. It is clear that using more genetic markers of many species from around the globe and/or using different approaches, such as next generation sequencing (NGS,) are necessary for understanding the Cosmocercidae phylogeny.

Research on the life cycle of this group of nematodes, shows a lack in the number of life cycle studies. Of the few studies, the number of found male specimens in comparison to the number of females is suspiciously low. In the study by Stångberg et al. (2020), authors studied the sexual dimorphism and sex ratio of various nematodes. The experiment found that increased intrasexual competition led to the occurrence of sex specific evolution (Stångberg et al., 2020). A similar study conducted by Fritzsch et al. (2014) found that males were less sensitive to intrasexual competition. Females, however, were found to be more competitive and evolved twice the rate the males did. The authors studied only two factors that may cause the difference in the sex ratio (Fritzsche et al. 2014; Stångberg et al. 2020), nonetheless, this is very understudied but could explain the differences in the number of found male specimens in comparison to females. There are no studies conducted on the difference in the sex ratio in Cosmocercidae and one of the future prospective studies may include a comprehensive study of the difference between male and female sex ratios together with their life cycles.

There are about 169 southern African species of frogs with 130 endemic to South Africa (Leballo et al. 2013) Based on the diversity in host species and host specificity of members of the genus *Cosmocerca*, the potential for high diversity in species of Cosmocercidae is very high. This might become clearer in future studies.

4.2 Conclusion

Using comparative literature, this study found species of cosmocercin nematodes in South Africa to be understudied. Species recorded herein, confirm diversity in cosmocercins and contribute information regarding the diversity of the Afrotropical Cosmocercinae.

Of the 206 analysed nematodes, three new species are in peer review. These species were found to be host specific in spite of two hosts, *C. boettgeri* and *K. senegalensis*, occurring in the same site location. Thus it would appear that species of Cosmocercidae might be much more diverse and more host specific than previously recorded. Species of *Aplectana* were found in more anuran hosts than previously listed. This adds to the host list of cosmocercin nematodes of the Afrotropical region.

Molecular analyses of the present study revealed rather high diversity of Cosmocercinae in South African amphibians. Unfortunately, no molecular data of *Aplectana* spp. from South Africa is available to confirm species of *Aplectana* in this study, as either a previously described or new species.

Morphologically different characteristics and phylogenetic analyses confirm the diversity of Cosmocercinae in South Africa. Therefore, we conclude that Cosmocercin nematodes being limited to only five species is highly unlikely.

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Appendix 1: Copy of permits

Limpopo permit

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
ECONOMIC DEVELOPMENT, ENVIRONMENT & TOURISM

DO SCIENTIFIC RESEARCH ON AMPHIBIAN

(Issued in terms of the provisions of the Limpopo Environmental Management Act 2003, Act no.7 of 2003).
In terms of and subject to the provisions of the abovementioned legislation and the regulation framed thereunder, the holder of this permit is hereby authorized to catch and/or collect the species and number of invertebrates specified on the table below for scientific purpose in the property mentioned on this permit.

Permit Holder		
Name	PROF. LOUIS HEYNS DU PREEZ	
Trade Name	N/A	
ID/Passport Number	6207095025081	
Address [Physical / Postal]	5 SCHUBART ST POTCHEFSTROOM 2531	P.O.BOX 19278 NOORDBRUG POTCHEFSTROOM 2522

Permit Details			
Permit No :	ZA/LP/94378	Stamp:	CITES & PERMIT MANAGEMENT ENVIRONMENTAL AFFAIRS LIMPOPO PROVINCE
Reference No :	CPM/22391/2018		
Date Issued :	2019-02-01		
Valid until :	2020-02-01		
Paid (ZAR):	R 60.00		
Receipt No :	FC 139065		

Farm Name / Organization	District	Province	Country
	N/A	Limpopo	South Africa

See Special Condition

Species Name	Scientific Name	Quantity	Note

	2019-02-01	2019-02-01	
Printed by: Seakamela TL	Printed Date:	Effective Date:	Signature of Permit Holder I acknowledge, accept and understand fully the permit conditions as described.

WILDLIFE TRADE & REGULATION

Cnr Dorp and Suid Street, Polokwane, 0699 P.O. Box 55464, Polokwane, 0700
Tel: +27 15 290 7171/7173-78 Fax: +27 15 295 5018 Website:www.ledet.gov.za Email: permits@ledet.gov.za

The heartland of southern Africa - development is about people!

