

A revision of the taxonomy and phylogenetic background of the South African genus *Kazimierzus* Plisko, 2006 (Oligochaeta: Kazimierzidae)

By

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ABSTRACT

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Species identity and distribution information is important in conservation and monitoring of biodiversity. Megadrile fauna is very diverse in Southern Africa with most of the fauna demonstrating high levels of endemism, closely related species often separated by subtle morphological characters. Natural and various fertile biotopes of South Africa have a diverse terrestrial megadrile fauna, but to date, taxonomic and systematic studies of most taxa are incomplete. Such studies are vital as they contribute to the understanding of evolutionary processes and also provide information for conservation.

To date, South African indigenous megadrile accredited to three strict indigenous families, Microchaetidae (s.str.), Tritogeniidae and Kazimierzidae, differ morphologically and differ also in their geographical distribution. The representatives of *Kazimierzus*, the only genus in Kazimierzidae, are known from the Western and South-Western Atlantic coast of South Africa, the area known for endemism in other invertebrate fauna and often associated with diverse flora. Considering these factors *Kazimierzus* was selected for the present study.

The selected 25 *Kazimierzus* species occur in specific biotopes, their distribution is highly restricted to selected places, and most of them do not overlap. The study aimed to employ integrative taxonomy (morphology and molecular) to carry out a taxonomic revision of this genus and the objectives were:

1. to evaluate the validity of described species and provide an identification key to all species of *Kazimierzus*;
2. to determine the distribution of the genus *Kazimierzus*, in order to provide reliable data on species diversity, geographical distribution in Southern Africa and factors contributing to their distribution limits; this information is vital for conservation planning because earthworms can be used as bio-indicators of soil ecosystem health and
3. to investigate phylogenetic relationships within the genus.

Kazimierzus was originally accredited to the family Microchaetidae (s. lato). Family Kazimierzidae was erected to accommodate *Kazimierzus* species because their morphology and anatomy were found to be different from the Microchaetidae (s. str) and also their geographic distribution confirmed separation from the other families. Four new species were described and a key to all species of *Kazimierzus* was constructed.

DNA was extracted from twelve species and mitochondrial gene fragments (COI) were amplified and sequenced. Bayesian and maximum likelihood were used to determine phylogenetic relationships among species. The results validated the twelve species known currently. Cryptic diversity was observed in *K. occidualis* with genetic divergence greater than 12 % among populations. *Kazimierzus franciscus* and *K. ljunstroemi* have a low genetic variability suggesting close relatedness or probably conspecificity.

A group of specimens from Clanwilliam are morphologically identical to *K. sophieae* but are genetically distinct and may belong to an undescribed taxon. These observations highlighted the importance of integrative taxonomy in earthworms in order to present reliable taxonomic and biogeographic data.

A species distribution model was used to predict the distribution patterns of *K. hamerae*. Potentially new habitats were predicted along the Atlantic Ocean, in the southern part, as well as, in the north-western part of Namibia. The occurrence in Namibia was tested in the south, unfortunately no *Kazimierzus* specimens were found in the region where sampling was done. In addition, the model demonstrated that precipitation in the driest month is the most relevant predictor in spatial distribution patterns of *K. hamerae*.

Because of the increasing awareness of the importance of earthworms in South Africa, indigenous earthworm species are given English common names. As such, all the species of *Kazimierzus* were given English common names. It is hoped that using common names may facilitate and increase the use of earthworms in conservation planning and environmental impact assessments. More farmers are showing interest in soil biology and it is hoped that, by giving earthworms English names, their importance and use will increase in the agricultural sector.

Keywords: earthworms, diversity, COI, distribution, common names, new species, Microchaetidae, Kazimierzidae.

IQOQA

Ukongiwa nokuqaphelwa kwemvelo kuncike olwazini ngokwahlukahlukana kwezinhlobonhlobo zezilwane kanye nokusabalala kwazo. INingizimu Afrika inefa elicebile lemisundu ehlala emhlabathini. Ingxenye enkulu yalezilwanyana zinezinga elikhulu lemisinsi yendabuko bese futhi iba nezimpawu ezincane ukuyihlukanisa naleyo ehlobene nayo kakhulu. Izinkangala namahlathi ase Ningizimu Afrika anezinhlobonhlobo eziningi ezahlukahlukene zemisundi kodwa kuze kube manje, ulwazi lwethu olujulile ngemisundu alikapheleli. Izifundo ezijulile ngemisundu ziyintuba yokunezezela olwazini ngemvelaphi yemisundu okungaphinde futhi kusize ngolwazi lokongiwa kwayo.

Uhlobo lwemisundu olungaphansi kohla olubizwa nge *Kazimierzus* lutholakala eNtshona Koloni kanye naseNhla Koloni. Okwamanje kunezinhlobo ezingamashumi amabili nanhlanu zemisundu ezitholakala ngaphansi kwaloluhla kanti nokusabalala kwazo lezinhlobonhlobo kwahlukene. Lolucwaningo lwaluhlose ukuhlanganisa ukufundwa kwemisundu ngezinhlobonhlobo zayo ukuze kubuyekezwe ulwazi kanye nolibofuzo lohla i-*Kazimierzus*.

Izinhloso:

1. ukuqinisekisa ulwazi ngezinhlobo zemisundu eyaziwayo ukuthi ngabe luyilo ngempela yini bese kuphendlwa umgwaqosiseko wokuhlukanisa izinhlobo zemisundu ezingaphansi kwe-*Kazimierzus*;

2. ukuthatha izinqumo ngokusabalala kwemisundu etholakala ngaphansi kohla i-*Kazimierzus*. Lesisenzo sibalulekile ukujulisa ulwazi ngokwahlukahluka kwalezizilwane, ukusabalala kwezindawo lapho zitholakala khona eNingizimu Afrika kanye nezimbangela zokwahlukahluhana kwazo;

3. ukuhlola ukuhlobana kwalezinhllobonhlobo zemisundu ngokolibofuzo. Lolulwazi lubalulekile kubagcinimvelo ngoba imisundu ingasetshenziswa ukubona uma umhlaba usesimweni esihle ngokwesimo somhlabathi.

Umndenikazi omusha wemisundu obizwa nge-*Kazimierzidae* wabe ususungulwa ukuze kuncikiswe imisundu etholakala kuhlu i-*Kazimierzus* ngenxa yikuthi yona yayihlukile kuleyo etholakala kumndenikazi i-*Microchaetidae* kanti futhi nokusabalala kwayo kwehlukile. Imisundu emine emisha okwatholakala ukuthi yayingakaze itholakale ngaphambilini, yabe isiqanjwa amagama ngokosiko kanti kwabe sekubuye kwenziwa umgwaqosiseko wokuqamba imisundi engaphansi kohla i-*Kazimierzus*. Ucwangingo ngokolibofuzo lwenziwa kwimisundu eyishumi nambili kusetshenziswa isiqephu se gene COI. Kwabe sekusetshenziswa izindlela ezimbili i-Bayesian ne Maximum likelihood ukuqinisekisa ukuhlobana phakathi kwalemisundu. Imiphumela yaqinisekisa ukwahlukana kwemisundu eyishumi nambili evele eyaziwayo. Kuhlobo lwemisundu ebizwa ngo *Kazimierzus occidualis* kwatholakala ukuthi kukhona neminye imisundu ecashe ngaphansi kwaloluhlobo kodwa eyehlukile ngenxa yokuthi ukwehluka ngokolibofuzo kwakungaphezulu kwamaphesenti ayishumi nambili. Izinhlobo ezimbili zemisundu i-*Kazimierzus franciscus* ne *ljungstroemi* kwatholakala ukuthi ngokolibofuzo ukuhlobana kwazo kusondelene kakhulu okuveza ukuthi bahlobene kakhulu noma basondelene kakhulu.

Izinhlobo zemisundu eyatholakala e-Clanwilliam kwatholakala ukuthu zefana nemisundu i *K. sophieae* kodwa ngokocwangingo lolibofuzo bahlukene kakhulu, okungase kuchaze ukuthi lena yimisundu engakanikwa igama ngokwesayensi. Lezizinkomba zagcizelela ukubaluleka kokufunda imisundu ngokuhlanganisa izinhlobo ezahlukahlukeni ukubheka ubuhlobo phakathi kwayo ukuze sibe nolwazi olunzulu ngezinhlobonhlobo zemisundu kanye nangokwehlukana kwezindawo lapho itholakala khona.

Izindawo ezintsha okungathekiselwa ukuthi imisundi ingase itholakale khona imisundi i - *K. hamerae*, zatholakala ngasogwini lolwandlekazi i-Atlantic Ocean, ezindaweni ezingaseningizimu kanye nangasenyakatho-ntshonalanga yezwe lase-Namibia. Ukutholakala

kwalemisundu e-Namibia, kwaqinisekiswa eningizimu yezwe lase Namibia, ngeshwa akutholakalanga ngisho nolulodwa uhlobo lwe – *Kazimierzus* kulezozindawo okwakumbiwa kuzo. Phezukwalokho, ucubungulo lwakhombisa ukuthi ukutholakala kwezimvula enkwindla iyonandlela enohlonzo ukubikezela ngokusabalala kwe – *K. hamerae*. Ngenxa yokudlondlobala kolwazi ngokubaluleka kwemisundu eNingizimu Afrika, imisundu isinikezwe amagama eSingisi engabizwa ngawo ngokuwayelekile. Ngenxa yalokho, yonke imisundu engaphansi komndeni i-*Kazimierzus* yabe isinikezwa amagama eSingisi. Ukupha imisundu amagama ajwayelekile kuletha ithuba lokuthi imisundu ingase isetshenziswe ezinhlelweni zokulondolozwa kwemvelo kanye nasekuhlolweni kwemithelela yokwakha kwimvelo. Abanikazi bamapulazi bafuna ukwazi ngempilo engaphansi komhlaba abatshale kuwe kanti ukunika imisundu amagama ajwayelekile kungenza kunyuke ukusebenziswa nokubaluleka kwayo kwezolimo.

Keywords: imisundu, ukwehlukahlukana, COI, ukusabalala, amagama ajwayelekile, imisundu emisha, Microchaetidae, Kazimierzidae.

PREFACE

The work described in this thesis was carried out in the Discipline of Genetics in the School of Life Sciences, University of KwaZulu-Natal, Westville campus, between 2016 and 2018, under the supervision of Dr Oliver Tendayi Zishiri, Prof Tarombera Mwabvu and Dr Jadwiga Danuta Plisko.

This study represents original work by the author. The work has not been submitted in any form for any degree or diploma to any other tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1: PLAGIARISM

I, Thembeke Clara Nxele declare the following

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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 - b. where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed:

Date:

DECLARATION 2: PUBLICATIONS

Publication 1: This study was published in 2016 in the journal *African Invertebrates*, Pensoft Publishers.

Nxele TC, Plisko JD, Mwabvu T, Zishiri OT (2016) A new family Kazimierzidae for the genus *Kazimierzus*, earlier recorded to the composite Microchaetidae (Annelida, Oligochaeta). *African Invertebrates* 57 (2): 111–117.

Publication 2: This study was published in 2017 in the journal *Zootaxa* Magnolia Press.

Nxele TC, Plisko JD, Mwabvu T, Zishiri OT (Submitted) Four new earthworm species of *Kazimierzus* Plisko, 2006 (Clitellata, Kazimierzidae). *Zootaxa* 4353 (1): 187–194.

Publication 3: This study was submitted in 2018 to the journal *African Invertebrates* and is under review.

Nxele TC, Plisko JD, Mwabvu T, Zishiri OT (2017) Molecular phylogeny of *Kazimierzus* Plisko, 2006 (Clitellata, Kazimierzidae) from the Western and Northern Cape Province inferred from mitochondrial DNA sequences

Signed:

Date:

DEDICATION

This work is dedicated to my late father, Julius Bota Bambalele, my grandmother Nondyebo Bambalele–Makaula and my brother Sipho Justice Bambalele for the love and support they provided at different stages of my life. You are missed everyday.

Sindane

Bhese

Vezi

Manzini

Nomandla

Mnukwambi

Qaphuna

Sobhalabhala ka Mnyamana

Mpungushe! Nyathi emnyama eyawela umfula phezu kwe Ngwangwani!

Nozulu, izulu elibi elabuyisa abafazi ekuthezeni

Majolóbomvu njengentolwane

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To God alone be the glory, for granting me courage and determination and above all blessing me with good health to finish this study. Relevant acknowledgments have been given at the end of each chapter of this thesis. However, I would like to express my appreciation to a number of people for their contribution to this thesis as a whole.

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My mom Nompumelelo Bambalele, this is a small gesture compared to what she has done for me, her prayers and love keeps me going.

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My husband was forced to develop interest in earthworms as he had to read many drafts of chapters of this thesis and helped translate the abstract to IsiZulu. His support made it possible for me to complete this project.

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Mike Mostovski commented on some chapters of this thesis

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GLOSSARY:

(The glossary is adopted after Gate (1972), Reynolds (1977), Blakemore (2010), Plisko and Nxele (2015)).

ac clitellate: individuals with no clitellum.

aestivation: a period of inactivity, or dormancy resulting from unfavourable environmental conditions (usually a lack of moisture).

ampulla (pl. ampullae): dilated ental end of spermatheca, forming a distal chamber.

asetal: with no setae (peristomium and anal segment do not have setae).

calciferous glands: glands located on the gut (oesophageal or intestinal), and serving various functions (for example controlling pH balance of the digestive system and secreting calcium carbonate).

coelom: a body cavity between the body wall and internal organs, accommodating the alimentary canal, vascular and excretory systems, and other organs.

clitellum: a part of the body wall that is formed from glandular cells at maturity and that is involved with forming a cocoon. In the final state of its formation it can be annular or saddle-shaped, but the shape varies according to the state of maturity of the individual. The shape and setting on the segments are significant at family, genus and species level. Appears in full capacity on mature individuals. Two types are recognised (Fig. 1):

1. **saddle-shaped:** covering only the dorso-lateral part of the body.
2. **annular:** ring-shaped, encircling the body.

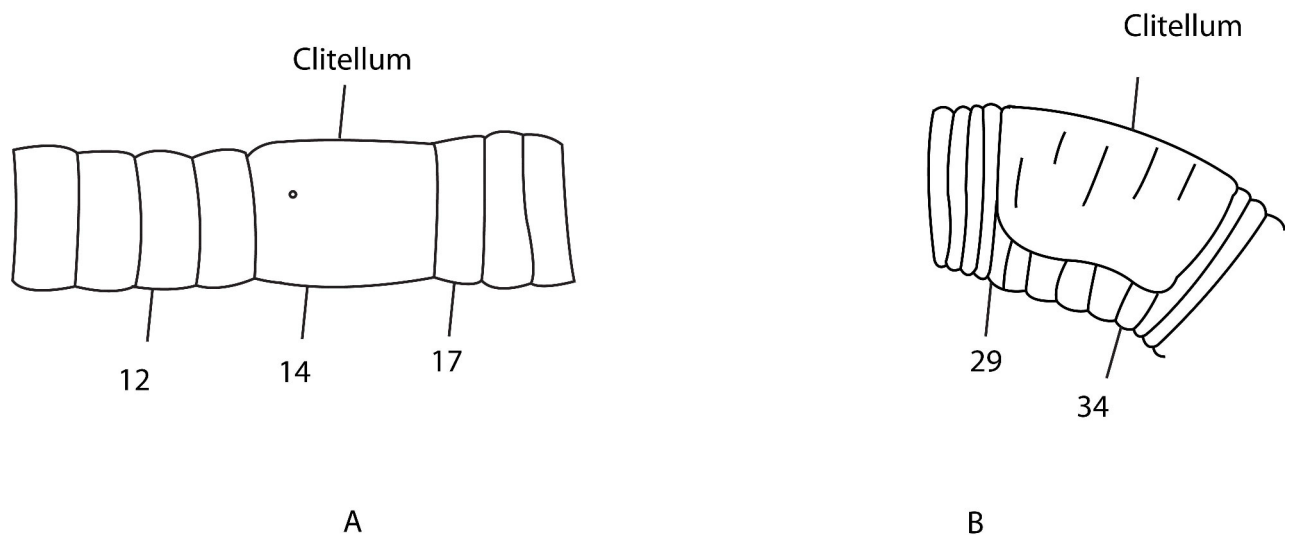


Figure 1. Clitellum: (A) annular; (B) saddle-shaped.

dorsal vessel: blood vessel located above the alimentary canal.

endemic: restricted to a certain region or part of region, native.

epilobous: a shape of prostomium with a tongue partly dividing the peristomium, may be open or closed

female pores: the external pores of the oviducts; may be paired or singular. Their position is characteristic to families and genera.

genital glands: internal glandular swellings, located in separated segments, usually around.

genital markings: glandular swellings, pits or grooves. In megascolecids variously arranged external sucker-like discs, pits, grooves, often correlated with position of spermathecal pores; are of great value for species identification.

gizzard (crop): a part of the digestive system, usually muscularised; however, in some species may be weakly muscularised or not at all. Its location in segments is an important generic and species character.

hermaphrodite: having both male and female reproductive organs in one individual. Earthworms are hermaphroditic.

holandric: a condition characterised by the presence of two pairs of testes and their funnels occurring successively in segments 10 and 11 (cf. proandric and metandric).

holandric condition: when testes and male funnels occur in segments 10 and 11.

holandry: a state of being holandric.

holoic: refers to the excretory system when the nephridia (stomate, exoic) are paired in segments (with the exception of the first and last segment, and in some species also omitting some of the segments). A state of the excretory system. A replacement for a term ‘meganephridial’ or shortened for ‘holonephridial’ (used in some papers) (cf. meroic).

intersegmental furrow: a division of segments; a border between two following segments where the epidermis is thinnest.

intestine: part of a digestive system. Commencement of intestine is an important character in all earthworm groups. In some species a folding occurs (named the caecum) on the dorsal part of the intestine.

juveniles: not mature; individuals with no recognisable external feature indicating maturity, such as clitellum or tubercula pubertatis.

male funnels: the ental ends of the sperm ducts initiated at testes. Sometimes in older papers named ‘spermiducal funnels’. Temporarily aggregated sperm in male pores may reveal its presence by iridescence (very useful observation for the funnels’ location).

male pores: the external openings of vasa deferentia through which the sperm are discharged during mating; may be simple opening, or with some glandular swellings (tumescens) around pores, or expanding on neighbouring segments. Important generic and species character.

megadrile: the term used for the description of terrestrial Oligochaeta with ovaria in 13.

metandric: refers to a condition characterised by the presence of a single pair of testes and their funnels in segment 11 (cf. holandric and proandric).

metandric condition: when testes and male funnels occur in segment 11.

metandry: a state of being metandric.

nephridium – (pl. nephridia): excretory organs: large, paired in one segment are referred to as holoic; small, multiple in segment are meroic. Their shapes are one of the most important characters in earthworm taxonomy.

nephropore: the external opening of a nephridium.

oesophagus: a portion of the digestive organ between pharynx and crop, ending in an oesophageal valve.

ovaries: the organs producing ova (eggs). In megadrile usually in segment 13.

papillae: occurring on various segments as swollen, raised areas; circular, oval or of other various shapes. It is of different taxonomic value in different families, and is often a problematic character.

peristomium: the first part of the body supporting the prostomium and the mouth; first segment containing mouth; it is asexual.

proandric: refers to a condition characterised by the presence of a single pair of testes and their funnels in segment 10 (cf. holandric and metandric).

proandric condition: testes and male funnels occur only in segment 10.

prolobous: a shape of prostomium with tongue not dividing peristomium (see other shapes of prostomium: epilobous, tanylobous, zygodolobous).

prostomium: the anterior part of the body (a lobe) projecting above the mouth. The shape of the prostomium is species specific and used broadly for species classification. Types of prostomium (Fig. 2):

1. **zygodolobous:** when anterior lobe is just like extended little part of peristomium.
2. **prolobous:** a shape of prostomium with tongue not dividing peristomium .
3. **epilobous:** when a tongue partly divides the peristomium, may be open or closed.
4. **tanylobous:** when a tongue completely divides peristomium . There may not be clear lines dividing the peristomium, making the prostomium open or closed.

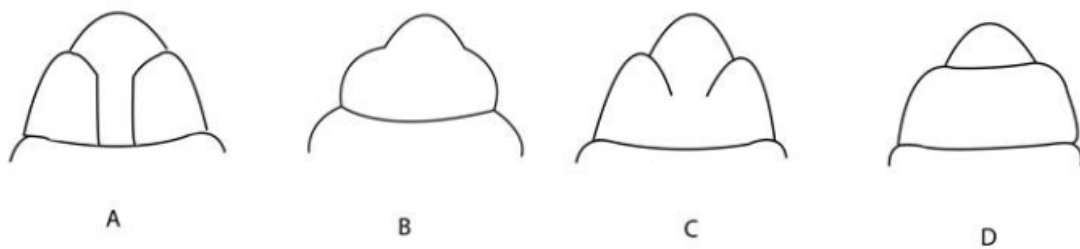


Figure 2. Prostomium: (A) tanylobous; (B) zygalobous; (C) epilobous; (D) prolobous.

secondary annulation: external demarcations of the segment occurring between intersegmental furrows and marking the segments. The term used to describe a loss of the uniformity in internal-external division in the preclitellar part of the body, something which occurs commonly in three South African indigenous families: Microchaetidae, Kazimierzidae and Tritogeniidae. Usually occurs on the anterior segments in front of the clitellum. The lack of uniformity creates difficulty in establishing the segment number and the position of internal and external characters, and special attention is therefore required during external observation and dissection.

segment (metamere): portion of the body externally divided by intersegmental furrows, internally separated by septa; a primary unit of segmentation. It may be a 'simple' part, internally divided by septa and externally demarcated by intersegmental furrows (as it is in acanthodrilids and megascolecids), or a primary unit superficially demarcated by shallow external furrows (as it is in microchaetids) when it is commonly termed 'secondary annulation' and sometimes a 'ringlet'.

segmentation: repeated transverse metameric internal and external divisions along the longitudinal axis, externally clearly marked by intersegmental furrows and internally by septa; simple.

semimature: an individual having partly developed some of the sexual characters in progress to maturity but with no external sexual features, such as clitellum, tubercula pubertatis or other maturity glands.

seminal vesicles: the storage sacs for an individual earthworm's own sperm.

septum (pl. septa): an internal partition between segments, dividing and supporting internal organs; usually delicate, thin, although in some segments much thickened to varying degrees. It is an important specific character in all earthworm groups.

seta (pl. setae): small, solid bristles to aid locomotion located at each segment (excluding only peristomium and last segment with anus). They are of several types, shapes, and functions: simple, genital, penial, associated with male pores, or genital papillae or certain other parts of the body. Each state has a particular taxonomical value at family, genus and species level (Fig. 3).

- When there are only eight setae per segment (Acanthodrilidae, Eudrilidae, Glossoscolecidae, Lumbricidae, Microchaetidae, Ocnerodrilidae, Tritogeniidae and Kazimierzidae) such condition is usually termed a 'lumbricine arrangement', or 'setae lumbricine'. Usually setae are arranged in regular lines. Exceptions include some species from the family Glossoscolecidae, where at the caudal part of the body the setae are arranged in a peculiar set-up, e.g. quincunx
- The condition with more than eight setae on each segment is termed a 'perichaetine arrangement' or 'setae perichaetine' as it occurs in Megascolecidae.

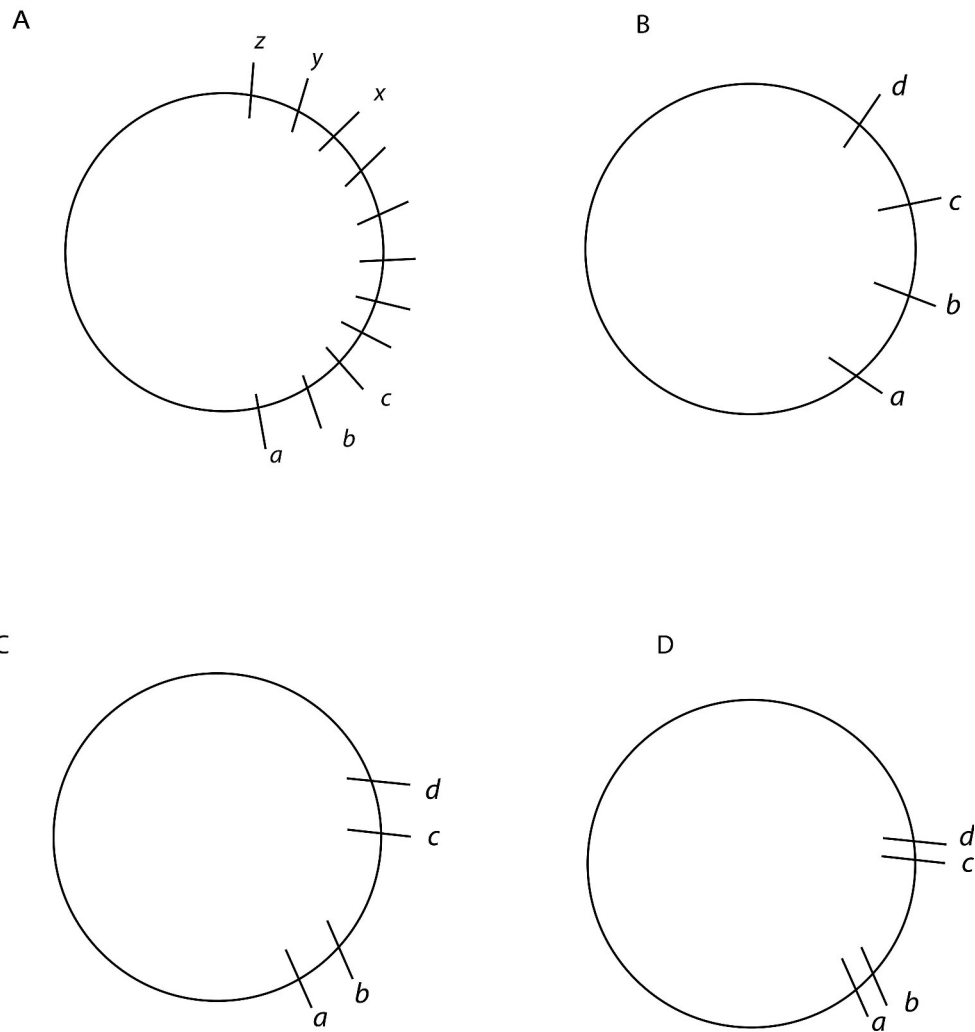


Figure 3. Setal arrangements: (A) perichaetine arrangement; (B–D) lumbricine arrangement: (B) separated in eight rows; (C) widely paired; (D) closely paired.

spermatheca (pl. spermathecae): a genital male organ into which sperm from the partner is deposited during copulation, and stored until release for reproduction, when laying the cocoon. Shape, size and location are important for species recognition. They may be simple (in Lumbricidae, Microchaetidae, Kazimierzidae and Tritogeniidae) or with diverticulae (in Acanthodrilidae and Megascolecidae). In Eudrilidae they are combined with an ovarian duct, forming a specific organ.

tanylobous: a shape of prostomium when a tongue completely divides peristomium (see also other shapes of prostomium: epilobous, prolobous, zygalobous).

testis (pl. testes): male organ for sperm production. May occur only in one segment: 10 (proandric condition) or 11 (metandric); or in two segments, 10 and 11 (holandric).

testis sac: sac containing one testis or a pair of testes.

tubercula pubertatis (sing. tuberculum): the paired glandular swellings located near the ventro-lateral margins of the saddle-shaped clitellum. They are of various sizes and shapes; on immature species they may not be completely developed, or may be absent.

typhlosole: a longitudinal fold in the gut projecting into its lumen; shape and commencement are species features.

zygalobous: a shape of prostomium where the lobe is just like the extended little part of the peristomium. (cf. **epilobous**, **prolobous**, **tanylobous**).

CHAPTER 1

Introduction

1.1 Rationale for research

South African earthworm fauna was first given attention by Rapp (1849) when he described the genus *Microchaetus* from specimens collected from the Cape. The genus *Tritogenia* was described by Kinberg (1849) whilst *Geogenia* was described by Kinberg in 1867 and revised by Plisko in 2006. Later, Plisko (1992, 1996, 2006) described *Proandricus*, *Michalakus* and *Kazimierzus*; all under Microchaetidae s. lato. The number of genera in Microchaetidae increased and it became a “catch-all” taxon. Partial revisions by Plisko (2013) have attempted to solve the taxonomic problems in this family.

The genus *Kazimierzus* occurs in the Western and Northern Cape provinces in South Africa, possibly extending into Namibia; its distribution does not overlap with any other microchaetid genus (Plisko 2012). *Kazimierzus* comprises 21 valid species; the distribution of the species is poorly known for most of them have only been collected from their type locality and some being represented by a single specimen only (for example, *Kazimierzus peringueyi* and *Kazimierzus sirgeli*). The members of the genus prefer undisturbed habitats and have low natural dispersal ability (Plisko 1995, 2000; Nxele 2014). The distribution pattern observed in *Kazimierzus* may be influenced by the type of soil, vegetation (Succulent Karoo Biome) or habitat transformation although this has never been tested. All the species have been described using traditional morphology-based approach.

Traditional morphology based taxonomy is difficult in this group because in some species morphological characters overlap and diagnosis is based on subtle changes while in other species morphological characters are distinct. It is possible that some known species names are synonymous. Western and Northern Cape have been poorly sampled (Plisko 2006) and consequently there is a high possibility that new *Kazimierzus* species, especially along the Atlantic area can be found. This makes a revision of this group a necessity.

Recognising the difficulty in morphological diagnoses of some species, the revision of *Kazimierzus* includes both morphological and molecular genetic characters. The revision of

this genus gives greater understanding of the species diversity and geographical distribution of constituent taxa, for example, checking if certain species extend their ranges to Namibia. This information is vital for conservation planning, given earthworms role as potential bio-indicators in soil ecosystems.

1.2 Aim

This study aims to provide a taxonomic revision of *Kazimierzus* based on the integrative morphological and molecular approach.

1.3 Objectives

The objectives are:

1. to evaluate validity of described species and provide an identification key to all species of *Kazimierzus*;
2. to determine the distribution of the genus *Kazimierzus*, in order to provide reliable data on species diversity, geographical distribution in Southern Africa and factors contributing to their distribution limits; this information is vital for conservation planning because earthworms can be used as bio-indicators of soil ecosystem health and
3. to investigate phylogenetic relationships within the genus.

1.4 Thesis structure

Each chapter is self-contained, with an introduction, materials and methods, taxonomy/results and discussions.

Chapter 2 discusses the rationale and gives a background of *Kazimierzus*.

Chapter 3 focuses on separating *Kazimierzus* species from Microchaetidae (s. lato) and accommodating them into a new family, Kazimierzidae.

Chapter 4 describes new species.

Chapter 5 is devoted to thorough descriptions of all species and provides a key to species identification.

Chapter 6 uses molecular data to compliment morphological data in assessing the validity of species and evaluating their relationships.

Chapter 7 discusses the use of predictive species niche model to predicts the distribution of *Kazimierzus hamerae* to determine the possible boundaries of this taxon.

Chapter 8 provides common names for all *Kazimierzus* species so that they may be used widely by general public and may be incorporated into conservation planning as well as into environmental studies.

Chapter 9 provides the main conclusions of the study and proposes direction of future research.

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CHAPTER 2

The study background

2.1 Introduction

South Africa is one of the world's megadiverse countries in terms of biodiversity (Steenkamp and Smith 2006). A large proportion of the economy is supported by biodiversity and many people rely on ecosystem services for food, water, shelter, jobs and medicine. Several ecosystem services, for example, nutrient cycling or soil structure maintenance (Barrios 2007; Lavelle et al. 2006) depend on soil. Soil biodiversity is the driving force behind the regulation of these services (Lavelle et al. 2006). It is therefore important for soils to remain healthy to support human activities; however, the contribution of soil fauna to soil health is often overlooked (Louw et al. 2014). Soil fauna in South Africa are poorly understood (Janion-Scheepers et al. 2016) with only well-known groups receiving most attention. The increase in environmental pressures means that biodiversity research is inevitably important and more research is needed to manage ecosystem services and their functioning (Hamer 2010; Crouch and Smith 2011). Knowledge of soil fauna is important for sustainable land use and food security.

Among soil-dwelling organisms, earthworms are known to contribute more to the soil macroinvertebrate biomass (Edwards 2004; Decaëns et al. 2013). Their influence in the soil ecosystem is well documented (Lavelle 1988; Lavelle et al. 2006; Bhadauria et al. 2000; Shuster et al. 2002; Salome et al. 2011). Lavelle et al. (2006) reported that earthworms convert large pieces of organic matter into rich humus in form of casts, thus improving soil fertility and quality, and influence soil formation. As such, nutrients that are released from decomposition of organic matter, including nitrates and phosphates, become available in an accessible form to plants and other organisms (Lavelle et al. 2006; Pey et al. 2014). Although their contribution is well known, there is a paucity of knowledge pertaining to earthworm characterisation and identification. Given the gap in knowledge of the taxonomy of earthworms, there is a high probability of finding novel species. Earthworms are vulnerable to habitat transformation due to their poor dispersal ability which results in high levels of

endemism. The distribution of species in Southern Africa is also poorly understood hence the urgency to describe earthworm diversity and their distribution patterns.

However, earthworm fauna in South Africa has been well studied compared to other African countries (Csuzdi et al 2015). Although the South African fauna is better studied, the systematics studies of most taxa are incomplete.

2.1.1 Taxonomic characters used in the taxonomic classification of indigenous South African earthworms

(amended from Plisko 2006a)

External characters include: body dimension (length and width) and shape; body colour; number of segments; body segmentation; setae arrangement; shape and location of nephridial pores; position, shape and location of female and male pores; location and number of spermathecal pores; papillae presence or absence, number and shape of papillae and the connection with genital glands; and the location and shape of clitellum and tubercula pubertatis.

Internal characters include: specific thickness of septa, whether thickened or thin; location and shape of gizzard; shape and position of calciferous glands; shape and initiation of intestine; initiation and termination of typhlosole; dorsal blood vessel with its dorso-ventral vessels location; number, shape and location of nephridia; shape and location of ovaria; confirmation of holandric character, position, shape, enclosed or free state of male funnels; location, shape and characteristics of seminal vesicles; shape, number and location of spermathecae; and shape, location and characteristics of genital glands. An illustration of these characters is in appendix (Figure A1).

2.1.2 *Kazimierzus* Plisko, 2006, current taxonomic position

The genus *Kazimierzus* has 25 species (*K. alipentus* (Plisko, 1998), *K. circulatus* (Plisko, 1998), *K. crousi* (Pickford, 1975), *K. davidi* (Plisko, 1998), *K. franciscus* (Pickford, 1975), *K. guntheri* (Pickford, 1975), *K. hamerae* (Plisko, 1998), *K. imitatus* (Plisko, 1998), *K. kleinoodi* Nxele & Plisko, 2017, *K. ljunstroemi* (Pickford, 1975), *K. metandrus* (Plisko, 1998), *K.*

nietvoorbiji Nxele & Plisko, 2017, *K. nieuwoudtvillensis* Nxele & Plisko, 2017, *K. obscurus* (Plisko, 1998), *K. occidualis* (Plisko, 1998), *K. occiduus* (Plisko, 1998), *K. pauli* (Plisko, 1998), *K. pearsonianus* (Pickford, 1975), *K. pentus* (Plisko, 1998), *K. peringueyi* (Michaelsen, 1913), *K. phumlani* Nxele & Plisko, 2017, *K. rosai* (Michaelsen, 1908), *K. senarius* (Plisko, 1998), *K. sirgeli* (Plisko, 1996) and *K. sophieae* (Plisko, 2002)). All the species have been recorded only in the Western and Northern Cape Provinces, South Africa, along the Atlantic coast (Figure 1).

Kazimierzus peringueyi and *K. sirgeli* are known only from one type specimen while 64 % species (*alipentus*, *circulatus*, *davidi*, *guntheri*, *imitatus*, *kleinoodi*, *lungstroemi*, *nietvoorbiji*, *nieuwoudtvillensis*, *obscurus*, *occidualis*, *pauli*, *pentus*, *phumlani*, *rosai* and *sophieae*) are known only from their type localities. Almost all the species in this genus are restricted to their type locality.

Morphological convergence among the *Kazimierzus* species is strong. In some species morphological characters overlap with very little morphological differentiation among species. This is not surprising given that in earthworm taxonomy, some anatomical and morphological characters that are used may overlap in various taxa (Fernandez et al. 2012). The reduction of posterior male funnel size and their enclosure in seminal vesicles in *Kazimierzus sirgeli* resulted in incorrect assignment to *Proandricus* (Plisko 2006b). The question then is whether the species names are different or they are synonyms.

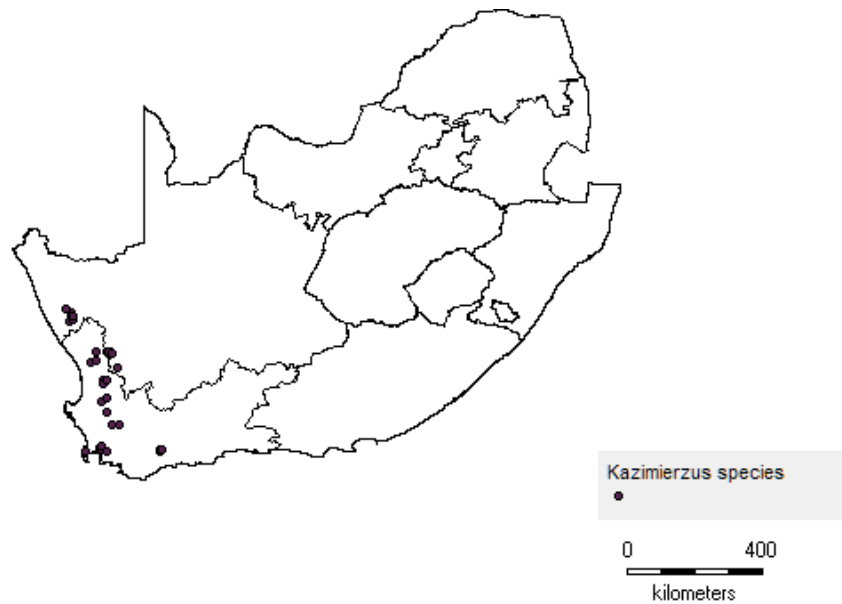


Figure 1. Distribution of *Kazimierzus* in South Africa

The taxonomy of the South African earthworms has been based largely on external and internal morphological characters. However, some of these characters are not taxonomically informative because they show intraspecific variation (Decaens et al. 2013; Loongyai et al. 2011; Briones et al. 2009). The simplicity and plasticity of characters used in identifying species cause challenges in traditional earthworm taxonomy (Novo et al. 2011; Csuzdi and Zicsi 2003). Both internal and external characters change with developmental stages of earthworms (Novo et al. 2011), for example, testes or spermathecae. Furthermore, the major problem with conventional systematics and taxonomic study of earthworms worldwide as mentioned by Blakemore et al. (2010) is the lack of taxonomists, missing type material and the plasticity of morphological characters. Fortunately, South African indigenous megadrile earthworms have many taxa having types. Selected characters that are used to differentiate among the indigenous families are presented in Table 1.