KwaZulu Natal permit

EZEMVELO KZN WILDLIFE

Conservation, Partnerships & Ecotourism

ORIGINAL

ORDINARY PERMIT

Fee:	R 50,00	Permit No:	OP 4095/2019
Receipt No:	3523/2019	Contact:	Permits Office

This permit is issued in pursuance of the provisions of the Nature Conservation Ordinance No 15 of 1974 , Chapter 7 and the Regulations framed thereunder.

The permit is issued to:

ID Number: 8905155123088

Mr Edward Charles Netherlands
Private Bag X6001
Potchefstroom
2530

Residential Address
Room G16 Building E6
Potchefstroom Campus
NWU
Potchefstroom
2531

Province: North West Province

In the capacity of Researcher

To Collect the following species of Amphibians Invertebrates and Reptiles

AMPHIBIANS *(AMPHIBIA sp.)*

10 per site per locality All non -red listed frogs to collect draw blood and release, transport keep a maximum of 5 frogs for parasite life cycle experiments. throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Kosi Bay Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Ukahlamba Drakensberg Park., Hluhluwe-iMfolozi Park, Sodwana Bay, iSimangaliso Wetland Park and Mkhuze Game Reserve.

REPTILES

10 per site per locality All non - red listed reptiles collect transport draw blood and release collect transport keep a maximum of 2 reptiles for parasite life cycle experiments. throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Kosi Bay Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Hluhluwe-iMfolozi Park, Sodwana Bay, iSimangaliso Wetland Park and Mkhuze Game Reserve.

Please read the Terms and Conditions under which this Permit is issued

ISSUED at PIETERMARITZBURG, KwaZulu-Natal, on 13 Dec 2019

for CHIEF EXECUTIVE

Permit Holder

EZEMVELO KZN WILDLIFE PERMITS OFFICE
PO Box 13053, Cascades, 3202, Pietermaritzburg, KwaZulu-Natal
Tel +27 33 845 1320 / 1324. Fax: +27 33 845 1747. Fax to Email: 086 529 3320
Email: permits@kznwildlife.com . Website: www.kznwildlife.com

OP 4095/2019 *Page 1 of 3*

EZEMVELO KZN WILDLIFE

Conservation, Partnerships & Ecotourism

ORIGINAL

ORDINARY PERMIT

Fee:	R 50,00	Permit No:	OP 4095/2019
Receipt No:	3523/2019	Contact:	Permits Office

This permit is issued in pursuance of the provisions of the Nature Conservation Ordinance No 15 of 1974 , Chapter 7 and the Regulations framed thereunder.

The permit is issued to:

ID Number: 8905155123088

Mr Edward Charles Netherlands
Private Bag X6001
Potchefstroom
2530

Residential Address
Room G16 Building E6
Potchefstroom Campus
NWU
Potchefstroom
2531

Province: North West Province

In the capacity of Researcher

To Collect the following species of Amphibians Invertebrates and Reptiles

AMPHIBIANS *(AMPHIBIA sp.)*

10 per site per locality All non -red listed frogs to collect draw blood and release, transport keep a maximum of 5 frogs for parasite life cycle experiments. throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Kosi Bay Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Ukahlamba Drakensberg Park., Hluhluwe-iMfolozi Park, Sodwana Bay, iSimangaliso Wetland Park and Mkhuze Game Reserve.

REPTILES

10 per site per locality All non - red listed reptiles collect transport draw blood and release collect transport keep a maximum of 2 reptiles for parasite life cycle experiments. throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Kosi Bay Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Hluhluwe-iMfolozi Park, Sodwana Bay, iSimangaliso Wetland Park and Mkhuze Game Reserve.

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Permit Holder

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PO Box 13053, Cascades. 3202, Pietermaritzburg, KwaZulu-Natal.
Tel +27 33 845 1320 / 1324. Fax: +27 33 845 1747. Fax to Email: 086 529 3320
Email: permits@kznwildlife.com Website: www.kznwildlife.com

OP 4095/2019 *Page 1 of 3*

ORIGINAL

EZEMVELO KZN WILDLIFE

Conservation, Partnerships & Ecotourism

museum/herbarium. Holotype specimens, and half the number of paratype specimens, of any new species MUST BE DEPOSITED with a recognised South African museum/herbarium, and may only leave South Africa on a loan basis. These specimens are to be deposited in the SA museums within a year of publishing the description of the new species. The holder shall provide the Chief Executive Officer, KZNNCS with the name of the museum at which the specimens have been lodged, and the accession number of each specimen. This condition relates to unavoidable by-catch of non-target organisms as well.

8. A copy or copies of any publication arising from the authority herein contained will be made available to E KZN Wildlife.

9. Should renewal of this permit be desired, a minimum of one month's notice is required.

10. (I) Reserving accommodation within E KZNWildlife areas is entirely the responsibility of the permit holder. Booking is obtainable at the Central Booking Office, Telephone 033 8451000.(II) Any assistance required from Board staff will be subject to other demands on the Officer's time and must be arranged in advance with him/her.

11. Holders shall provide the Chief Executive, with a named list of every specimen collected (including the class, order, family, gender and age), the geographical co-ordinates (to seconds accuracy) of each collection locality and dates of collection, as laid out in the following table. A Global Positioning System with the WGS84 Datum should be used wherever possible to determine the geographical co-ordinates of the collection sites; please state the method used.

12. SPECIMEN - COLLECTION DATE - SPECIES - LOCALITY - LATITUDE - LONGITUDE (museum Accession) (ddmmyy) (Seconds Accuracy) (Seconds Accuracy).
Holders are requested to provide additional information, such as the habitat in which each specimen was collected and abundance or relative abundance data (providing standardised sampling methods are used) with the list.

14. No collecting is permitted in the wilderness areas within the Protected Area. For confirmation of boundaries of the wilderness area contact the Officer in Charge.

15. No specimens collected or captured or exported under this permit may be sold.

16. If possible, dead by-catch is to be distributed to the relevant experts for those groups in South Africa under the same conditions of this permit. Please enclose a copy of this permit and the locality and field data associated with the by-catch with the by-catch specimens.

17. This permit/licence/certificate is issued subject to compliance with all other relevant legislation and does not preclude the permit holder from adherence thereto.

Please read the Terms and Conditions under which this Permit is issued

ISSUED at PIETERMARITZBURG, KwaZulu-Natal, on 13 Dec 2019

for CHIEF EXECUTIVE

Permit Holder

EZEMVELO KZN WILDLIFE PERMITS OFFICE
PO Box 13053, Cascades, 3202, Pietermaritzburg, KwaZulu-Natal.
Tel +27 33 845 1320 / 1324. Fax: +27 33 845 1747. Fax to Email: 086 529 3320
Email: permits@kznwildlife.com. Website: www.kznwildlife.com

Appendix 2: Measurement tables

Aplectana sp. 1

females / host №	№	Body length	Body width	lat alae	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %	
	1	4619	309	114	44	54	425	57	131	128	230	493	513	2380	51,53	
	2	4794	327	108	41	50	421	53	118	128	200	482	505	2350	49,02	
	3	5222	338	124	50	55	442	58	130	139	226	530	508	2564	49,10	
Mean		4878,33	324,67	115,33	45,00	53,00	429,33	56,00	126,33	131,67	218,67	501,67	508,67	2431,33	49,84	
Min		4619	309	108	41	50	421	53	118	128	200	482	505	2350	50,88	
Max		5222	338	124	50	55	442	58	131	139	230	530	513	2564	49,10	
SD		310,22	14,64	8,08	4,58	2,65	11,15	2,65	7,23	6,35	16,29	25,15	4,04	115,87	37,35	
males / host №	№	Body length	Body width	lat alae	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculum	Left spicule	Right spicule
96	1	2784	303	127	26	34	301	32	71	80	69	368	199	73	254	264

Aplectana sp. 2

females / host №	№	Body length	Body width	lat. Alae	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %
551	1	2432	129	128	41	46	372	41	95	91	172	339	337	1183	48,64
	2	3607	219	169	23	44	425	51	118	123	156	416	416	1909	52,92
	3	3303	179	240	46	47	400	45	111	113	225	387	382	1817	55,01
	4	2692	150	183	37	45	377	41	104	98	184	361	291	1491	55,39
	5	3715	232	216	46	47	434	52	115	122	167	437	376	2047	55,10
	6	3364	228	123	35	45	411	48	113	115	152	404	376	1796	53,39
	7	2612	151	122	39	41	374	40	101	100	151	354	344	1406	53,83
	8	3877	240	195	39	55	430	50	120	130	158	437	364	2067	53,31
557	9	3969	236	114	56	71	475	67	129	144	215	436	438	2055	51,78
	10	4400,0	324,0	241,0	62,0	75,0	467,0	65,0	130,0	152,0	222,0	477,0	510,0	2240,0	50,91
	11	3751	256	145	46	51	437	57	109	136	240	449	421	1940	51,72
	12	3735	253	a	48	57	397	62	117	137	181	456	404	1991	53,31
	13	4139	298	200	43	66	474	64	136	145	209	499	460	2130	51,46
	14	4455	304	192	58	76	500	69	127	157	258	443	488	2263	50,80
	15	4060	268	181	48	64	451	58	124	153	164	477	415	2079	51,21
Mean		3607,40	231,13	174,93	44,47	55,33	428,27	54,00	116,60	127,73	190,27	424,80	401,47	1894,27	52,58
Min		2432	129	114	23	41	372	40	95	91	151	339	291	1183	49
Max		4455	324	241	62	76	500	69	136	157	258	499	510	2263	55
SD		623,83	58,11	43,02	9,77	12,08	39,98	9,94	11,53	21,12	35,00	47,55	58,28	311,50	1,88