Table 1. Main characters separating the three major indigenous earthworm families

Family	Dorsal blood vessel	Oesophageal gizzard	Excretory system	Testes	Seminal vesicles
Kazimierzidae	Simple	Segment 7	Meganephridia, J-shaped	Holandric, metandric, proandric	One or two pairs extending backwards
Tritogeniidae	Double	Segment 6–7, 9	Meronephridia	Holandric	One or two pairs not extending
Microchaetidae	Double	Segment 7	Meganephridia, V-shaped	Holandric, proandric	One or two pairs rarely extending

2.1.3 Molecular data and earthworms

Advances in molecular phylogenetic techniques have uncovered an unexpected number of new taxa that would otherwise not have been discovered using morphology alone. It has led to improved phylogenetic analyses in earthworms (Minamiya et al. 2011a). Molecular tools seem to be the most appropriate to unravel some of the taxonomic problems (Fernandez et al. 2012). DNA barcoding (which uses COI) has been used progressively for species discovery and species identification (Hebert et al. 2003). The method has many advantages, it can be used at any life stage, the method is faster and cheaper than traditional morphological identifications and it may be easily available to anyone who has knowledge and sequencing facilities (Decaens et al. 2013). However, molecular studies of earthworms are better developed in the Lumbricidae and Megascolecidae (Blakemore et al. 2010). Two studies have used molecular data of South African indigenous species (James and Davidson 2012 and Dominguez et al. 2015). James and Davidson (2012) used South African species when they

studied the relationships among different families of the world whilst Dominguez et al (2015) used South African species as an outgroup in their study of lumbricid species. The fact that only two studies have used molecular data of South African species highlight the urgent need to study molecular phylogenetics of our species.

2.2 Literature survey

A search was conducted using the Web of Science search engine to survey for published literature wherein molecular markers were used to recognise species and to determine evolutionary relationships in earthworms. The molecular phylogenetic study of earthworms reached the 10th anniversary in 2012 and a critique was produced by Chang and James (2011). This present survey was conducted for the period between 2009 and 2017 to highlight the rapid growth in that period. The search terms used included: “earthworms and DNA”; “mitochondria DNA and earthworms”; “nuclear DNA and earthworms” and “earthworm species identification through DNA”. Studies that used DNA sequences for species recognition, phylogenetics and population genetics were selected.

2.3 Results and Discussion

A total of 79 references were recovered (Table 2.2). All taxonomic levels have been studied in earthworms using mitochondria markers, nuclear markers and microsatellites. Most studies are at species and genus levels (Figure 2). Mitochondria DNA markers were more frequently used compared to nuclear with COI being the most popular marker (Figure 3). According to Hebert et al. (2003) the advantages of using COI are that the primers used for this gene enable recovery of its 5’ end from most animals and possesses a wide range of phylogenetic signal. According to Boyer et al. (2012) mitochondrial genes are preferentially targeted because of their greater amplification success due to their higher number of copies per cell compared to single copy nuclear genes. Furthermore, mitochondrial DNA (mtDNA) evolves more rapidly than nuclear DNA, which allows it to be widely used in molecular taxonomy of animals to differentiate species (Loongyai et al. 2009; Otomo et al. 2009; Hebert et al. 2003).

Table 2. References wherein DNA markers were used for species recognition and evolutionary relationships in earthworms.

Source	Taxonomic level	DNA sequence markers
Briones et al. 2009	Family	COI, 16S
Chang et al. 2009	Species	COI
Minamiya et al. 2009	Species	COI, 16S
Novo et al. 2009	Species	COI
Pérez-Losada et al. 2009	Species	COII, 12S, 16S, ND1, tRNA, 28S
Rougerie et al. 2009	Species	COI
Voua Otomo et al. 2009	Species	COI
Blakemore et al. 2010	Species	COI
James et al. 2010	Species	COI
Knott & Haimi 2010	Species	COI
Novo et al. 2010	Species	COI, 16S, 28S, tRNAs
Richard et al. 2010	Species	COI
Bantaowong et al. 2011	Species	COI
Boyer et al. 2011	Species	COI, 16S
Buckley et al. 2011	Species	16S, 28S
Dupont et al. 2011	Species	AFLP
Fernández et al. 2011a	Species	COI, H3
Fernández et al. 2011b	Species	COI, COII, 28S, H3
Loongyai et al. 2011	Species	COI, 16S
Minamiya et al. 2011b	Species	COI
Novo et al. 2011	Genera	COI, 16S, 18S, 28S, H3, H4
Pérez-Losada et al. 2011	Genera	16S, 12S, ND1, COII, 28S
Bartz et al. 2012	Species	COI
Blakemore 2012	Species	COI
Fernández et al. 2012	Species	COI, COII, H3, 28S
James & Davidson 2012	Family	28S, 18S, 16S
Klarica et al. 2012	Species	12S, 16S, COI, COII
Lang et al. 2012	Species	COI
Novo et al. 2012	Species	COI, 16S rRNA, 16S tRNA, H3, H4
Pérez-Losada et al. 2012	Genera	COI
Shen 2012	Species	COI
Shen et al. 2012	Species	EcoRI, TaqI
Zhang et al. 2012	Species	16S, 28S
Donnelly et al. 2013	Species	COI, microsatellites
Prasankok et al. 2013	Species	COI
Shen et al. 2013	Species	COI
Siqueira et al. 2013	Species	COI, 5.8S, ITS1, ITS2
Sun et al. 2013	Species	COI
Voua Otomo et al. 2013a	Species	COI
Voua Otomo et al. 2013b	Species	COI
Chang et al. 2014	Species	COI

Donnelly et al. 2014	Species	COI
Rorat et al. 2014	Species	COI
Shekhovtsov et al. 2014	Species	COX1, COX2, ITS2
Shen et al. 2014	Species	COI
Torres-Leguizamon et al. 2014	Species	COI, microsatellites
Atopkin & Gennady 2015	Species	COI, 16S
Blakemore 2015	Species	COI
Decaëns et al. 2015	Species	COI
Domínguez et al. 2015	Genera	28S, 18S, 16S, 12S, ND1, COII, tRNA Asn, Asp, Val, Leu, Ala, Ser
Dupont et al. 2015	Species	COI
Iwona et al. 2015	Species	COI, ATP6
Jiang et al. 2015	Species	COI
Martinsson et al. 2015	Species	COI
Moreau et al. 2015	Species	COII, 12S, 16S, 28S
Shen et al. 2015	Species	COI
Sun et al. 2015	Species	COI
Szederjesi & Csuzdi 2015	Species	COI
Aspe et al. 2016	Species	COI, 16S, 28S H3
Cervantes et al. 2016	Species	COI
Decaëns et al. 2016	Species	COI
Hong & Csuzdi 2016	Species	COI
Jirapatrasilp et al. 2016	Species	COI, 16S
Kvist 2016	Phylum	COX1
Marchán et al. 2016	Species	COI, 16S, 28S, H3
Paoletti et al. 2016	Species	COI
Rombke et al. 2016	Species	COI
Shekhovtsov et al. 2016a	Species	COX1, ITS2
Shekhovtsov et al. 2016b	Species	COX1
Shen et al. 2016	Species	COI
Spurgeon et al. 2016	Species	COI
Jeratthitikul et al. 2017	Species	COI
Kim et al. 2017	Species	16S, COI
Latif et al. 2017	Species	COI
Martinsson & Erseus 2017	Species	COI, H3
Martinsson et al. 2017	Species	COI, ITS2, H3
Razafindrakoto et al. 2017	Species	COI
Sun et al. 2017	Species	COI, COII, ND1, 12S, 16S, 18S
Wang & Shih 2017	Species	COI

Briones et al. (2009) reported that COI and 16S rRNA have limited discriminatory power above the genus level. However, according to Loongyai et al. (2009); Pop et al. (2003) and Klarica et al. (2012) DNA barcodes based on COI and 16S rDNA genes seem to be suitable

for species and generic delimitation. Furthermore, several studies have demonstrated that COI is useful in revealing cryptic species (Pérez-Losada et al. 2009; Rougerie et al. 2009; Chang et al. 2007).

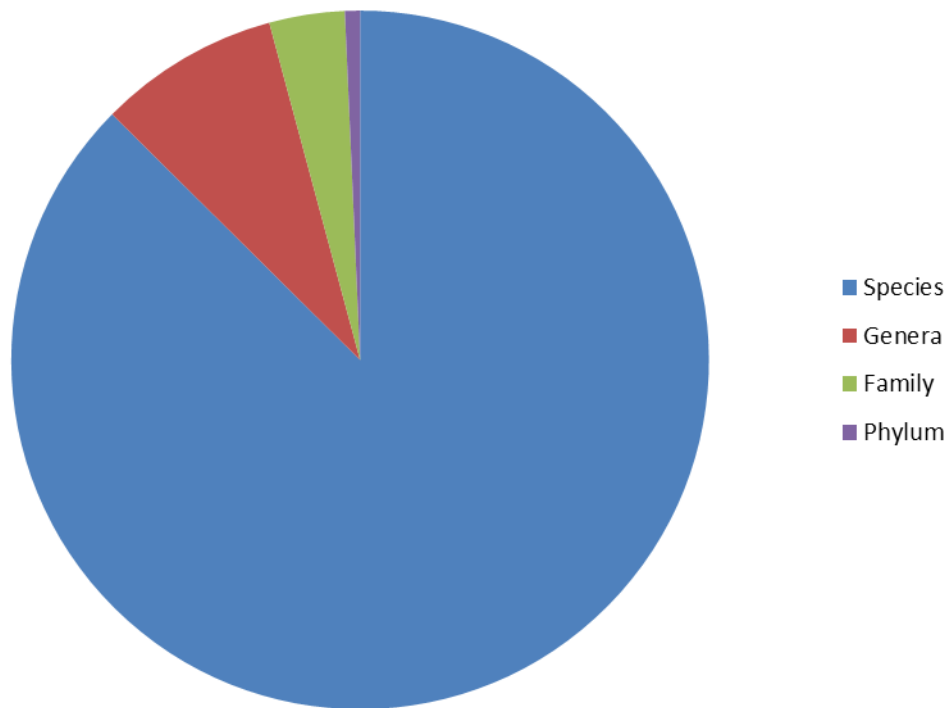


Figure 2. Taxonomic levels studied using DNA markers in earthworms

12S rRNA has been proposed for species identification because it has a lower intraspecific genetic distances compared to protein coding genes COI and COII (Klarica et al. 2012). The studies that included South African species used both mitochondria and nuclear genes and showed that when used together the genes resolve relationships better. Other studies have also used mitochondrial genes above genus level with success (Briones et al. 2009).

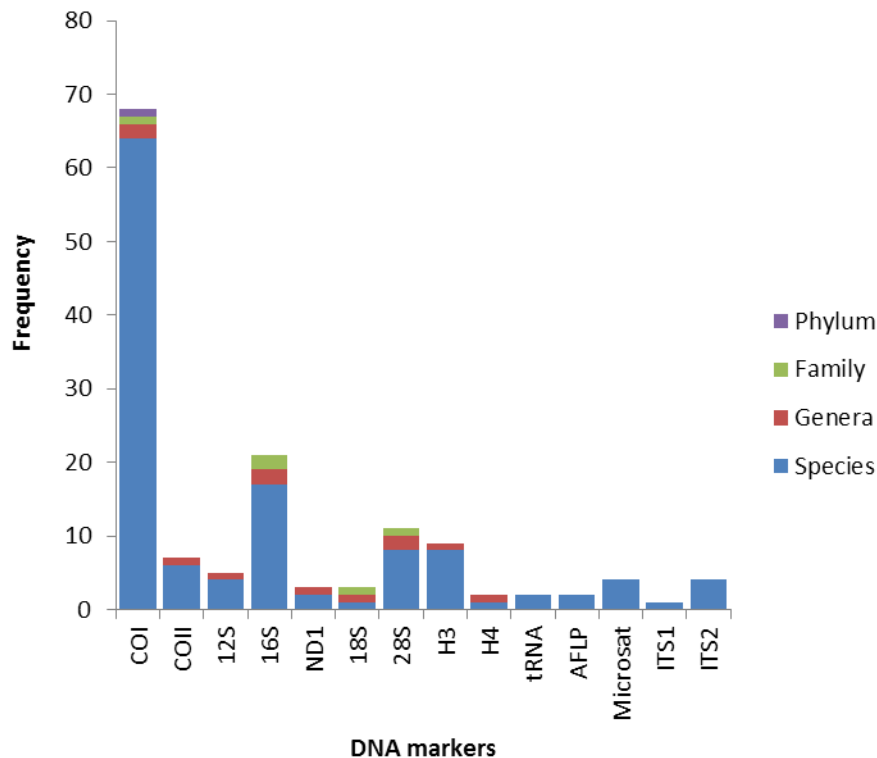


Figure 3. Frequency of use of DNA markers in earthworm taxonomy

Generally, nDNA encoding genes are more conserved relative to the mitochondrial genes, hence, they are generally more suited for studying higher taxonomic levels. As such earthworm taxonomic studies on higher taxonomic level should be done based on 18S rRNA and 28S rRNA (James & Davidson 2012; Pop et al. 2003; Pop et al. 2007). However, these genes have been used at lower taxonomic levels as well (Novo et al. 2010; Novo et al. 2011; Buckley et al. 2011; Pérez-Losada et al. 2009; Marchán et al. 2016; Sun et al. 2017). Both larger 28S rRNA and smaller 18S rRNA are characterized by a combination of highly conserved and rapidly evolving segments (Hwang and Kim 1999). Therefore, variable segments of the 18S rRNA and 28S rRNA highlighted by Raupach et al. (2010) may account for part of their utility at genus and species level.

Substitution rates vary within and among the genes making them suited for both higher and lower level taxonomic studies (Danforth et al. 2005). In addition, six nuclear genes (tRNA, AFLP, H3, H4, ITS1 and ITS2), ND1 as well as microsatellites have been used in earthworm taxonomy at lower taxonomic level.

2.4 Conclusion

The popularity of mtDNA over nDNA in taxonomic studies of earthworms may be attributed to the former's properties or because most studies are focusing on lower level taxonomic categories. Based on the sequence evolution rates, rapidly evolving mtDNA would be better suited to separate recently diverged taxa compared to the slow evolving nDNA regions. There may be a correlation between low taxonomic levels and sampling effort. A study that focuses on a genus or species is unlikely to sample a large geographic range due to the low vagility and strict habitat specialization of earthworms. On the other hand, a taxonomic study on the Clitellata as a whole would require more sampling effort and costs because members of the group may be located in different countries. South Africa is lagging behind, there is a huge gap in molecular studies on indigenous earthworms and more effort is required in this area of research.

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CHAPTER 3

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A new family Kazimierzidae for the genus *Kazimierzus*, earlier recorded to the composite Microchaetidae (Annelida, Oligochaeta)

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3.1 Abstract

A review of the genus *Kazimierzus* Plisko, 2006, based on available type material enriched by study of selected specimens from the earthworm collection gathered at the NMSA and literature, revealed that the species presently accredited to this genus are characterized by unique features and clearly differ from the species of the other genera endorsed to

Microchaetidae. Basing on this discovery the genus *Kazimierzus* is separated from Microchaetidae and Kazimierzidae fam. n. is erected to accommodate the 21 species of this genus. Species accredited to the new family Kazimierzidae are listed, their peculiar characters and specific distribution discussed.

Keywords: *Kazimierzus*, Oligochaeta, South Africa, megadrile, earthworms, indigenous species, Afrotropical region.

3.2 Introduction

Twenty of the studied species of the genus *Kazimierzus* Plisko, 2006 initially were described in the composite genus *Microchaetus* Rapp, 1849 what at that time incorporated the majority of the known South African indigenous earthworm species. The only *K. sirgeli* (Plisko, 1996) was described in *Proandricus* Plisko, 1992 although its exceptional characters were noted, several times underlined (Plisko 1996, 2002, 2003) and the species was transferred to this genus (Plisko 2006). *K. rosai* (Michaelsen, 1908) and *K. peringueyi* (Michaelsen, 1913) are the oldest species of this genus. Over sixty years later, the five species: *crousi*, *franciscus*, *guntheri*, *ljungstroemi* and *personianus* were described by Pickford (1975) also in *Microchaetus*. Plisko (1998) described other species: *alipentus*, *circulatus*, *davidi*, *hamerae*, *imitatus*, *metandrus*, *obscurus*, *occidualis*, *occiduus*, *pauli*, *pentus*, *senarius* and *sophieae* (Plisko 2002), placed them as well in *Microchaetus*, although pointed their distinctive features and debated species' exceptional position in this genus. Re-examination of the distinctive characters observed in the 21 species occurring in limited area of the Western Cape permitted Plisko (2006) to erect for them a new genus *Kazimierzus*. The earlier debates on systematic position of the species accredited to *Kazimierzus* and note on species' restricted geographical occurrence in the Western Cape (Plisko 1998) lead to present review of this genus. Closer assessment of the generic characters observed in the species ascribed to *Kazimierzus* revealed that these, differing from the features occurring in the genera *Microchaetus* Rapp, 1849, s. str., *Geogenia* Kinberg, 1867 and *Proandricus* Plisko, 1992, accredited to Microchaetidae (summarized in Table 1) indicate inconsistency in the family. Thus a new family Kazimierzidae is erected to accommodate these species.

3.3 Material and methods

In order to evaluate the status of the genus *Kazimierzus* the following material was examined: the types of *K. franciscus* (SAM 21542), *K. guntheri* (SAM 21543), *K. ljungstroemi* (SAM 21541) and *K. pearsonianus* (SAM 21544) kindly loaned by Iziko, the South African Museum, Cape Town to the KZN Museum, Pietermaritzburg; the types and other material of fourteen species: *alipentus*, *circulatus*, *davidi*, *hamerae*, *imitatus*, *metandrus*, *obscurus*, *occidualis*, *occiduus*, *pauli*, *pentus*, *senarius*, *sirgeli* and *sophieae* gathered at the NMSA collection. Although the types of *K. crousi* (Pickford, 1975), *K. peringueyi* (Michaelsen, 1913) and *K. rosai* (Michaelsen, 1908) accredited to this genus by Plisko (2006) were not studied, the literature has assisted in establishing their identity and indicated their specific generic features.

The characters investigated were: the dorsal blood vessel; excretory system with its shape of nephridial bladders; seminal vesicles, considering their number, position, shape and extension; male funnels condition; anterior septa: their number and thickness, with special attention to septum 6/7 and 9/10; body shape, dimensions and the number of segments. The earthworms were examined under a stereo dissecting microscope. Diagnoses and species descriptions follow Plisko (1996, 1998, 2002).