Aplectana cf *capensis* sp. 1

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %		
359	1	5782	552	200	57	68	543	80	155	197	287	600	517	3908	67,59		
	2	6486	436	206	43	58	521	68	148	157	279	587	576	3275	50,49		
	3	6937	461	186	43	54	516	70	139	160	209	571	598	3556	51,26		
	4	6861	468	200	51	62	511	63	134	158	177	594	648	3452	50,31		
Mean		6516,50	479,25	198,00	48,50	60,50	522,75	70,25	144,00	168,00	238,00	588,00	584,75	3547,75	54,44		
Min		5782	436	186	43	54	511	63	134	157	177	571	517	3275	56,64		
Max		6937	552	206	57	68	543	80	155	197	287	600	648	3908	56,34		
SD		527,86	50,41	8,49	6,81	5,97	14,10	7,14	9,35	19,37	53,68	12,52	54,29	266,71	50,53		
males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculm	tail point	Left spicule	Right spicule
		2891,00	262,00	155,00	28,00	30,00	345,00	30,00	81,00	67,00	96,00	401,00	240,00	44,00	a	221,00	275,00

Aplectana cf *capensis* sp. 2

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %		
238	1	6155	380	167	48	58	429	62	134	144	237	491	465	3027	49,18		
	2	6111	366	178	43	60	413	62	126	136	195	500	452	3079	50,38		
	3	6262	405	175	40	52	450	68	133	149	195	505	470	3035	48,47		
240	4	5607	271	190	38	53	376	49	119	105	190	416	442	2421	43,18		
	5	5682	301	235	48	55	407	55	110	132	178	464	363	2683	47,22		
	6	4994	259	147	35	50	360	49	116	106	159	450	441	a	#VALUE!		
	7	5368	275	168	52	54	434	57	131	123	224	424	324	2794	52,05		
241	8	5598	327	157	42	58	439	61	126	140	191	492	460	2988	53,38		
242	9	7076	420	135	43	60	461	58	125	126	232	519	478	3812	53,87		
	10	6470,0	412,0	153,0	47,0	56,0	412,0	58,0	119,0	119,0	206,0	523,0	436,0	3268,0	50,51		
	11	3510	188	144	34	39	370	48	113	108	150	411	343	1939	55,24		
Mean		5712,09	327,64	168,09	42,73	54,09	413,73	57,00	122,91	126,18	196,09	472,27	424,91	2904,60	50,85		
Min		3510	188	135	34	39	360	48	110	105	150	411	324	1939	55,24		
Max		7076	420	235	52	60	461	68	134	149	237	523	478	3812	53,87		
SD		927,39	75,33	27,52	5,71	5,96	33,32	6,34	8,10	15,52	27,81	41,42	54,61	500,26	53,94		
males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculm	tail point	Left spicule	Right spicule
466		2234	201	85	20	26	264	26	62	72	94	348	224	70	a	249,00	262,00
240		2441	179	129	34	27	322	35	71	79	145	332	181	42	a	191,00	171,00
		2355	160	122	32	28	287	26	66	68	128	319	160	40	a	186,00	178,00
Mean		2343,33	180,00	112,00	28,67	27,00	291,00	29,00	66,33	73,00	122,33	333,00	188,33	50,67	#DIV/0!	208,67	203,67
Min		2234	160	85	20	26	264	26	62	68	94	319	160	40	0	186	171
Max		2441	201	129	34	28	322	35	71	79	145	348	224	70	0	249	262
SD		103,99	20,52	23,64	7,57	1,00	29,21	5,20	4,51	5,57	25,97	14,53	32,62	16,77	#DIV/0!	35,02	50,64