Acronyms and abbreviations used in this paper: SAM – Iziko, South African Museum collection, Cape Town; NMSA – KwaZulu-Natal Museum, collection and South Africa database.

3.4 Taxonomy

Kazimierzidae Nxele & Plisko, fam. n.

<http://zoobank.org/3C9091CD-990A-448E-8153-B88671266761>

Type genus. *Kazimierzus* Plisko, 2006: 46.

Diagnosis. Dorsal blood vessel simple throughout the body, rarely enlarging in segments 8 or 9. Excretory system holoic with nephridial bladders proclinate J-shaped. Testes arranged in holandric (male funnels in segments 10 and 11) or metandric (male funnels in segment 11) condition, enclosed or free. Seminal vesicles one or two pairs, confined to one or two

segments (in 11 or 12, or in 11 and 12); the latter pair may be extended backwards, behind segment 12 (sometimes to segment 30). Spermathecae testicular or post testicular. Oesophageal gizzard in 7, muscular. Some of the preclitellar septa 4/5–9/10 variably thickened. Calciferous glands not stalked, in one or two segments (9, 10 or 11): encircling oesophagus with vestigial medial and dorsal grooves, or dorsoventral, paired, with obvious medial and dorsal grooves. Secondary annulation of preclitellar segments present; segment 1 and 2 fused appearing as one segment, 4–9, 10 ringleted with 2 or 3 ringlets, annulated or not. Setae minute, eight per segment in four pairs.

Description. Pigmented or not; alive violetish-grey or grey; preserved whitish grey. Body length not exceeding 350 mm, and 2–15 mm wide at tubercula pubertatis. Average number of segments 100–550. Setae minute; on preclitellar segments visible on various segments or easily visible on papillae, on post clitellar segments in regular rows. Female pores paired, in 14. Clitellum saddle-shaped. Tubercula pubertatis variable in shape and location. Papillae present, located variably. Spermathecal pores located in or behind testis segments. Vasa deferentia paired in holandric, one pair in metandric species. Genital glands various in size and position. Spermathecal ampullae with variably shaped ducts.

Distribution. All species presently accredited to *Kazimierzus* are known from a limited area in the western and south-western Atlantic coast of South Africa. The distribution of these species is poorly known as the most known species have only been collected from their type localities, some species represented only by a holotype. The species are known from variable biotopes: wet, muddy soil, or very dry soil; collected between hard rocks in mountain areas characterized by winter-fall and associated with topography of western escarpment and neighbouring Namaqualand, bordering the Atlantic seaboard. Species occurrence may be expected to continue from the Northern Cape Province to the neighbouring Namibia. The distribution pattern observed in *Kazimierzus* may be influenced by the soil, vegetation (Succulent Karoo Biome) or habitat transformation although this has never been tested.

Remarks. There is currently one genus, *Kazimierzus*, in this family comprising the following species: *K. alipentus* (Plisko, 1998); *K. circulatus* (Plisko, 1998); *K. crousi* (Pickford, 1975); *K. davidi* (Plisko, 1998); *K. franciscus* (Pickford, 1975); *K. guntheri* (Pickford, 1975); *K. hamerae* (Plisko, 1998); *K. imitatus* (Plisko, 1998); *K. ljungstroemi* (Pickford, 1975); *K. metandrus* (Plisko, 1998); *K. obscurus* (Plisko, 1998); *K. occidualis* (Plisko, 1998); *K.*

occiduus (Plisko, 1998); *K. pauli* (Plisko, 1998); *K. pearsonianus* (Pickford, 1975); *K. pentus* (Plisko, 1998); *K. peringueyi* (Michaelsen, 1913); *K. rosai* (Michaelsen, 1908); *K. senarius* (Plisko, 1998); *K. sirgeli* (Plisko, 1996); *K. sophieae* (Plisko, 2002).

Table 1. Diagnostic characters for Kazimierzidae fam. n. and Microchaetidae.

Kazimierzidae fam. n. (with one genus <i>Kazimierzus</i>)	Microchaetidae (with three genera: <i>Microchaetus</i> , <i>Geogenia</i> , <i>Proandricus</i>)
Dorsal blood vessel simple through the whole body length	Dorsal blood vessel double in some preclitellar segments
Seminal vesicles confined to one or two segments or extending backwards up to segment 30	Seminal vesicles confined to one or two segments, not extending backwards
Nephridial bladders J-shaped	Nephridial bladders V-shaped
Septa 6/7 often thickened	Septa 6/7 often missing,
Endemic to limited regions in the western and south-western coastal Atlantic parts of South Africa	Microchaetidae (s.str.) do not occur in the area where are Kazimierzidae

3.5 Discussion

The 21 indigenous megadrile species occurring in south-western Atlantic coast of South Africa accredited by Plisko (2006) to genus *Kazimierzus* evidently differ from the other members of the composite family Microchaetidae (*s. lato*) and deserve a family of their own. A study of types and other material together with findings and opinion of earlier authors confirm clear differences between Microchaetidae (*s. str.*) as it now stands and a new family Kazimierzidae.

Particular characters noted in the studied species are diagnostic and they also attracted attention of earlier researchers. Pickford (1975) found that the seminal vesicles extended backwards, and the 9/10 septum thicker than other septa, and declared this as a character unique to *K. ljunstroemi*. When more material became available, Plisko (1998) observed

that these features occur in most species known from certain areas of south-western Atlantic coast of South Africa (mainly Namaqualand in Western Cape). The backwards extension of seminal vesicles into more than two segments was also found in other species collected in these areas but not in the species from other parts of South Africa (Plisko 1998).

The circulatory system in Kazimierzidae differs from that in the Microchaetidae. It is a single tube through the length of the whole body and was declared as exclusive to *Kazimierzus* by Plisko (2006). The dorsal blood vessel in the Microchaetidae is double in anterior segments, simple when passing septa and forming a cordiform structure in segments 8, 9 noted by Plisko (1991, 1992, 2000, 2002, 2003, 2005, 2006, 2013).

The excretory system in the Microchaetidae is holoic with V-shaped nephridial bladders unlike in studied species where the excretory system although holoic the nephridial bladders are proclinate J-shaped. It should be noted that in *K. ljunstroemi* the nephridial bladders may be not seen clearly as J-shaped although their curve-shape suggests its similarity to a J-shape. The septum 6/7 is thickened in the Kazimierzidae whilst in the Microchaetidae it is much thinner or missing in some microchaetid species. The Kazimierzidae specimens are small to medium size, rarely extending more than 350 mm in length, while in some of microchaetids the body length may reach 2 m in addition the segment number (100–550) differs from the species in the Microchaetidae. Although the majority of the Kazimierzidae species is holandric, the metandric character was observed, whilst in the Microchaetidae no species is metandric.

Pickford (1975) and Plisko (1996, 1998, 1992, 2008) noted these unique features, characteristic for the species occurring in Western Cape but never observed in *Geogenia*, *Proandricus* and *Microchaetus* (*s.str.*) in the composite Microchaetidae. This was highlighted by Plisko (2006) when a new genus *Kazimierzus* was erected for these 21 species. The current review of this genus confirms earlier supporting observations and leads to proposed erection of the new family Kazimierzidae.

In South African soils there are various megadrile species accredited to indigenous families Microchaetidae, Tritogeniidae, Acanthodrilinae, presently newly erected Kazimierzidae and also representatives of foreign Lumbricidae, Glossoscolecidae, Megascolecidae, Eudrilidae, Ocnerodrilidae and Benhamiinae (Nxele et al. 2015, Plisko and Nxele 2015, Janion–Scheepers et al. 2016). The majority of species not native to South African soils occur in

variable biotopes; these may have been transported intentionally or accidentally by humans, or dispersed naturally. These taxa may be found in natural biotopes and also in cultivated, agriculture lands, in undisturbed areas and in polluted areas (Plisko 2010). South African endemic species tend to have a restricted distribution and occur in natural, undisturbed or less disturbed biotopes, mostly in primary grasslands and forests (Plisko 1995, 2000; Nxele 2014). The species presently assigned to Kazimierzidae occur in the area known for endemism in its invertebrate fauna and associated with diverse flora. Kazimierzidae species are endemic to small areas restricted in the western and south-western Atlantic coast of South Africa. This geographical distribution and their unique characters lead to increased interest in erection of a new family to accommodate this genus. The re-assessment of generic characters for the new family Kazimierzidae compared with features occurring in the microchaetid genera *Microchaetus* Rapp, 1849 (*s. str.*), *Geogenia* Kinberg, 1867 and *Proandricus* Plisko, 1992 accredited to Microchaetidae is clearly presented in Table 1.

The restricted range of the Kazimierzidae species makes them vulnerable to habitat transformation due to their poor dispersal ability. The restricted distribution due to specific ecological requirements may lead to speciation. The species distribution of earthworms in Southern Africa is presently poorly known hence the urgency for extended study on earthworm diversity and their distribution patterns. Extensive earthworm collection in the western Atlantic coast may bring more data on this and other taxa. Furthermore, planned molecular studies on the megadriles occurring in the southern Africa may bring clarity to earthworm taxonomy.

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CHAPTER 4

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Four new earthworm species of *Kazimierzus* Plisko, 2006 (Clitellata, Kazimierzidae)

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4.1 Abstract

Four new species of *Kazimierzus* are described from the Western Cape and Northern Cape, South Africa: *K. kleinoodi* sp. n., *K. nietvoorbiji* sp. n., *K. nieuwoudtvillensis* sp. n., and *K. phumlani* sp. n. *Kazimierzus kleinoodi* is distinguished from the other species of this genus by the position of the calciferous glands in 9 and paired spermathecae in five segments. The position of the spermathecae in 11/12–15/16 as well as the position of the calciferous glands in 11 separates *K. nietvoorbiji* from the other new species. *Kazimierzus nieuwoudtvillensis* is distinguished from the others by the position of the clitellum (12–25) and spermathecae in four rows. The combination of the position of the clitellum, three rows of spermathecae and the position of the calciferous glands in 10 distinguish *K. phumlani* from the other species.

Key words: Afrotropical, taxonomy, Oligochaeta, Western Cape, Northern Cape, South Africa, new species.

4.2 Introduction

Although the African earthworm fauna is poorly known, South African earthworms have been studied better. The knowledge of South African earthworm fauna continues to increase as new taxa are being discovered. Apart from the previously known family Microchaetidae, two new endemic South African families have been recognised recently: Tritogeniidae Plisko, 2013 and Kazimierzidae Nxele et al., 2016. The genus *Kazimierzus* was erected by Plisko (2006) for species of Microchaetidae that are restricted to the Western Cape and Namaqualand areas, along the Atlantic region, and that differed in several characters from all other Microchaetidae: dorsal blood vessel simple throughout (vs. double in anterior segments), anterior septa thickened (vs. thin or absent), nephridial bladders J-shaped (vs. V-shaped), and seminal vesicles extending backwards (vs. confined to 1-2 segments). These differences from all other Microchaetidae, together with its restricted area, led Nxele et al. (2016), in a taxonomic revision of the genus, to accommodate the species of *Kazimierzus* in a new family, named Kazimierzidae Nxele et al., 2016.

To-date, 21 species of *Kazimierzus* have been described (Nxele et al. 2016). After a new collection campaign was carried out in the Western Cape and Northern Cape in 2011, the study of the large earthworm collection resulted in the discovery of four new species of *Kazimierzus*, described in this paper.

4.3 Material and methods

Qualitative sampling was carried out in the Western and Northern Cape provinces of South Africa during the rainy season in 2011. Earthworms were collected by digging up soil to one meter depth and by hand-sorting to remove all earthworms in the extracted soil samples. Collected specimens were anesthetized in 20% ethanol solution, fixed in formalin, then preserved in 75 % ethanol; a subset was preserved in 96% ethanol for further DNA analysis. Anatomical examinations were conducted under a stereomicroscope subsequent to dorsal dissection of specimens in order to expose the internal organs. Diagnoses and descriptions followed Plisko (1996, 1998, 2002) and Pickford (1975). Juveniles that were collected with adult type material are added under additional material for DNA analysis. All studied material is currently housed at the KwaZulu-Natal Museum Oligochaeta Collection.

4.4 Results

Family **Kazimierzidae** Nxele & Plisko, 2016

Genus *Kazimierzus* Plisko, 2006

Kazimierzus kleinoodi Nxele & Plisko, sp. n.

(Figure 1)

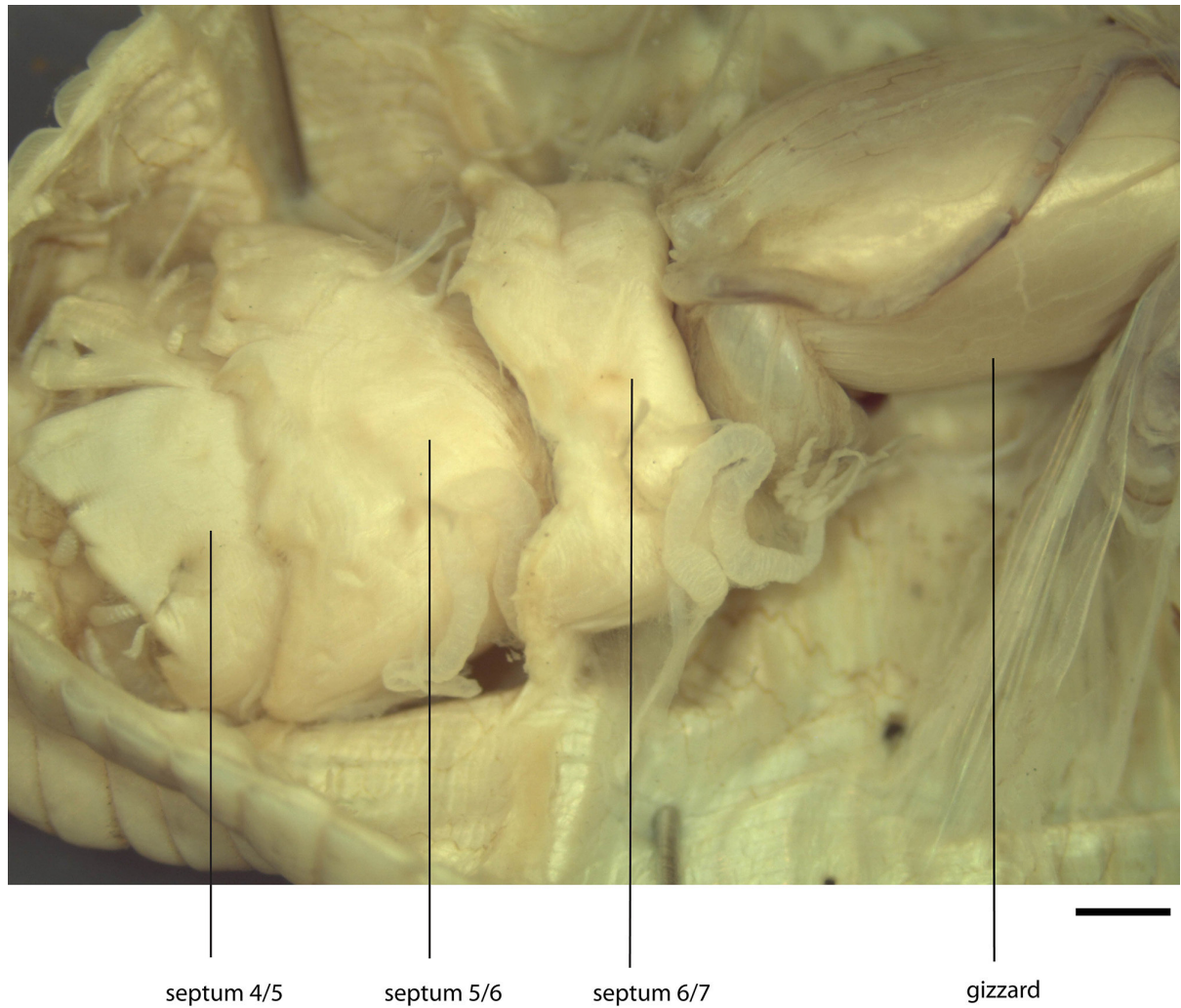


Figure 1. *Kazimierzus kleinoodi* sp. n. Dorsal view, anterior end showing muscular septa and a large well-developed gizzard; anterior end on the left. Scale bar 1 mm.

Description

External: *General:* Body cylindrical. *Colour:* Whitish grey. *Body Dimension:* Holotype: > 110 mm long, abscised, width 8 mm at tubercula pubertatis; paratype: > 250 mm in length, abscised, width 8 mm. *Segment number:* Holotype > 200, paratype > 300. *Prostomium:* prolobous. *Segmentation:* Preclitellar segments with secondary annulations: segment 1 and 2 simple, 3 with superficial line, 4–6 divided by one furrow, the anterior annulus again subdivided giving four annuli per segment; 7, 8, 9 also with annuli subdivided to make a total of 5, 6, 8 annulations, respectively. *Setae:* aa<bc: ab=cd:cd<bc minute, closely paired especially on papillae. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* Not observed, presumably at intersegmental furrows of 12/13–16/17, corresponding to the number and location of the spermathecae. *Clitellum:* Saddle-shaped on 12–27, 28, segmented, appears as a band of discolouration. *Tubercula pubertatis:* Not clear on 17,18–21. *Papillae:* On 10–28 or 10–26 on paratype.

Internal: *Septa:* 4/5, 5/6, 6/7 thickened, muscular (Fig. 1); other septa thin. *Gizzard:* Well developed in 7, globular, muscular. *Calciferous glands:* In 9, fused dorsally. *Intestine:* Origin in 12. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, male funnels in separate sacs, closely paired. *Seminal vesicles:* Very small, one pair in 11. *Spermathecae:* Near cd setal line, one pair per segment in 12–16 near intersegmental furrows 12/13–16/17, one pair per segment, small, round-shaped with long diverticulum. *Genital glands:* Present on 10–26 or 28.

Etymology: Refers to the type locality, Kleinoed Farm.

Material examined: South Africa: Western Cape: Holotype: Stellenbosch, Kleinoed Farm near stream in wet seepage area (33.99646S 18.87353E), 199 m asl, 30.viii.2011, one clitellate, NMSA/Olig.04987b; Paratype: one clitellate, NMSA/Olig.04987a; Additional material: five juveniles, NMSA/Olig.04987c,e. All material collected in one locality by JDP, S James, G Schultz.

Remarks: This species is similar to *K. pearsonianus* (Pickford, 1975) with muscular septa at 4/5–6/7 but differs by the position of calciferous glands in segment 9 (in 10 in *K.*

pearsonianus). *K. kleinoodi* sp. n. has five pairs of spermathecae whilst *K. pearsonianus* has three. Furthermore, the clitellum in *K. kleinoodi* is on 12–27 and extends slightly to 28 in the paratype, but it covers segments 11–28 in *pearsonianus*. *K. nietvoorbiji* sp. n. and *K. nieuwoudtvillensis* sp. n. (see below) also have thickened septa 4/5–6/7 but differs from *K. kleinoodi* in the number of spermathecae (5, 6, 4 respectively). Some of the characters specific for this species are indicated in Table 1.

***Kazimierzus nietvoorbiji* Nxele & Plisko, sp. n.**

Description

External: *General:* Body cylindrical. *Colour:* Pale grey. *Body dimension:* Holotype: > 145 mm, abscised, width 9 mm at tubercula; paratypes: 145–282 mm in length, width 4–6 mm. *Segment number:* Holotype > 260, paratypes > 290. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations: segments 1–3 simple with grooves, segments 4–7 with four annuli of same size, segments 8 and 9 with five primary annuli, each subdivided to make a total of 10 annulations; from segment 10 on and in postclitellar randomly annulated. *Setae:* aa<bc: ab=cd small and closely paired. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* Not observed. *Clitellum:* Saddle-shaped on 12, 13–28, segmented, dorsal borders well marked. *Tubercula pubertatis:* On 17, 18–26, not clearly marked. *Papillae:* On 8–30 as small swellings around setae.

Internal: *Septa:* 4/5, 5/6, 6/7 thickened, muscular; other septa thin. *Gizzard:* Well-developed in 7, globular, muscular, attached to a large crop which is also in segment 7. *Calciferous glands:* In 11, fused dorsally. *Intestine:* Origin in 13. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, male funnels in separate sacs, closely paired. *Seminal vesicles:* In 11 and 12, the pair in 11 close to the calciferous gland. *Spermathecae:* Near *cd* setal line, one pair per segment close to intersegmental furrow 10/11–15/16 in holotype, 11/12–15/16 in adult paratypes, one pair per segment, small round-shaped. *Genital glands:* Present, different shapes and sizes on 10–28 or 29 or 30.

Etymology: Refers to the type locality, Nietvoorbij Research Farm.

Material examined: South Africa: Western Cape: Holotype: Stellenbosch, Nietvoorbij Research Farm, hillside in native bush (33.9079S 18.86883E), 259 m asl, 31.viii.2011, one clitellate, NMSA/Olig.04988a; Paratypes: one clitellate, NMSA/Olig.04988d; four clitellate, NMSA/Olig.04802; Additional material: two juveniles NMSA/Olig.04988b,c. All material collected in same locality by JDP, S James, T Nxele, P Madonda.

Remarks: This species is similar to *K. franciscus* (Pickford, 1975) and *K. crousi* (Pickford, 1975) in the position of calciferous glands. However, *K. nietvoorbiji* differs from the two species in the position of the clitellum (14–23 in *K. crousi*, 13,14–26 in *K. franciscus* and 12, 13–28 in *K. nietvoorbiji*) and number and position of spermathecae (13/14–16/17 in *K. crousi*, 13/14–15/16 in *K. franciscus* and 10/11–15/16 in *K. nietvoorbiji*). Some of the characters specific for this species are summarized in Table 1.

Table 1. Comparison of *K. kleinoodi* sp. n., *K. nietvoorbiji* sp. n., *K. nieuwoudtvillensis* sp. n., and *K. phumlani* sp. n. with morphologically similar species.

Species	Clitellum	Thickened septa	Calciferous glands	Spermathecae	Seminal vesicles	Segments 1 and 2
<i>K. kleinoodi</i> sp. n.	12–27,28	4/5–6/7	in 9, fused	paired, 12/13–16/17: 5 segments	11–12	not fused
<i>K. pearsonianus</i> (Pickford, 1975)	11–28	4/5–6/7	in 10, fused	multiple, 13/14–15/16: 3 segments	11–13	not fused
<i>K. nietvoorbiji</i> sp. n.	12,13–28	4/5–6/7	in 11, fused	paired, 10/11–15/16: 6 segments	11–12	not fused
<i>K. franciscus</i> (Pickford, 1975)	13,14–26	4/5–6/7	in 11, fused	multiple, 13/14–15/16: 3 segments	11–12	fused
<i>K. crousi</i> (Pickford 1975)	14–23	4/5–6/7	in 10–11, fused	multiple, 13/14–16/17: 4 segments	11–12	not fused
<i>K. nieuwoudtvillensis</i> sp. n.	12–25	4/5–9/10	in 10–11, fused	multiple, 12/13–15/16: 4 segments	11–15 or 16	fused
<i>K. peringueyi</i> (Michaelsen, 1913)	12,13–25	4/5–8/9	in 10, fused	multiple, 12/13–16/17: 5 segments	11–12	not fused
<i>K. phumlani</i> sp. n.	12–22	4/5–9/10	in 10, paired	multiple, 13/14–15/16: 3 segments	11–12	fused
<i>K. occidualis</i> (Plisko, 1998)	12–22	4/5–5/6, 7/8–9/10	in 10–11, paired	multiple, 13/14–15/16: 3 segments	11–13	fused

Kazimierzus nieuwoodtvillensis Nxele & Plisko, sp. n.

(Figure 2)

Description

External: *General:* Body cylindrical. *Colour:* Light violet dorsally and whitish grey ventrally. *Body dimension:* Holotype: > 215 mm, abscised, width 18 mm at tubercula; paratypes: 175–182 mm in length, width 6 mm. *Segment number:* > 320. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations: segments 1–2 simple and fused, appearing as one segment, 3 simple, segments 4–7 with annulus subdivided to make four annuli of equal size, 8–9 with six annuli. *Setae:* aa<bc: ab>cd closely paired, on papillae, minute. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* Not observed. *Clitellum:* Saddle-shaped on 12–25, segmented, dorsal borders well marked. *Tubercula pubertatis:* On 17–20, distinct, clearly marked. *Papillae:* On 10–25, 26, slight swellings on setae.

Internal: *Septa:* 4/5, 5/6, 6/7 thickened, muscular, 7/8, 8/9, 9/10 also thickened with 7/8 stronger than 8/9, 9/10; other septa thin. *Gizzard:* Well developed in 7, globular, muscular. *Calciferous glands:* In 10–11, fused dorsally. *Intestine:* Origin in 13. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, male funnels in separate sacs, closely associated with seminal vesicles. *Seminal vesicles:* In 11 and 12, the posterior pair large, extending to 15 on the left side and 16 on right. *Spermathecae:* On both *ab* and *cd* setal lines, multiple per segment in 12/13–15/16, in rows, medium and round, different numbers per segment (Fig. 2). *Genital glands:* Present, different shapes and sizes on 10–26.

Etymology: Type locality, named after Nieuwoudtville town.

Material Examined: South Africa: Holotype: Northern Cape: Nieuwoudtville, south of Nieuwoudtville in flat lands, roadside deep soil (31.43191S 19.13628E), 719 m asl, 05.ix.2011, one clitellate, NMSA/Olig.04990a; Paratype: two clitellate, NMSA/Olig.04796;

Additional material: four juveniles, NMSA/Olig.04990b, c. All material collected in one locality by JDP, S James, T Nxele, P Madonda.

Remarks: This species is similar to *K. peringueyi* (Michaelsen, 1913) in appearance but differs in spermathecae; they are in five segments in *peringueyi* but in four in *nieuwoudtvillensis* (Table 1). Michaelsen highlighted that the clitellum in *peringueyi* starts partly on 13, in *nieuwoudtvillensis* the clitellum is on 12–25.

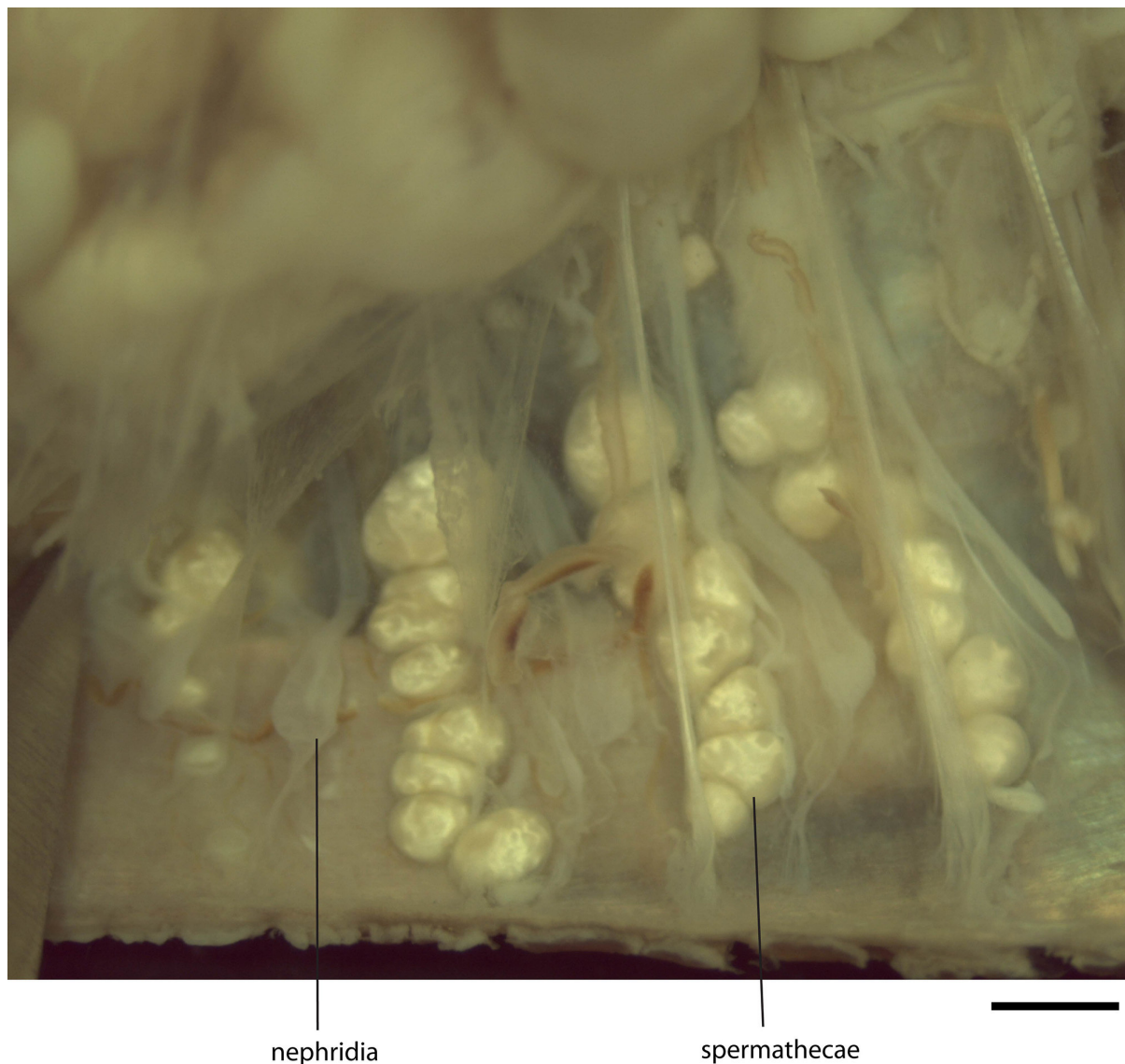


Figure 2. *Kazimierzus nieuwoudtvillensis* sp. n. Internal view with rows of spermathecae; anterior end on the left. Scale bar 1 mm.

Kazimierzus phumlani Nxele & Plisko, sp. n.

(Figure 3)

Description

External: *General:* Body cylindrical. *Colour:* Whitish grey. *Body dimension:* Holotype: > 60 mm, abscised, width 6 mm at tubercula. *Segment number:* > 105. *Prostomium:* Prolobous, small. *Segmentation:* Preclitellar segments with secondary annulations: segments 1–2 simple and fused, appearing as one segment, 3 simple, segments 4–7 with two annuli, 8–9 with second annuli appearing smaller. *Setae:* aa<bc: ab>cd, closely paired, minute. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* Not observed. *Clitellum:* Saddle-shaped on 12–22, segmented, dorsal borders well-marked. *Tubercula pubertatis:* On 17–20, band in clitellar tissue. *Papillae:* On 13–27.

Internal: *Septa:* 4/5, 5/6 thickened, strong, 6/7, 7/8, 8/9, 9/10 thickened but less so; other septa thin. *Gizzard:* Very small in 7. *Calciferous glands:* In 10, separated dorsally and ventrally. *Intestine:* Origin in 13. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, male funnels in separate sacs, both pairs iridescent in clitellate holotype. *Seminal vesicles:* In 11 and 12, one pair per segment, pair in 12 larger. *Spermathecae:* Between *ab* and *cd* setal lines, multiple per segment in 13/14–15/16, 1 or 2 at each side, round-shaped. *Genital glands:* Large (Fig. 3) on 13–27.

Etymology: Named after Phumlani Madonda (KwaZulu-Natal Museum- technical assistant) who helped in the collection of this species.

Material examined: South Africa: Western Cape: Holotype: Doringbos, 25 km N of Doringbos on R364 (31.77182S 19.29114E), 734 m, 05.ix.2011, one clitellate, NMSA/Olig.04951/2e; Additional material: six juveniles, NMSA/Olig.04951/2b, c. All material collected in the same locality by JDP, S James, T Nxele, P Madonda.

Remarks: This species is similar to *K. occidualis* (Plisko, 1998), Both species have clitellum on 12–22, spermathecae in segments 13/14–15/16. However, *K. occidualis* has calciferous

glands in 10–11 with septum 10/11 in the middle of glands whilst *K. phumlani* has calciferous glands confined to segment 10. In *K. occidualis* the anterior pair of seminal vesicles is confined to segment 11, the posterior pair may extend through 12 and 13, while in *K. phumlani* the anterior pair is confined to segment 11 and the posterior pair to 12. The complex of characters specific for this species is shown in Table 1.

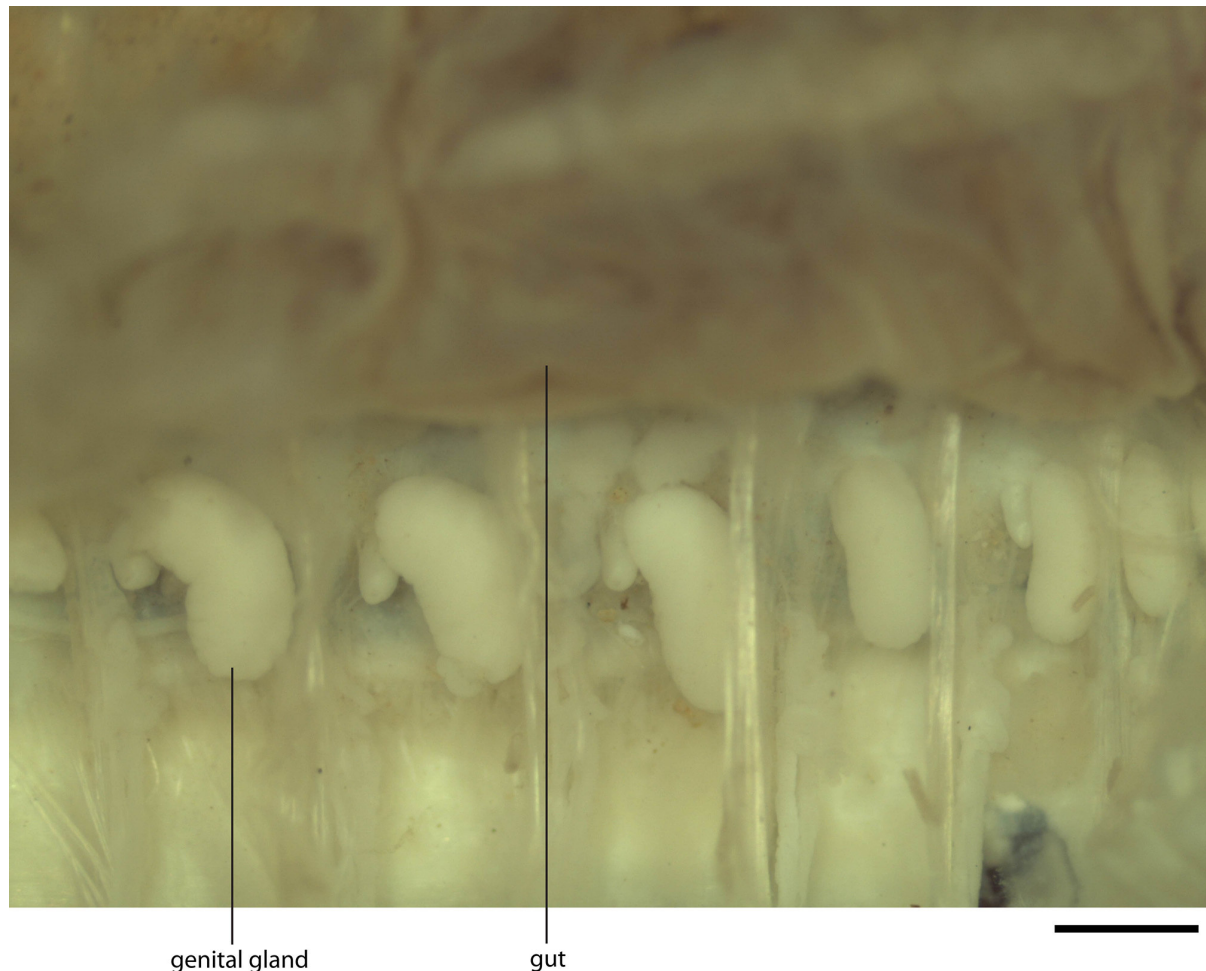


Figure 3. *Kazimierzus phumlani* sp. n. Internal view of large genital glands; anterior end on the left. Scale bar 1 mm.

4.5 Discussion

The species described in the present study occur in the Western Cape and Northern Cape provinces of South Africa, in an area with topographical diversity and complex botanical biota. These new species, having excretory system holoic with J-shaped bladders and dorsal blood vessel simple throughout the body, share the characters diagnostic of the family

Kazimierzidae. The holandric condition, similar to the majority of the previously known species of this genus, also supports their accreditation to this family. In two species, *K. nieuwoudtvillensis* sp. n. and *K. phumlani* sp. n., preclitellar segments have secondary annulations, but segments 1 and 2 are simple and fused; they appear externally as one segment. This peculiar condition has been noted in some of the previously known *Kazimierzus* species. The extension of seminal vesicles behind segment 12 was listed in Nxele et al. (2016) as one of the family-diagnostic characters that separate Kazimierzidae from Microchaetidae s.str. This condition, which occurs not in all species of the family, was found in one of the new species, *K. nieuwoudtvillensis* sp. n.; in the other three new species, seminal vesicles are confined to segments 11 and 12. The backward extension of the seminal vesicle, sometimes as far as to segment 28, has been observed in eleven previously known species: *K. alipentus* (Plisko, 1998), *K. hamerae* (Plisko, 1998), *K. metandrus* (Plisko, 1998), *K. obscurus* (Plisko, 1998), *K. occidualis*, *K. occiduus* (Plisko, 1998), *K. pentus* (Plisko, 1998), *K. pearsonianus*, *K. imitatus* (Plisko, 1998), *K. rosai* Michaelsen, 1908, *K. senarius* (Plisko, 1998). The thickening of septum 9/10 was initially noted in *K. ljunstroemi* by Pickford (1975, there as *Microchaetus ljunstroemi*) and thought to be specific for this species. However, this character was later found in four other species (*K. circulatus* (Plisko, 1998), *K. hamerae*, *K. occidualis* and *K. davidi* (Plisko, 1998)) and the occurrence of thickened septa in post-gizzard septa was validated by Plisko (1998) as customary in *Kazimierzus*. In two of the new species, *K. nieuwoudtvillensis* and *K. phumlani*, septum 9/10 is also thickened, although in the other two new species, *K. kleinoodi* and *K. nietvoorbiji*, all septa behind the gizzard are thin. *Kazimierzus kleinoodi* and *K. nietvoorbiji* are similar in external appearance with clitellum covering segments 12–27,28 and 12, 13–28, but they differ internally in size and location of the calciferous glands, seminal vesicles and spermathecae. The presence of spermathecae in 13/14 in all new species confirms earlier observations (Plisko 1998) in the majority of species accredited to *Kazimierzus*. The similarities and differences of some key characters, e.g. clitellum, septa, spermathecae, calciferous glands and seminal vesicles, noted in four new species and in similar species discussed above, are provided in Table 1.