Aplectana cf *macintoshii* sp. 1

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %	
87	1	3509	219	108	36	46	401	48	106	121	209	407	361	1998	56,94	
	2	3608	256	111	31	42	399	44	104	123	172	386	384	1871	51,86	
	3	3345	213	124	33	38	406	49	104	119	199	379	389	1766	52,80	
	4	3377	289	109	36	45	347	44	110	102	167	363	353	1646	48,74	
	5	3419	228	116	37	47	368	46	105	120	128	386	323	1685	49,28	
86	6	3829	228	114	30	40	338	47	100	125	175	324	313	2472	64,56	
	7	2585	197	92	27	32	334	38	90	104	119	325	287	1675	1675,00	
	8	2643	245	45	27	33	348	52	97	121	104	318	278	0	0,00	
	9	2776	234	56	35	37	333	44	101	125	194	328	282	1532	55,19	
Mean		3232,33	234,33	97,22	32,44	40,00	363,78	45,78	101,89	117,78	163,00	357,33	330,00	1627,22	50,34	
Min		2585	197	45	27	32	333	38	90	102	104	318	278	0	0,00	
Max		3829	289	124	37	47	406	52	110	125	209	407	389	2472	64,56	
SD		449,55	26,78	27,95	3,88	5,48	30,53	3,96	5,82	8,64	37,51	33,87	43,42	670,14	149,07	

males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculm	Left spicule	Right spicule
87	1	1190	106	139	25	20	239	26	51	60	102	226	145	35	166	187
	2	1355	116	143	23	23	284	29	69	71	84	221	152	33	193	193
	3	1785	160	n	21	23	259	32	66	76	124	294	238	42	210	224
	4	1823	152	47	23	28	262	28	70	85	99	245	296	38	236	200
	5	1868	169	68	27	23	289	32	65	80	104	278	187	73	210	203
	6	1787	158	82	30	23	288	26	69	81	198	303	224	81	210	208
Mean		1634,67	143,50	95,80	24,83	23,33	270,17	28,83	65,00	75,50	118,50	261,17	207,00	50,33	204,17	202,50
Min		1190	106	47	21	20	239	26	51	60	84	221	145	33	166	187
Max		1868	169	143	30	28	289	32	70	85	198	303	296	81	236	224
SD		286,94	7,07	2,83	1,41	2,12	31,82	2,12	12,73	7,78	12,73	3,54	4,95	1,41	19,09	4,24

Aplectana cf *macintoshii* sp. 2

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %	
428	1	4619	309	114	44	54	425	57	131	128	230	493	513	2380	51,53	
	2	4794	327	108	41	50	421	53	118	128	200	482	505	2350	49,02	
	3	5222	338	124	50	55	442	58	130	139	226	530	508	2564	49,10	
	4	6008	396	120	44	55	465	58	124	130	213	507	593	2854	47,50	
	5	5070	375	132	45	51	450	54	103	131	a	543	a	2734	53,93	
429	6	2145	156	83	37	45	335	41	82	94	167	308	309	985	45,92	
	7	2649	184	75	46	38	338	40	95	95	173	332	363	1311	49,49	
	8	4312	307	111	43	51	363	50	109	120	169	427	447	2201	51,04	
	9	3093	240	98	40	47	356	39	85	101	140	370	354	1657	53,57	
	10	3512,0	257,0	78,0	33,0	56,0	349,0	43,0	96,0	106,0	209,0	355,0	401,0	1778,0	50,63	
	11	4284	310	112	43	51	359	52	105	119	168	419	429	2206	51,49	
Mean		4155,27	290,82	105,00	42,36	50,27	391,18	49,55	107,09	117,36	189,50	433,27	442,20	2092,73	50,36	
Min		2145	156	75	33	38	335	39	82	94	140	308	309	985	45,92	
Max		6008	396	132	50	56	465	58	131	139	230	543	593	2854	47,50	
SD		1179,99	74,80	19,11	4,57	5,31	49,35	7,45	17,07	15,79	30,05	83,12	88,01	592,72	50,23	
males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculm	Left spicule	Right spicule
		1687	189	95	29	28	284	31	62	63	115	325	187	45	183,00	196,00
		1599	146	163	22	27	281	27	56	62	129	299	228	43	170,00	186,00