4.6 Acknowledgements

The KwaZulu-Natal Museum is acknowledged for the support continuously given for the advancement of the study of earthworms in Southern Africa. Dr S. James (University of Iowa, USA), P. Madonda (KwaZulu-Natal Museum) and G. Schultz (the owner of the Kleinood Farm in Stellenbosch), are thanked for their contribution in the field collection in 2011. Csaba Csuzdi and Malalatiana Razafindrakoto are thanked for the comments and suggestions in reviewing this manuscript. The research on the South African megadrile is financially supported in part by the National Research Foundation, South Africa, through the Incentive Funding for Rated Researchers programme.

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CHAPTER 5

A taxonomic revision of the species of *Kazimierzus* Plisko, 2006, (Clitellata, Kazimierzidae) with an identification key to all species

5.1 Abstract

The KwaZulu-Natal Museum's collection is being augmented continuously. The taxonomy though has remained unstable with phylogenetic relationships among taxa being unclear due to low number of morphological characters available for separating the different species. With new material from the Western and Northern Cape provinces, *Kazimierzus* species were revised. The genus was originally described under Microchaetidae s. lato, with most species in *Microchaetus*. A phylogeny was constructed but was not completely resolved. A reduction of one pair of seminal vesicles is noted in *K. alipentus*, *imitatus*, *metandrus*, *pauli*, *pearsonianus* and *sirgeli*. Out of the 25 *Kazimierzus* species known currently, *K. metandrus* has a metandric condition; *K. sirgeli* has a proandric condition whilst the remaining 23 species are holandric. The combination of character states for *metandrus* is not consistent with other *Kazimierzus* species and as such a new genus is suggested to accommodate this species. A functional guide to separate species of *Kazimierzus* is provided.

Keywords: *Kazimierzus*, metandric, proandric, earthworms, taxonomy, Western Cape, Northern Cape, South Africa.

5.2 Introduction

Earthworms are a major part of soil macrofauna. Earthworms contribute a significant part of biomass in the soil (Decaëns et al. 2013; Edwards 2004). They are detritivores that modify the soil and regulate resource availability and thereby act as ecosystem engineers (Jouquet et al. 2006). Given that food production depends on agriculture, a better understanding of biodiversity and the biophysical regulations of soil fertility is needed. Therefore, study and observation of a complex range of environmental indicators is required. Proper knowledge of

the soil organisms is therefore essential for understanding the complexities of soil ecosystems (Cortet et al. 1999; Fragoso et al. 1999).

Despite the important role of earthworms in soil processes, the taxonomy of earthworms is poorly studied and the assignment of taxa is debatable. As such, several species are probably awaiting description and described species require revision using new characters and advanced techniques such as molecular tools. In South Africa earthworm fauna have been studied for many years but Plisko and Nxele (2015) emphasised that the currently known taxa, as well as, those that have not been described need greater attention. The genus *Kazimierzus* Plisko, 2006 contains 25 nominal species. Members of this genus are distributed across the Western and Northern Cape provinces (Plisko 1998; Nxele et al 2016). The species boundaries are controversial and some species are known only from their type localities. In recent years there has been increasing awareness of earthworms in South Africa and there is hope that they will be included in environmental and agricultural studies, however, their identification to species level remains problematic. Here a key to all species of *Kazimierzus* is provided to reduce misidentification of taxa.

5.3 Material and methods

5.3.1 Studied material

All studied material is at the KwaZulu-Natal Museum, Oligochaeta collection. *Kazimierzus crousi* was not examined, Pickford (1975) reported that original material is damaged and in pieces, hence, no loan of this material was requested. Published data were used for the assessment of the specific features. Information from literature was also used for *K. peringueyi* (Michaelsen, 1913) and *K. rosai* (Michaelsen, 1908), although Michaelsen (1913) reported that the type specimen of *K. peringueyi* is in the South African Museum, the material has not been found there. Anatomical examinations were conducted under a stereomicroscope subsequent to dorsal dissection of specimens in order to expose the internal organs. Characters constituting this family are discussed in chapter three and a glossary is provided. Colour of specimens refers to the colour of alcohol preserved specimens.

The collectors' abbreviations are as follows: BRS – B.R. Stuckenberg; JDP – J.D. Plisko; TL – T. Liversage.

5.3.2 Character scoring

All specimens were examined using a Wild Heerbrugg stereo-microscope and identified according to the classifications by Plisko (1996, 1998, 2002, 2006), Pickford (1975) and Michaelsen (1908, 1913). The following characters were studied: body length, number of segments, prostomium, segmentation, setae, nephridial pores, female pores, spermathecal pores, clitellum, tubercula pubertatis, papillae, septa, gizzard, calciferous glands, intestine, typhlosole, blood vessels, nephridia, testicular funnels, seminal vesicles, spermathecae and genital glands. Eight morphological characters were scored for 25 *Kazimierzus* species.

5.3.3 Data analysis

A phylogeny was constructed for this group using morphological characters (Table 1). *Microchaetus papillatus* (family Microchaetidae) was used as an outgroup, based on the material housed at the KwaZulu-Natal Museum (NMSA/OLIG. 05012). The character matrix was analysed with PAUP* 4.0b10 (Swofford 2003) software package. The parsimony uninformative characters were excluded and 1000 bootstraps were performed. The maximum number of trees found per bootstrap replicate was limited to 500. For each bootstrap replicate tree search, 10 different starting trees were used to start branch swapping. Each of these trees was produced with a random taxon addition order. The bootstrap consensus tree (majority-rule consensus tree) was computed from the best trees found.

Table 1. Characters and character states used in the phylogenetic analysis with absent/present states.

1	Position of calciferous glands: 0 – in 10; 1 – not in 10
2	Calciferous glands: 0 – fused; 1 – paired
3	Seminal vesicles: 0 – extend backward; 1 – not extending
4	Seminal vesicles: 0 – one pair reduced; 1 – no reduction in pairs
5	Position of spermathecae: 0 – in three segments; 1 – in more than three segments
6	Number of spermathecae: 0 – a pair per segment; 1 – multiple per segment
7	Male funnels: 0 – holandric; 1 – not holandric
8	Septa 9/10: 0 – thickened; 1 – thin

5.4 Results

5.4.1 Phylogeny

The parsimony analysis resulted in 100 most parsimonious trees with tree length of the most parsimonious tree = 66 steps (CI = 0.296, RI = 0.672). All characters were parsimony-informative. Bootstrap values above 75 % were considered significant support (Hillis & Bull 1993), while values below 50 % were not shown on the tree. In general, some branches of the cladogram were strongly supported with most branches supported by more than 75 % (Figure 1). The branching of the cladogram is interesting, *K. crousi*, *kleinoodi*, *nietvoorbiji* and *sirgeli* separated from the other species; the branch with *kleinoodi*, *nietvoorbiji* and *sirgeli* is also well supported (99 % bootstrap value). Given that these species are similar, the sharing of the clade by *K. alipentus*, *imitatus* and *pauli* is not surprising.

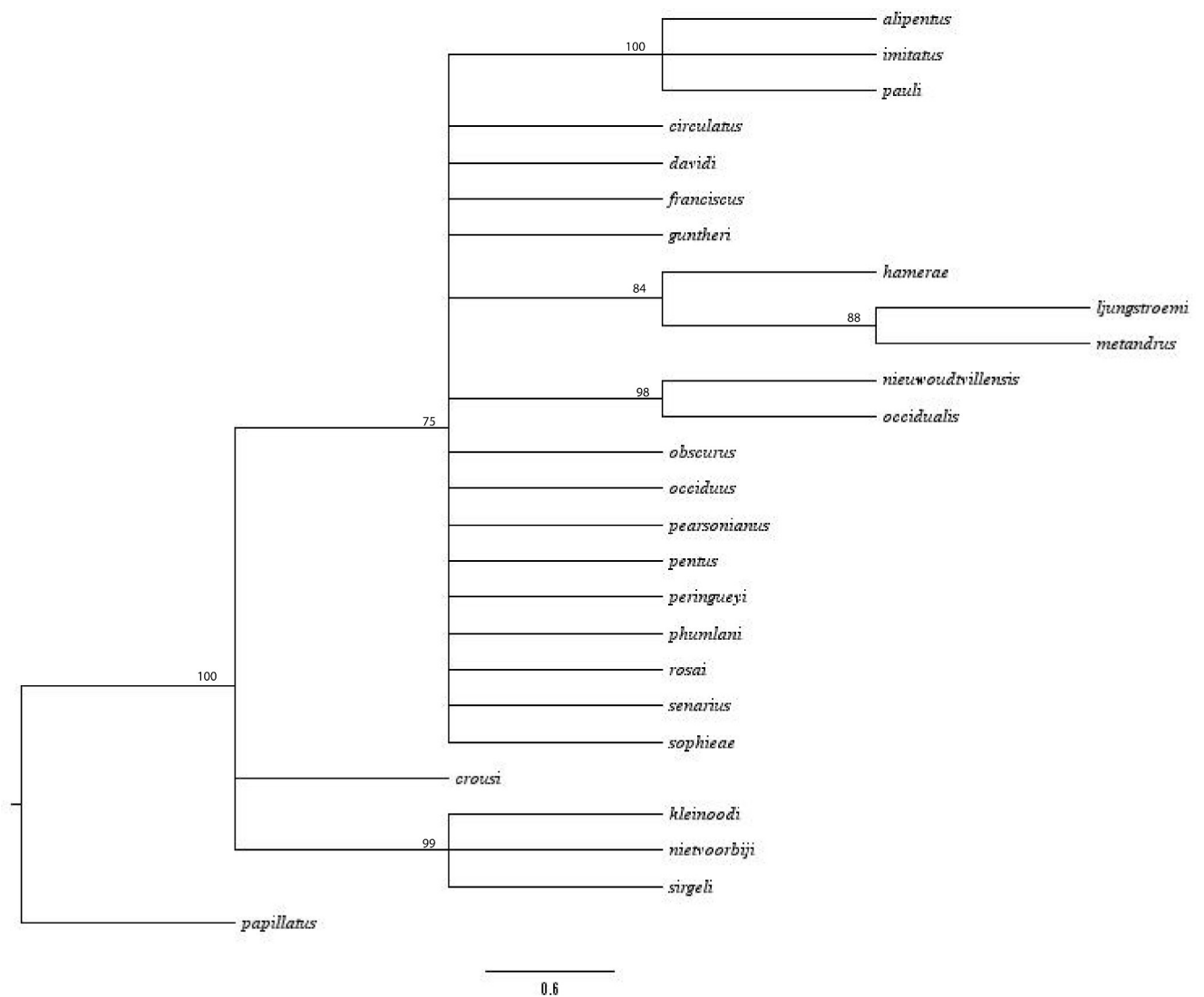


Figure 1. Maximun Parsimony consensus tree of *Kazimierzus* species. Values annotated onto branches represent bootstrap support values, only the bootstrap support values higher than 50 % are presented.

5.4.2 Taxonomy

Family *Kazimierzidae* Nxele & Plisko, 2016

Genus *Kazimierzus* Plisko, 2006

Type species *Kazimierzus hamerae* (Plisko, 1998)

Identification key to the species of *Kazimierzus* Plisko, 2006

1. Seminal vesicles confined to two segments, not extending backwards 2
 - Seminal vesicles not confined to two segments, extends backwards 11
2. Calciferous glands paired 3
 - Calciferous glands fused4
3. Spermathecae in four segments (near septa 12/13–15/16), multiple, clitellum in 12–24,25 *dauidi* (Plisko, 1998)
 - Spermathecae in three segments (near septa 13/14–15/16), multiple, clitellum in 12–22 *phumlani* Nxele & Plisko, 2017
4. Spermathecae multiple, not paired, in 3 segments 5
 - Spermathecae paired or multiple, in more than 3 segments 6
5. Calciferous glands fused in 11, septa 4/5–6/7 thickened..... *franciscus* (Pickford, 1975)
 - Calciferous glands fused in 10, 3 septa thickened *guntheri* (Pickford, 1975)
6. Spermathecae in four segments 7
 - Spermathecae in five or six segments9
7. Three thickened septa (4/5–6/7), calciferous glands fused in 10-11, clitellum on 14–23, spermathecae multiple near septa 13/14–16/17 *crousi* (Pickford, 1975)
 - More than three thickened septa, calciferous glands fused in 0 or 10, clitellum on 12–24, spermathecae paired 8
8. Septa 4/5–9/10 thickened, calciferous glands fused in 10, spermathecae paired near septa 12/13–15/16, clitellum on 12,13–23, male funnels not in proandric condition *circulatus* (Plisko, 1998)
 - Septa 4/5 5/6, 7/8 8/9 thickened, calciferous glands fused in 9, spermathecae paired near septa 12/13–15/16, clitellum on 12–24, male funnels in proandric condition

.....	<i>sirgeli</i> (Plisko, 1996)
9. Spermathecae in five segments (near septa 12/13–16/17), paired or multiple, clitellum between 12–28, three or five thickened septa, calciferous glands fused in 9 or 10	10
– Spermathecae in six segments (near septa 10/11–15/16), paired, clitellum on 12,13–28, three thickened septa (4/5 5/6 6/7), calciferous glands fused in 11.....	
.....	<i>nietvoorbiji</i> Nxele & Plisko, 2017
10. Clitellum on 12–27,28, three septa thickened (4/5 5/6 6/7), calciferous glands fused in 9, spermathecae paired near septa 12/13–16/17	<i>kleinoodi</i> Nxele & Plisko, 2017
– Clitellum on 12,13–25, five septa thickened (4/5 5/6 6/7 7/8 8/9), calciferous glands fused in 10, spermathecae multiple near septa 12/13–16/17	<i>peringueyi</i> (Michaelsen, 1913)
11. Calciferous glands fused	12
– Calciferous glands paired	13
12. Three septa (4/5 5/6 6/7) thickened, clitellum on 11,12–28, calciferous gland fused in 10, spermathecae multiple in three segments (near septa 13/14–15/16), reduction in anterior pair of seminal vesicles	<i>pearsonianus</i> (Pickford, 1975)
– Six septa thickened (4/5–9/10), clitellum on 12–25, calciferous glands fused in 10–11, spermathecae multiple in four segments (near septa 12/13–15/16), anterior pair of seminal vesicles not reduced	<i>nieuwoudtvillensis</i> Nxele & Plisko, 2017
13. Spermathecae paired per segment	14
– Spermathecae multiple per segment	18
14. Male funnels in holandric condition	15
– Male funnels in metandric condition	<i>metandrus</i> (Plisko, 1998)
15. Spermathecae in three segments	16
– Spermathecae in four segments	17

16. Septa 4/5–9/10 thickened, calciferous glands paired in 10, posterior pair of seminal vesicles extends to 23	<i>ljungstroemi</i> (Pickford, 1975)
– Septa 4/5–8/9 thickened, clitellum in 12,13–22,23, calciferous glands paired in 10, posterior pair of seminal vesicles extends to 24	<i>sophieae</i> (Plisko, 2002)
17. Clitellum on 12,13–22,23,24, calciferous glands paired in 10, 4/5–9/10 septa thickened, seminal vesicles extends to 24, spermathecae paired in four segments near septa 12/13–15/16	<i>hamerae</i> (Plisko, 1998)
– Clitellum on 15–24, calciferous glands in 10–11, three septa (4/5 5/6 6/7) thickened, seminal vesicles extends beyond 24, spermathecae paired in four segments near septa 12/13–15/16	<i>rosai</i> (Michaelsen, 1908)
18. Reduction in seminal vesicles	19
– No reduction in seminal vesicles	21
19. Spermathecae multiple in five segments (near septa 11/12–15/16), seminal vesicles extends to 14, clitellum on 12,13–24,25	<i>alipentus</i> (Plisko, 1998)
– Spermathecae multiple in six segments (near septa 11/12–16/17), seminal vesicles extends to 15 or 17, clitellum on 12–28	20
20. Clitellum on 12–26, reduction in posterior seminal vesicles, commence in 10/11 and extends to 15	<i>imitatus</i> (Plisko, 1998)
– Clitellum on 12–28, seminal vesicles extends to 17	<i>pauli</i> (Plisko, 1998)
21. Septa 9/10 thickened	22
– Septa 9/10 not thickened	24
22. Spermathecae multiple in 5 segments near septa 11/12–16/17, seminal vesicles extends one or more segments, calciferous glands paired in 10, clitellum between 12–28	23
– Spermathecae multiple in 3 segments near septa 13/14–15/16, seminal vesicles extends one segment, calciferous glands paired in 10–11, clitellum on 12–22 ...	<i>occidualis</i> (Plisko, 1998)

23. Clitellum in 12–26, spermathecae multiple in five segments near septa 11/12–15/16, seminal vesicles extends to 15 *pentus* (Plisko, 1998)
- Clitellum on 12,13–27,28, spermathecae multiple in five segments near septa 12/13–16/17, seminal vesicles extends one segment *obscurus* (Plisko, 1998)
24. Clitellum on 12,13–23, septa 4/5–8/9 thickened, spermathecae multiple in three segments near septa 12/13–14/15, seminal vesicles extends to 24 *occiduus* (Plisko, 1998)
- Clitellum on 12–26, septa 4/5 5/6, 7/8 8/9 thickened, spermathecae multiple in six segments near septa 11/12–16/17, seminal vesicles extends to 15 *senarius* (Plisko, 1998)

Kazimierzus alipentus (Plisko, 1998)

Microchaetus alipentus: Plisko 1998: 255; 2003: 281; 2006:50; Nxele et al. 2016: 112.

Description:

External: *General:* Body cylindrical. *Colour:* Dorsally dark violet, ventrally whitish-grey. *Body dimension:* Holotype: 193 mm, width 4 mm at tubercula pubertatis; paratypes: 85–185 mm, 4–5 mm wide at tubercula pubertatis. *Segment number:* Holotype 407, paratypes 290–360. *Prostomium:* Prolobous, small. *Segmentation:* Preclitellar segments with secondary annulation: segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–7 with 2 simple ringlets similar in size and appearance, 8–9 with 2 irregularly annulated ringlets, second shorter, 10 and postclitellar segments simple. *Setae:* Closely paired, $ab = cd$, postclitellarly $aa < bc < dd$, first pairs of ab on 5. *Nephridial pores:* Small, more visible on clitellar segments, first pair in intersegmental furrow 3/4. *Female pores:* On segment 14, above ab setae. *Male pores:* Not observed. *Spermathecal pores:* In 11/12–15/16 intersegmental furrows. *Clitellum:* Saddle-shaped, segmented, on 12–25, one paratype had clitella tissue in segment 11, clitellum not clearly bordered anteriorly and posteriorly. *Tubercula pubertatis:* Flat glandular bands, segmented, overlapping clitellum ventrally, on 17–23. *Papillae:* Paired swellings, small, well visible on setae on 10–29.