Cosmocerca sp. 1

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva	vulva %		
386	1	1984	101	71	17	28	240	30	70	66	125	256	138	993	50,05		
	2	2228	98	188	27	28	262	31	71	65	152	295	255	594	26,66		
	3	2140	105	72	22	29	265	28	70	66	117	276	196	894	41,78		
	4	2026	99		22	24	240	33	77	63		248	184		0,00		
413	5	5719	204	55	25	25	320	33	87	78	161	338	289	2588	45,25		
	6	3016	105	82	23	31	340	31	80	71	178	295	272	1544	51,19		
	7	5924	202	48	25	31	316	29	93	80	167	352	362	1956	33,02		
	8	6119		126	24	34	359	39	93	94	166	539	318	2861	46,76		
	9	3303	120	42	23	23	293	32	83	73	168	393	258	1691	51,20		
376	10	4724	323	127	42	38	433	47	120	128	212	487	408	2456	51,99		
413	11	3806	138	80	26	28	324	34	77	84	183	424	358	1950	51,23		
	12	3245	140	83	26	26	293	36	89	75	178	385	305	1691	52,11		
	13	3138	131	88	27	24	300	31	82	69	165	373	303	1637	52,17		
	14	3043	132	95	23	20	293	28	83	78	157	391	305	1563	51,36		
	15	3 372	133	47	29	30	287	32	78	68	192	391	312	1 681	49,85		
178	16	4 737	209	84	26	26	313	38	82	87	183	468	326	2 398	50,62		
	17	5 281	232	110	27	35	303	37	89	96	190	534	384	2 593	49,10		
376	18	4 154	255	161	29	35	412	45	104	118	202	442	364	2 144	51,61		
	19	6 700	395	171	40	58	433	71	127	150	227	581	505	3 405	50,82		
	20	4 962	284	42	36	49	468	47	105	123	206	552	478	2 610	52,60		
	21	5 175	274	156	37	32	465	56	121	123	212	531	477	2 721	52,58		
	22	4 814	282	152	36	51	432	52	108	119	212	524	340	2 495	51,83		
	23	6 448	392	144	41	57	470	61	140	151	276	535	453	3 362	52,14		
	24	5 483	305	141	38	44	457	57	117	134	234	552	453	2 889	52,69		
	25	5 169	317	286	40	46	473	60	126	129	244	518	440	2 845	55,04		
	26	5 187	284	165	36	40	433	55	117	122	243	545	443	2 692	51,90		
	27	4 967	278	159	36	47	440	56	110	126	223	541	370	2 643	53,21		
	28	4 825	253	149	31	48	450	59	120	120	233	512	479	2 500	51,81		
	29	5 405	297	146	36	41	486	52	119	130	292	547	506	2 811	52,01		
	30	6 694	443	144	32	53	478	69	141	164	242	525	479	3 352	50,07		
Mean		4459,60	225,21	117,72	30,07	36,03	369,27	43,63	99,30	101,67	197,93	445,00	358,67	2260,66	47,76		
Min		1984	98	42	17	20	240	28	70	63	117	248	138	594	0		
Max		6700	443	286	42	58	486	71	141	164	292	581	506	3405	55		
SD		1417,76	100,05	54,92	6,87	10,93	83,68	13,25	21,48	30,53	41,42	104,05	99,67	731,82	10,80		
males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculm	tail point	Left spicule	Right spicule
386	1	1064	72	37	11	13	379	36	81	71	121	365	127	60	11,00	64	66
387	2	2284	105	38	18	16	208	21	43	45	112	220	133	60	22,00	61	61
413	3	1616	100	29	23	17	217	20	54	44	104	323	121	82	22,00	76	83
178	4	1444	95	30	22	14	198	20	47	42	105	245	146	89	18,00	84	86
Mean		1602,00	93,00	33,50	18,50	15,00	250,50	24,25	56,25	50,50	110,50	288,25	131,75	72,75	18,25	71,25	74,00
Min		1064	72	29	11	13	198	20	43	42	104	220	121	60	11	61	61
Max		2284	105	38	23	17	379	36	81	71	121	365	146	89	22	84	86
SD		509,81	23,33	0,71	4,95	2,12	120,92	10,61	26,87	18,38	6,36	102,53	4,24	0,00	7,78	2,12	3,54