Internal: *Septa:* 4/5 5/6, 7/8 8/9 thickened moderately, similar in size and appearance, other septa thin. *Gizzard:* In 7, cylindrical. *Calciferous glands:* In 10, separated ventrally and dorsally. *Intestine:* Originates in 13. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment with J-shaped bladders. *Male funnels:* Holandric arrangement, iridescent. *Seminal vesicles:* Starts in 10/11 and extends to 14 and in one paratype it extends to 15, in one specimen the anterior is missing. *Spermathecae:* Near septa 11/12–15/16, in segments 12–16, multiple per segment, small round bladders with thin necks, different sizes and number per segment. *Ovaries:* Not observed. *Genital glands:* Various sizes, round-shaped, associated with *ab* seta and corresponding with papillae, in 10–30.

Material examined: Western Cape: *Holotype:* 7 km SE Cirtusdal (32°36'S:19°01'E), rocky hillside near road R 303, 14 August 1997, clitellate, NMSA/Olig.02644; Paratypes collected together with holotype: 4 clitellate, NMSA/Olig.02645; 6 juveniles, NMSA/Olig.02601. All material collected by JDP & TL.

Remarks: The dimensions are slightly different from the original description and this was observed in most specimens and may be due to the fact that some specimens are in a fragile condition. No new material was collected for this species. This species is known only from the type locality. This species is similar to *pentus*, as the names suggest, they have five rows of spermathecae but differs in that *alipentus* has a reduced pair of seminal vesicles while *pentus* has both pairs well developed.

Kazimierzus circulatus (Plisko, 1998)

Microchaetus circulatus: Plisko 1998: 260; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description:

External: *General:* Body cylindrical. *Colour:* Yellowish-white. *Body dimension:* Holotype: > 100 mm, abscised, width 4 mm at tubercula pubertatis; paratypes: 50–110 mm, clitellate abscised at 50 mm; new material is 70–122 mm long with a width of 4–6 mm at tubercula

pubertatis. *Segment number*: Holotype >155, paratypes 284–320, new material have up to 336 segments. *Prostomium*: Prolobous, small. *Segmentation*: Preclitellar segments with secondary annulation, segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–8 with 2 simple ringlets similar in size and appearance, 9 with 2 ringlets, first longer than second, 10 and the following segments simple. *Setae*: Minute, closely paired, $ab = cd$, $aa > bc$. *Nephridial pores*: Visible above cd setal lines. *Female pores*: Not observed. *Male pores*: Not observed. *Spermathecal pores*: In 12/13–15/16 intersegmental furrows. *Clitellum*: Saddle-shaped, segmented, on 12,13–22,23, clearly bordered anteriorly and posteriorly. *Tubercula pubertatis*: Not distinct from clitellum, on clitella tissue, very faint, on 17–22. *Papillae*: Slight swellings on setae, on holotype on 10–23, on other specimens 10–26.

Internal: *Septa*: 4/5 5/6, 7/8 8/9 9/10 thickened moderately, similar in size and appearance, 6/7 thickened less, other septa thin. *Gizzard*: In 7, cylindrical mostly but elongated in some, muscular, anteriorly softened. *Calciferous glands*: In 10, fused dorsally and ventrally. *Intestine*: Originates in 13. *Typhlosole*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body, in segments 8–10 moderately enlarged compared to anterior part. *Nephridia*: Meganephridia, one pair per segment with J-shaped bladders. *Male funnels*: Holandric arrangement, 2 pairs of large, free funnels, close to seminal vesicles. *Seminal vesicles*: Two pairs in 11 and 12. Large sized pouches showing internal chambering. *Spermathecae*: One pair per segment, small ampullae with thin neck longer than ampulla, near septa 12/13–15/16. Spermathecae iridescent. *Ovaries*: Not observed. *Genital glands*: One pair per segment, large, finger-like glands, in 10–25,26. All associated with ab setae and papillae.

Material examined (Old): Northern Cape: *Holotype*: 19 km NE of Garies, Studer's Pass (30° 24'S:18°05'E) at *ca.* 1100 m, from water seepage at side of the road, 30 August 1989, clitellate, NMSA/Olig.02535; Paratypes collected together with holotype, 1 clitellate abscised and 11 juveniles, another juvenile is in pieces, NMSA/Olig.00943. All collected by BRS.

(New): Northern Cape, Western Cape: Liliefontein, on roadside between Liliefontein and Kamieskroon (30.30223S: 18.05556E) at *ca.* 1180 m, 03 September 2011, 4 clitellate, 3 juveniles, NMSA/Olig.04799; Vredendal (31.65226S: 18.57652E) at *ca.* 6 m, roadside bush in red sand soil, 04 September 2011, 3 clitellate, 2 juveniles, NMSA/Olig.04805; Studer's Pass

(30.42762S: 18.06224E) at *ca* 833 m, wet ravine on S/E side of the road, 03 September 2011, 3 clitellate, NMSA/Olig.04942/3a,b,c. All collected by S James, JDP, T Nxele and P Madonda.

Remarks: Holotype and paratypes are slightly decomposed. Similar to *rosai*, having four pairs of spermathecae with long necks, near septa 12/13–15/16, and calciferous glands encircling oesophagus. Differs in the position of clitellum and the shape of the tubercula pubertatis. This species has been collected in both the Western and Northern Cape.

Kazimierzus crousi (Pickford, 1975)

Microchaetus crousi: Pickford 1975: 42; Reynolds & Cook 1976: 91; Plisko 1992: 339; 1998: 252; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* The preserved specimen is unpigmented. *Body dimension:* > 135 mm, abscised, width 6 mm. *Segment number:* Not known. *Prostomium:* a rudimentary upper lip overhung by segment 1. *Segmentation:* Segments 1, 2 and 3 simple, 4 and 5 divided by one major furrow, the anterior annulus again subdivided, 6–9 divided into two major annuli and each of these divided into two secondary annuli, annulation decreasing posteriorly. *Setae:* Closely paired, on the second annulus, $aa = bc$, $dd = ca$. 0.3 *u* in middle region. *Nephridial pores:* On the anterior margin of the segments below setal line *c*, the first pair on 4. *Female pores:* Not visible, presumably on 14. *Male pores:* Not visible, presumably at 18/19. *Spermathecal pores:* Not visible, presumably at intersegments 13/14–16/17, corresponding to the location of the spermathecae. *Clitellum:* Not clearly indicated, on 14–23. *Tubercula pubertatis:* On 16–21. *Papillae:* On 10–28 (left) or 10–27 (right).

Internal: *Septa:* 4/5 5/6 6/7 strongly thickened. *Gizzard:* In 7. *Calciferous glands:* Fused dorsally and ventrally in 10–11. *Intestine:* Begins in 14. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Undivided, communicating with moniliform "hearts" in 7–11. *Nephridia:* One pair of meganephridia per segment, the tightly coiled tubules of the ental region form a tuft situated immediately behind the septum and close to the mid-ventral line.

Male funnels: Holandric, folded and fluted but clearly not fully developed and without iridescence. *Seminal vesicles*: Two pairs from 10/11 and 11/12 into 11 and 12, small and undeveloped but of equal size. *Spermathecae*: In 13/14–16/17, multiple, one or two at four successive intersegments, the spermathecae are obviously not fully developed. *Ovaries*: Not seen. *Genital glands*: Short, stout tubular or sausage-shaped glands situated immediately beside the ventral setal bundles on 10–28 (left) or 10–27 (right).

Remarks: According to Pickford (1975), the holotype is in a fragile condition. The paratypes were badly damaged and in pieces, no attempt was made to study their anatomy. This species is similar to *franciscus* in the position of calciferous glands but differs in the position of the clitellum.

Kazimierzus davidi (Plisko, 1998)

Microchaetus davidi: Plisko 1998: 263; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description:

External: *General*: Body cylindrical. *Colour*: Anterior segments dorsally dark grey, ventrally whitish-grey. *Body dimension*: Holotype: > 114 mm, abscised, width 5 mm at tubercula pubertatis; paratype > 115 mm, abscised, width 5 mm; juveniles 70–100 mm, 4–5 mm wide. *Segment number*: Holotype > 228, paratypes > 140. *Prostomium*: Prolobous. *Segmentation*: Secondary annulation present on preclitellar segments, segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, nearly as long as the first two segments, 4–6 with 2 simple ringlets, similar in size and appearance, 7–9 with 2 irregularly annulated ringlets, 9 with second ringlet shorter than first, 10 and postclitellar segments irregularly annulated. *Setae*: Closely paired, minute, $ab = cd$, postclitellarly $aa < bc < dd$, first pairs of ab on 4. *Nephridial pores*: better visible on clitellar segments, first pair in intersegmental furrow 3/4 below cd setal lines. *Female pores*: On segment 14, large. *Male pores*: Not observed. *Spermathecal pores*: In 4 intersegmental furrows: 12/13 13/14 14/15 15/16, multiple. *Clitellum*: Saddle-shaped, on segments 12–24,25, well developed, segmented, clear borders anteriorly and posteriorly. *Tubercula pubertatis*: Very faint rectangular band, inside clitellar

tissue, on 18–20. *Papillae*: Not observed but may be on 10–26 in association with genital glands.

Internal: *Septa*: 4/5 5/6 muscular, 6/7–9/10 thickened much but less than the anterior septa, other septa thin. *Gizzard*: In 7, cylindrical. *Calciferous glands*: Paired in 10, separated widely ventrally, with narrow groove dorsally. *Intestine*: Originates in 13. *Typhlosome*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia one pair per segment of tightly coiled lateral tubules, with J-shaped bladders. *Male funnels*: Holandric, small funnels in 10 and 11, iridescent. *Seminal vesicles*: Small sacs, similar in size and shape, confined to segments 11 and 12. *Spermathecae*: Near septa 12/13–15/16, in four segments, multiple, number and shape vary. *Ovaries*: Not observed. *Genital glands*: Variable shapes and sizes, some large others small, in 10–26.

Material examined: Northern Cape: *Holotype*: 16 km E of Kamieskroon (30°13'S:17°55'E), near road R 355, 17 August 1997, clitellate, NMSA/Olig.02627; Paratypes collected together with holotype: 1 clitellate abscised, NMSA/Olig.02626; 5 juveniles, NMSA/Olig.02628. All collected by JDP & TL.

Remarks: Papillae and genital glands in the original description are on 10–23 but I found the genital glands to be on 10–26 in the present study. Some juveniles are damaged, there are posterior pieces that could not be assigned to anterior parts. This species is similar to *rosai* in four segments with spermathecae but differs in that *davidi* has spermathecae in rows per segment while *rosai* has one pair per segment. Furthermore *davidi* has seminal vesicles confined to segments 11 and 12, and not extending backwards as in *rosai*.

***Kazimierzus franciscus* (Pickford, 1975)**

Microchaetus franciscus: Pickford 1975: 46; Reynolds & Cook 1976: 103; Plisko 1992: 339; 1993b: 230; 1998: 339; 2003: 281; 2006: 50. Nxele et al. 2016: 112.

Description

External: *General*: Body cylindrical. *Colour*: whitish-grey. *Body dimension*: Paratype: > 270 mm, abscised, width 5 mm. *Segment number*: > 474. *Prostomium*: Prolobous. *Segmentation*:

Secondary annulation present on preclitellar segments. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–7 with 2 simple ringlets, 8–9 has ringlets further subdividing into secondary annuli. *Setae*: Closely paired, minute, difficult to trace, only better visible from 10, $aa=bc$, $ab < cd$. *Nephridial pores*: Minute, not easily traced below *c* setal line. *Female pores*: Not observed. *Male pores*: Not observed. *Spermathecal pores*: Not observed. *Clitellum*: Saddle-shaped, on segments 12–25,26 on paratype but on the additional material on 12,13–28,29, well developed. *Tubercula pubertatis*: On 17–20, not distinct. *Papillae*: Could not be traced but on tubercula pubertatis setae is slightly raised.

Internal: *Septa*: 4/5 5/6 6/7 thickened, other septa thin. *Gizzard*: In 7. *Calciferous glands*: In 11, fused. *Intestine*: Originates in 13. *Typhlosome*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia, one pair per segment of tightly coiled lateral tubules, with J-shaped bladders. *Male funnels*: Holandric, in 10 and 11. *Seminal vesicles*: Two pairs of medium size sacs, confined to segments 11 and 12. *Spermathecae*: Near septa 13/14–15/16, in three segments, multiple, size, shape and number is different per segment. *Ovaries*: Not observed. *Genital glands*: Variable shapes and sizes, some finger-like, some bent, in 10–27.

Material examined: Western Cape: Paratype: Swellendam District, Cape Province, Duivel's Bosch in the Langebergen near Swellendam, in loamy soil among rocks in bank of stream, mostly deep down between rock crannies, 12 December 1926, 1 clitellate, SAM 21542, FW Paish & GE Hutchinson leg; Swellendam (34°02'S: 20°26'E) 20 km W of; hard, dry soil near small reservoir, 29 November 1991, 1 clitellate, 2 semi mature, 9 juveniles, NMSA/Olig.01174. Collected by JDP & A Zicsi.

(NEW): Western Cape: Swellendam, Marloth NR, Duiwelbos Forest (33°59'56.7''S: 20°27'27.4''E), indigenous and rocky, 05 October 2015, 3 semi mature, NMSA/Olig.06959a,b,c; 4 juveniles, NMSA/Olig.06948. All collected by T Nxele, S Xaba & T Nene.

Remarks: The paratype is not in good condition and this may have been due to multiple dissections over the years. The number of segments occupied by the clitellum is more on new material, 12–29 compared to 12–26 on paratype. The intestine also commences in 13 not in

14 as originally stated by Pickford (1975). This species is similar to *crousi* and *nietvoorbiji* in the position of calciferous glands. However, the species differ in the position of the clitellum.

Kazimierzus guntheri (Pickford, 1975)

Microchaetus guntheri: Pickford 1975: 37; Reynolds & Cook 1976: 110; Plisko 1992: 339; 1993b: 235; 1998: 252; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Dark brown dorsally. *Body dimension:* Paratype: > 300 mm, abscised, width 5 mm. *Segment number:* > 500. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments highly annulated, segments 1–3 simple with irregular longitudinal grooves. Segment 4 divided into four primary annuli, the second further subdivided into two secondary annuli, 5 and 6 similar to 4 except that the second, third and fourth primary annuli are all subdivided, 7 and 8 each have five primary annuli. *Setae:* Closely paired, minute, difficult to trace because of annulation, $aa=bc$, $aa < bc$. *Nephridial pores:* Not observed. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* Not observed. *Clitellum:* Not developed on all specimens. *Tubercula pubertatis:* Very faint on 18–25, not distinct. *Papillae:* Could not be traced.

Internal: *Septa:* 4/5 5/6 6/7 thickened, other septa thin. *Gizzard:* In 7. *Calciferous glands:* Fused dorsally and ventrally in 10. *Intestine:* Originates in 13. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, coiled tubules with J-shaped bladders. *Male funnels:* Holandric arrangement, in 10 and 11. *Seminal vesicles:* Two pairs of small sacs confined to segments 11 and 12. *Spermathecae:* Near septa 13/14–15/16, multiple, different size, shape and number per segment. *Ovaries:* Not observed. *Genital glands:* Tubular glands in 10–28.

Material examined (OLD): Western Cape: Paratype: Paarl District, White River Valley. Bain's Kloof, marshy area with sedge-like vegetation by stream, 12 July 1926, immature specimen in three pieces, slightly decomposed, SAM 21543. Collected by E. R. Gunther & G. E. Pickford.

(NEW): Western Cape: Stellenbosch (33.93502S: 18.82794E) at *ca.* 113 m, Riparian area behind Horizon House off Devon Valley Road, 27 August 2011, NMSA/Olig.04986a–g. Collected by S James, JDP, T Nxele & P Madonda.

Remarks: The paratype is in very poor condition, decomposed, it is in pieces and studying its anatomy was difficult. Tertiary annulation is present, this is not common in *Kazimierzus* species. The intestine begins from septum 12/13, starting in segment 13 not in 14 as recorded in the first description by Pickford (1975). This species is similar to *pearsoniunus* by having multi spermathecae in three segments but *pearsoniunus* has reduction in seminal vesicles with only one pair which extends backwards, however, *guntheri* has both pairs but confined to 11 and 12, respectively.

Kazimierzus hamerae (Plisko, 1998)

Microchaetus hamerae: Plisko 1998: 265; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description:

External: *General:* Body cylindrical. *Colour:* Grey with cream white clitellum. *Body dimension:* Holotype: 110 mm long, 4 mm wide at tubercula pubertatis; paratypes 50–190 mm long, 4–5 mm wide at tubercula pubertatis. *Segment number:* Holotype 312, paratypes 294–469. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulation. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–6 with 2 simple ringlets similar in size and appearance, 7 with larger ringlets, 8–9 with 2 irregularly annulated ringlets, in 9, second ringlet is shorter than first, 10 and postclitellar segments simple. *Setae:* Closely paired, minute, $ab = cd$, postclitellarly $aa < bc < dd$. *Nephridial pores:* Clearly visible on clitellar segments, first pair in intersegmental furrow 3/4, much below cd setal lines. *Female pores:* On segment 14 between bc setae. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows 12/13–15/16. *Clitellum:* Saddle-shaped, clearly bordered anteriorly and posteriorly, on 12,13–22,23,24. *Tubercula pubertatis:* Flat, overlapping clitellum on 17–20. *Papillae:* Paired swellings associated with ab setae, on 10–23.

Internal: *Septa*: 4/5 5/6 much thickened, similar in size and appearance, 7/8 8/9 thickened slightly less than those of anterior segments, 9/10 slightly thickened on some specimens, other septa thin. *Gizzard*: In 7, cylindrical. *Calciferous glands*: Paired in 10, separated ventrally and dorsally. *Intestine*: Originates in 12. *Typhlosole*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia, one pair per segment of tightly coiled tubules with J-shaped bladders. *Male funnels*: Holandric arrangement, iridescence. *Seminal vesicles*: Two pairs of different size and shape, commence at septa 10/11 and 11/12. Anterior pair much smaller than posterior pair and confined to segment 11. Posterior pair form bulging pouches extending from segments 12–24, often different on left and right side. In holotype the sacs of second pair extend on left side to segment 17, on right side to segment 19. *Spermathecae*: Large, ovoid ampullae, with long, slender ducts, which enter body wall in 4 rows, near septa 12/13–15/16. Shape and size vary. *Ovaries*: Not observed. *Genital glands*: Variable in size and shape, correspond with papillae, in 10–23,24.

Material examined: (OLD): Western Cape, Northern Cape: *Holotype*: 10 km W Nieuwoudtville (31°22'S: 19°06'E), near road, from sandy, moist soil, 15 September 1994, clitellate, NMSA/Olig.02650. Paratypes (same data as holotype): 19 clitellate, 10 juveniles, NMSA/Olig.02006; 3 clitellate, NMSA/Olig.02007; 2 clitellate, NMSA/Olig.02008. Holotype and paratypes collected by JDP, BRS & M Hamer. Material collected by JDP & TL: Bokkeveldberge, on top of hill, near road, from muddy bank of local stream, 15 September 1997, 1 clitellate, NMSA/Olig.02651; 12 km and 7 km W of Nieuwoudtville, near road, from soaked soil, 15 September 1997, 4 clitellate, 2 juveniles, NMSA/Olig.02613; 4 clitellate, 2 juveniles, NMSA/Olig.02614; 10 km N Vanrhynsdorp (31°37'S:18°44'E), from dry bed of local stream, 15 September 1997, 5 clitellate, 5 juveniles, NMSA/Olig.02612; 24 km S Garies (30°33'S:17°59'E), near road N 7, from moist, sandy soil, 17 August 1997, 10 clitellate, NMSA/Olig.02623; 5 specimens not fully mature, NMSA/Olig.02624; 7 juveniles, NMSA/Olig.02625; 50 km N Clanwilliam (32°10'S:18°55'E), from sandy, moist soil, 18 August 1997, 1 clitellate, NMSA/Olig.02632; 2 abscised, NMSA/Olig.02633; 1 clitellate, NMSA/Olig.02634.