Cosmocerca sp. 2

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindrical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva			
	1	2797	205	239	49	56	364	50	93	118	167	237	359	1116			
	2	2951	250	288	27	52	398	49	100	120	124	381	317	1641			
0	3	3290	282	215	34	67	469	50	105	137	184	457	402	1720			
	4	3268	222	219	29	53	406	49	101	118	154	433	374				
	5	2525	193	218	25	53	373	44	93	105	158	368	345	1343			
	6	2241	154	222	25	38	368	35	81	86	110	361	323	1223			
	7	3061	227	192	28	49	385	48	98	117	151	392	381	1539			
	8	3115	299	159	29	50	394	40	88	130	142	390	308	1680			
	9	3184	273	138	29	57	371	48	98	119	130	352	375	1669			
	10	3868	332	205	33	67	416	61	121	145	166	422	360	1988			
	11	3684	231	293	35	63	412	52	98	130	138	462	455	1844			
	12	3781	242	238	29	57	467	57	110	124	169	477	361	2069			
	13	4242	297	254	30	62	447	62	124	146	181	440	410				
	14,00	3483,00	244,00	192,00	34,00	66,00	412,00	68,00	116,00	141,00	172,00	362,00	357,00	1784,00			
Mean		3311,83	249,67	212,08	30,00	56,83	410,00	51,17	102,75	124,83	154,58	409,67	370,92	1685,90			
Min		2241	154	138	25	38	368	35	81	86	110	352	308	1223			
Max		4242	332	293	35	67	469	68	124	146	184	477	455	2069			
SD		559,92	49,66	40,82	3,36	8,78	35,28	9,51	13,05	17,48	21,86	44,24	39,35	264,06			
CV		16,91	19,89	19,25	11,19	15,45	8,61	18,59	12,70	14,00	14,14	10,80	10,61	15,66			
males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindrical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculum	tail point	Left spicule	Right spicule
191	1	1939	200	174	12	19	326	42		81	239	362	154	114	13,00	94	96
399	3	2134	203	215	20	22	291	33	74	84	94	369	162	100	9,00	95	107
403	4	1779	177	216	15	18	267	32	67	76	91	328	130	90	12,00	90	92
177	2	2032	178	122	14	16	314	27	61	70	195	357	150	103	15,00	87	93
Mean		1971,00	189,50	181,75	15,25	18,75	299,50	33,50	67,33	77,75	154,75	354,00	149,00	101,75	12,25	91,50	97,00
Min		1779	177	122	12	16	267	27	61	70	91	328	130	90	9	87	92
Max		2134	203	216	20	22	326	42	74	84	239	369	162	114	15	95	107
SD		150,75	13,92	44,38	3,40	2,50	26,08	6,24	6,51	6,13	74,10	18,02	13,61	9,88	2,50	3,70	6,88
CV		0,08	0,07	0,24	0,22	0,13	0,09	0,19	0,10	0,08	0,48	0,05	0,09	0,10	0,20	0,04	0,07

Cosmocerca sp. 3

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva			
264	1	3457	371	291	30	33	413	49	114	136	152	418	131	1255	36,30		
	2	3228	340	324	31	58	414	53	115	129	188	446	449	1620	50,19		
0	3	3286	340	116	28	52	420	51	109	129	144	454	465	1672	50,88		
	4	3039	339	299	34	50	416	50	114	136	182	420	333	1630	53,64		
	5	3124	271	289	31	36	401	52	112	134	137	405	450	1512	48,40		
	6	3900	420	299	23	47	467	59	129	165	244	392	415	1932	49,54		
	7	3730	326	303	25	32	452	36	97	122	163	406	478	1842	49,38		
247	8	3293	270	338	31	44	449	50	123	132	123	438		1696	51,50		
	9	3177	292	317	34	40	386	52	126	148	118	420		1643	51,72		
	10	3069	251	342	31	46	371	52	106	127		421		1596	52,00		
	11	2890	230	119	29	43	370	43	109	127	123	389	401	1489	51,52		
	12	3150	300	302	31	40	358	50	114	145	158	386	426	1559	49,49		
	13	3076	305	328	34	45	403	49	113	137	175	403	220	1696	55,14		
	14,00	3371,00	324,00	344,00	33,00	47,00	355,00	52,00	112,00	133,00	144,00	427,00	433,00	1647,00	48,86		
	15,00	3106,00	312,00	289,00	39,00	45,00	385,00	49,00	105,00	131,00	143,00	404,00	443,00	1563,00	50,32		
	16	3128	302	324	41	41	420	41	112	136	139	425	404	1581	50,54		
	17	3712	342	306	41	50	428	50	116	138	211	444	486	1824	49,14		
	18,00	3161,00	298,00	283,00	35,00	44,00	397,00	48,00	113,00	132,00	190,00	395,00	408,00	1542,00	48,78		
	19,00	3050,00	278,00	264,00	32,00	40,00	408,00	44,00	109,00	135,00	184,00	362,00	346,00	1581,00	51,84		
	20	3191	271	316	32	43	776	47	105	118	217	390	469	1590	49,83		
	21	3185	255	234	47	56	426	51	119	141	179	377	475	1611	50,58		
	22	2979	251	271	39	45	408	44	114	122	177	371	403	1558	52,30		
	23	3074	268	313	32	40	422	46	115	122	197	408	406	1618	52,64		
	24	3172,0	314,0	271,0	43,0	49,0	412,0	53,0	113,0	134,0	183,0	400,0	451,0	1626,0	51,26		
	25	3002	293	272	27	39	361	48	110	128	195	362	389	1473	49,07		
	26	2789	283	367	30	38	393	54	105	129	193	392	428	1523	54,61		
	27	3141,0	309,0	321,0	29,0	42,0	416,0	53,0	115,0	137,0	203,0	401,0	425,0	1588,0	50,56		
	28	3007,0	308,0	295,0	32,0	45,0	398,0	51,0	112,0	130,0	217,0	393,0	415,0	1502,0	49,95		
	29	3006,0	286,0	307,0	34,0	43,0	387,0	50,0	112,0	130,0	188,0	381,0	435,0	1535,0	51,06		
	30	3025,0	308,0	287,0	31,0	46,0	387,0	55,0	109,0	128,0	192,0	373,0	430,0	1500,0	49,59		
Mean		3183,93	301,90	291,03	32,97	43,97	416,63	49,40	112,57	133,03	174,45	403,43	407,93	1600,13	50,26		
Min		2789	230	116	23	32	355	36	97	118	118	362	131	1255	45,00		
Max		3900	420	367	47	58	776	59	129	165	244	454	486	1932	49,54		
SD		243,66	38,98	54,44	5,28	5,88	72,98	4,52	6,31	8,96	31,59	24,28	76,94	123,62	50,74		
CV		7,65	12,91	18,71	16,00	13,36	17,52	9,14	5,61	6,74	18,11	6,02	18,86	7,73	100,95		

males / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	gubernaculm	tail point	Left spicule	Right spicule
231	1	2285	172	108	20	15	368	24	76	88	126	344	166	110	13,00	79	81
233	2	1532	149	110	11	17	317	31	67	70	104	299	134	105	17,00	95	103
247	3	1496	123	160	20	19	322	29	70	70	163	268	154	107	22,00	38	41
Mean		1771,00	148,00	126,00	17,00	17,00	335,67	28,00	71,00	76,00	131,00	303,67	151,33	107,33	17,33	70,67	75,00
Min		1496,00	123,00	108,00	11,00	15,00	317,00	24,00	67,00	70,00	104,00	268,00	134,00	105,00	13,00	38,00	41,00
Max		2285,00	172,00	160,00	20,00	19,00	368,00	31,00	76,00	88,00	163,00	344,00	166,00	110,00	22,00	95,00	103,00
SD		445,50	24,52	29,46	5,20	2,00	28,11	3,61	4,58	10,39	29,82	38,21	16,17	2,52	4,51	29,40	31,43
CV		0,25	0,17	0,23	0,31	0,12	0,08	0,13	0,06	0,14	0,23	0,13	0,11	0,02	0,26	0,42	0,42

Cosmocercidae gen sp.

females / host №	№	Body length	Body width	wing distance	pharyn L	pharyn W	cylindical L	cylindrical W	Oes bulb L	Oes Bulb W	nerve ring	excre pore	Tail	vulva
	1	2921	254	101	34	15	321	21	87	82	123	303	239	a
	2	2858	210	98	28	19	312	24	83	69	97	201	203	a
	3	2274	216	104	35	26	303	24	82	71	114	221	199	a
Mean		2684,33	226,67	101,00	32,33	20,00	312,00	23,00	84,00	74,00	111,33	241,67	213,67	#DIV/0!
Min		2274	210	98	28	15	303	21	82	69	97	201	199	0
Max		2921	254	104	35	26	321	24	87	82	123	303	239	0
SD		356,75	23,86	3,00	3,79	5,57	9,00	1,73	2,65	7,00	13,20	54,05	22,03	#DIV/0!