(NEW): Western Cape, Northern Cape: Eendekuil (32.68331S: 18.88159E) at *ca* 113 m, River crossing on R365 near Eendekuil, in road bank and in sandy soil under native bush, 07 September 2011, 1 abscised clitellate, 6 juveniles, NMSA/Olig.04786; Van Rhyn's Pass (31.37131S: 19.04739E) at *ca* 803 m, flats above Van Rhyn's pass, Nieuwoudtville side, 05 September 2011, 1 clitellate, 2 juveniles, NMSA/Olig.04818; Clanwilliam (32.22321S: 18.9217E) at *ca* 124 m, a few meters above water level of reservoir, 06 September 2011, 3 clitellate, NMSA/Olig.04790; Clanwilliam (32.21516S: 18.91328E) at *ca* 120 m, Old Cape Road above reservoir, 06 September 2011, 1 clitellate, NMSA/Olig.04817. Van Rhyn's Pass (31.37131S: 19.04739E) at *ca* 803 m, flats above Van Rhyn's pass, Nieuwoudtville side, 05 September 2011, 4 clitellate, NMSA/OLIG.04956 b,c,e,f. All new material collected by S James, JDP, T Nxele & P Madonda.

Remarks: There is high variation in the thickness of the anterior septa in this species. There is also variation in the extension of seminal vesicles with the left side pouch not extending the same as the right side and the number of segments occupied by the extended pouches varies among specimens. This species is widely distributed in the Western and Northern Cape provinces. *Kazimierzus hamerae* is similar to *rasai*, having paired spermathecae in four intersegmental furrows 12/13–15/16 and last pair of seminal vesicles extended backwards to posterior segments. However these species differ in the shape of calciferous glands, position of the clitellum and number of thickened septa.

Kazimierzus imitatus (Plisko, 1998)

Microchaetus imitatus: Plisko 1998: 268; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description:

External: *General:* Body cylindrical. *Colour:* Dorsally dark grey, ventrally whitish-grey. *Body dimension:* Holotype: 76 mm long, 4 mm wide at tubercula pubertatis; paratypes 76–88 mm long, 3–4 mm wide at tubercula pubertatis. *Segment number:* Holotype 265, paratypes 221–300. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulation. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple,

4–7 with 2 simple ringlets similar in size and appearance, 8–9 with 2 ringlets, second shorter than first, 10 and postclitellar segments simple. *Setae*: Closely paired, minute, $ab = cd$, postclitellarly $aa < bc < dd$. *Nephridial pores*: Not observed. *Female pores*: Not observed. *Male pores*: Not observed. *Spermathecal pores*: In intersegmental furrows: 11/12–16/17, multiple. *Clitellum*: Saddle-shaped, whitish-grey, anteriorly not clearly bordered, on 12,13–26. *Tubercula pubertatis*: Rectangular, broad bands, segmented, on 17–23,24, dorsally below clitellar edges. *Papillae*: Paired small swellings, on 10–16 and 24–26.

Internal: *Septa*: 4/5 5/6, 7/8 8/9 thickened, similar in size and appearance, other septa thin. *Gizzard*: In 7, well developed. *Calciferous glands*: In 10, separated dorsally and ventrally. *Intestine*: Originates in 13. *Typhlosole*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia, one pair per segment with J-shaped bladders. *Male funnels*: Holandric, well developed, iridescent. *Seminal vesicles*: One pair of sacs commencing in septum 10/11, extending through segments 12–15. Begins as small and increase in size. *Spermathecae*: In segments 12–16, near septa 11/12–16/17, multiple, small globular ampullae, with very thin necks, often deeply embedded in body tissue. Numbers of ampullae vary in the rows from 1–8 at each side. *Ovaries*: Not observed. *Genital glands*: Small glands associated with setae, and corresponding with papillae in segments 9–30.

Material examined: Western Cape: *Holotype*: 17 km NW Porterville (33°00'S: 19°00'E), from ditch near road 44, 13 August 1997, clitellate, NMSA/Olig.02670. Paratypes collected with holotype: 2 clitellate, 4 juveniles, NMSA/Olig.02673. All material collected by JDP & TL.

Remarks: Spermathecae ampullae varied in number amongst specimens. Similar to *pauli* by having multiple, small spermathecae in six rows. The two species differ in the position of the clitellum and the origin of intestine.

Kazimierzus kleinoodi Nxele & Plisko, 2017

Kazimierzus kleinoodi: Nxele et al. 2017: 188.

Description

External: *General:* Body cylindrical. *Colour:* Whitish grey. *Body dimension:* Holotype: > 110 mm, abscised, width 8 mm at tubercula; Paratype: > 250 mm in length, abscised, width 8 mm. *Segment number:* Holotype > 200, paratype > 300. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segment 1 and 2 simple. Segment 3 with superficial line, 4–6 divided by one furrow, the anterior annulus again subdivided giving four annuli per segment, 7, 8, 9 also with annuli subdivided to make a total of 5, 6, 8 annulations, respectively. *Setae:* $aa < bc$: $ab = cd$: $cd < bc$ minute, closely paired especially on papillae. *Nephridial pores:* Not observed. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* Not observed, presumably at intersegmental furrows of 12/13–16/17, corresponding to the number and location of the spermathecae. *Clitellum:* Saddle-shaped on 12–27, 28, segmented, appears as a band of discolouration. *Tubercula pubertatis:* Not clear on 17, 18–21. *Papillae:* On 10–28 or 10–26 on paratype.

Internal: *Septa:* 4/5 5/6 6/7 thickened, muscular, other septa thin. *Gizzard:* Well developed in 7, globular, muscular. *Calciferous glands:* In 9, fused dorsally. *Intestine:* Originates in 12. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, in separate sacs, closely paired. *Seminal vesicles:* Very small, one pair in 11. *Spermathecae:* Near *cd* setal line, one pair per segment in 12–16 near intersegmental furrows 12/13–16/17, one pair per segment, small, round-shaped with long diverticulum. *Ovaries:* Not observed. *Genital glands:* Present on 10–26 or 28.

Material examined: (NEW): Western Cape: Holotype: Stellenbosch, Kleinood Farm (33.99646S: 18.87353E) at ca 199 m, near stream in wet seepage area, 30 August 2011, clitellate, NMSA/Olig.04987b; Paratype and other material collected with holotype: 1 clitellate NMSA/Olig.04987a; 5 juveniles NMSA/Olig.04987c,e. All material collected by S James, JDP, T Nxele & P Madonda.

Remarks: Tertiary annulation was observed in this species. This species is similar to *pearsonianus* by having muscular septa at 4/5–6/7 but differs by the position of calciferous glands in segment 9 (in 10 in *pearsonianus*). *Kazimierzus kleinoodi* has five pairs of spermathecae whilst *pearsonianus* has three. Furthermore, the clitellum in *kleinoodi* is on 12–27 and extends slightly to 28 in the paratype, but it covers segments 11–28 in *pearsonianus*.

Kazimierzus nietvoorbiji and *nieuwoudtvillensis* also have thickened septa 4/5–6/7 but differs from *kleinoodi* in the number of spermathecae (5, 6, 4).

Microchaetus ljungstroemi (Pickford, 1975)

Microchaetus ljungstroemi: Pickford 1975: 50; Reynolds & Cook 1976: 129; Plisko 1992: 339; 1993: 235; 1998: 270; 2006: 50; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Whitish grey. *Body dimension:* Holotype: > 60 mm in length, abscised, width > 6 mm, specimen in three pieces. *Segment number:* Holotype > 130. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segment 1 and 2 fused. Segment 3 simple, 4–9 with two ringlets of similar size and 10 simple. *Setae:* Closely paired, first pair visible on the first segment, aa<bc: ab=cd. *Nephridial pores:* Below setal line c. *Female pores:* On 14 near ab. *Male pores:* Not observed. *Spermathecal pores:* Not observed. *Clitellum:* Not developed. *Tubercula pubertatis:* Not clear on 17–19. *Papillae:* On 10–23, slight raise in setae.

Internal: *Septa:* 4/5–9/10 thickened, 4/5 5/6 6/7, 9/10 moderately thickened, 7/8 8/9 muscular, other septa thin. *Gizzard:* In 7. *Calciferous glands:* Paired in 10. *Intestine:* Originates in 12. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, in 10 and 11. *Seminal vesicles:* In 11 and 12, with the posterior pair extending to 23. *Spermathecae:* In three segments, one pair per segment, near 13/14–15/16. *Ovaries:* Not observed. *Genital glands:* Present on 10–23.

Material examined: (OLD): Western Cape: Holotype: Tulbagh District, Great Winterhoek, at ca. 4000–5000 ft, 16 April 1916, semi mature, SAM 21541, collected by KH Barnard.

(NEW): Western Cape: Swellendam, Marloth NR, Koloniesbos Forest (33°59'41.0''S: 20°29'10.2''E) at ca 727 ft, indigenous & rocky, 05 October 2015, 8 semi mature NMSA/Olig.06960 a-h. Collected by T Nxele, S Xaba & T Nene.

Remarks: Prostomium is prolobous not zyglobous as originally stated by Pickford (1975), new material also confirms this. Male funnels of the holotype were removed in earlier dissections but the original description state that they have a holandric condition. Seminal vesicles were also earlier removed and kept in a small vial with the specimen. This species is similar to *phumlani* with septa 4/5–9/10 thickened but *ljungtroemi* has the posterior pair of seminal vesicles extending backwards whilst *phumlani* has both pairs in 11 and 12 respectively.

Kazimierzus metandrus (Plisko, 1998)

Microchaetus metandrus: Plisko 1998: 271; 2003: 281; 2006: 50; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Dorsally dark grey, ventrally whitish-grey. *Body dimension:* Holotype: 86 mm long, 4 mm wide at tubercula pubertatis; paratypes 60–92 mm, 5 mm at tubercula pubertatis. *Segment number:* Holotype 218, paratypes 131–236. *Prostomium:* Prolobous. *Segmentation:* Secondary annulation present on preclitellar segments. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–7 with 2 simple ringlets similar in size and appearance, 8–9 with 2 ringlets, second shorter than first. *Setae:* Closely paired, minute, first pair of *ab* on 3, *ab* = *cd*, postclitellarly *aa* < *bc* < *dd*. *Nephridial pores:* Between *ab* and *cd* setae, first pair in intersegmental furrow 3/4. *Female pores:* On segment 14 near *ab* setae. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows 13/14 14/15 15/16. *Clitellum:* Clearly bordered anteriorly and posteriorly, on segment 13–22. *Tubercula pubertatis:* Rectangular bands, segmented, overlapping clitellum on 17–20. *Papillae:* Minute swellings on 10–24.

Internal: *Septa:* 4/5–6/7 slightly thickened, 7/8 8/9 9/10, muscular, other septa thin. *Gizzard:* In 7, well developed. *Calciferous glands:* Paired, separated ventrally and dorsally, in segment 10. *Intestine:* Originates in 12. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, long coiled loops with V-shaped bladders. *Male funnels:* Metandric arrangement, one pair of well-

developed, iridescent funnels in segment 11. *Seminal vesicles*: One pair of highly lobulated pouches, commencing at septum 11/12, bulging backwards, extending to segments 21, 24 or in some individuals to 40. Some irregularities in shape and position of vesicles observed. In holotype a vesicle on right side anteriorly was overlapping intestine dorsally, while posteriorly was twisting to ventral side of body. In paratypes both vesicles extended ventrally under intestine. *Spermathecae*: Paired in segments 13–15 with short necks near septa 13/14–15/16, variable size. *Ovaries*: Not observed. *Genital glands*: Variable size and correspond with papillae, in 10–24 or 10–30 in one specimen.

Material examined: Western Cape: *Holotype*: 19 km N of Ceres (33°21'S: 19° 19'E), near road R 46 on hillside with proteas and rocky, sandy soil, 13 August 1997, clitellate, NMSA/Olig.02669; *Paratypes*: collected together with holotype, 1 clitellate, 8 juveniles, NMSA/Olig.02652; 18 km SE Porterville (33°00'S:19°00'E), from ditch near road 44, 18 August 1997, 1 clitellate, NMSA/Olig.02655. All material collected by JDP & TL.

Remarks: The number of segments for the holotype is 218 not 224 as stated by Plisko (1998). Characterised by metandry and possession of only the posterior pair of male funnels and the posterior pair of seminal vesicles. The metandric condition is unique to this species and suggests that *metandrus* is not closely related to any other species of *Kazimeirzus*.

Kazimierzus nietvoorbiji Nxele & Plisko, 2017

Kazimierzus nietvoorbiji: Nxele et al. 2017: 189.

Description

External: *General*: Body cylindrical. *Colour*: Pale grey. *Body dimension*: Holotype: > 145 mm, abscised, width 9 mm at tubercula pubertatis; paratypes 145–282 mm, width 4–6 mm. *Segment number*: Holotype > 260, paratypes > 290. *Prostomium*: Prolobous. *Segmentation*: Preclitellar segments with secondary annulations. Segments 1–3 simple with grooves. Segments 4–7 with four annuli of same size, segments 8 and 9 with five primary annuli, each subdivided to make a total of 10 annulations, from segment 10 on and in postclitellar

randomly annulated. *Setae*: $aa < bc$: $ab = cd$ small and closely paired. *Nephridial pores*: Small, first pair on 3/4. *Female pores*: Not observed. *Male pores*: Not observed. *Spermathecal pores*: Not observed but could be traced from inside to intersegmental furrows 10/11–15/16. *Clitellum*: Saddle-shaped on 12, 13–28, segmented, dorsal borders well marked. *Tubercula pubertatis*: On 17, 18–26, not clearly marked. *Papillae*: On 8–30 as small swellings around setae.

Internal: *Septa*: 4/5, 5/6, 6/7 thickened, muscular. Other septa thin. *Gizzard*: Well-developed in 7, globular, muscular, attached to a large crop which is also in segment 7. *Calciferous glands*: In 11, fused dorsally. *Intestine*: Originates in 13. *Typhlosole*: Commence with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Holoic, one pair per segment with J-shaped bladders. *Male funnels*: Holandric, male funnels in separate sacs, closely paired. *Seminal vesicles*: In 11 and 12, the pair in 11 close to the calciferous gland. *Spermathecae*: Near cd setal line, one pair per segment close to intersegmental furrow 10/11–15/16 in holotype, 11/12–15/16 in adult paratypes, one pair per segment, small round-shaped. *Genital glands*: Present, different shapes and sizes on 10–28 or 29 or 30.

Material examined: Western Cape: Stellenbosch, Nietvoorbij Research Farm (33.9079S: 18.86883E) at ca 259 m, hillside in native bush, 31 August 2011, clitellate, NMSA/Olig.04988a; Paratypes and other material collected together with holotype, 1 clitellate, NMSA/Olig.04988d; 4 clitellates, NMSA/Olig.04802; 2 juveniles NMSA/Olig.04988b,c. Collected by JDP, S James, T Nxele & P Madonda.

Remarks: Tertiary annulation observed. This species is similar to *franciscus* and *crousi* in the position of calciferous glands. However, *nietvoorbiji* differs from the two species in the position of the clitellum.

Kazimierzus nieuwooldtwillensis Nxele & Plisko, 2017

Kazimierzus nieuwooldtwillensis: Nxele et al. 2017: 190.

Description

External: *General:* Body cylindrical. *Colour:* Light violet dorsally and whitish grey ventrally. *Body dimension:* Holotype: > 215 mm, abscised, width 18 mm at tubercula; paratypes 175–182 mm in length, width 6 mm. *Segment number:* > 320. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1–2 simple and fused, appearing as one segment. Segment 3 simple, segments 4–7 with annulus subdivided to make four annuli of equal size, 8–9 with six annuli. *Setae:* $aa < bc$: $ab > cd$ closely paired, on papillae, minute. *Nephridial pores:* Small, better visible at clitella segments, first visible pair on 3/4. *Female pores:* Not observed. *Male pore:* Not observed. *Spermathecal pores:* Not observed. *Clitellum:* Saddle-shaped on 12–25, segmented, dorsal borders well marked. *Tubercula pubertatis:* On 17–20, distinct, clearly marked. *Papillae:* On 10–25, 26, slight swellings on setae.

Internal: *Septa:* 4/5, 5/6, 6/7 thickened, muscular, 7/8, 8/9, 9/10 also thickened with 7/8 stronger than 8/9, 9/10. Other septa thin. *Gizzard:* Well developed in 7, globular, muscular. *Calciferous glands:* In 10–11, fused dorsally. *Intestine:* Originates in 13. *Typhlosome:* Commence with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, male funnels in separate sacs, closely associated with seminal vesicles. *Seminal vesicles:* In 11 and 12, the posterior pair large, extending to 15 on the left side and 16 on right. *Spermathecae:* On both ab and cd setal lines, multiple per segment in 12/13–15/16, in rows, medium and round, different numbers per segment. *Genital glands:* Different shapes and sizes on 10–26.

Material examined: Northern Cape: Holotype: Nieuwoudtville (31.43191S: 19.13628E) at ca 719 m, south of Nieuwoudtville in flat lands, roadside deep soil, 05 September 2011, clitellate, NMSA/Olig.04990a; paratypes and other material collected together with holotype, 2 clitellates, NMSA/Olig.04796; 4 juveniles, NMSA/Olig.04990b, c. Collected by JDP, S James, T Nxele & P Madonda.

Remarks: Tertiary annulation observed. This species is similar to *peringueyi* in appearance but differs in number of segments occupied by spermathecae; five segments in *peringueyi* and four in *nieuwoudtvillensis*. Michaelsen (1913) highlighted that the clitellum in *peringueyi* starts partly on 13, in *nieuwoudtvillensis* the clitellum is on 12–25.

Kazimierzus obscurus (Plisko, 1998)

Microchaetus obscurus: Plisko 1998: 275; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Dorsally dark violet, ventrally whitish-grey. *Body dimension:* Holotype: 90 mm long, 5 mm wide at tubercula pubertatis; paratypes 90–105 mm long, 6 mm wide at tubercula pubertatis. *Segment number:* Holotype 212, paratypes 182–260. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–8 with 2 simple ringlets similar in size and appearance; 9 with 2 ringlets, second shorter than first. *Setae:* Closely paired, minute, first pair of *ab* on 3. Postclitellarly $ab = cd$, $aa < bc < dd$. *Nephridial pores:* Clearly visible on clitellar segments, first pair in intersegmental furrow 3/4 much below *cd* setae. *Female pores:* On segment 14 in front of *ab* setae. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows 12/13–16/17, minute. *Clitellum:* Indistinct, not fully developed, with no clear borders anteriorly and posteriorly, traced to be on 12,13–27,28. *Tubercula pubertatis:* Segmented, overlapping clitellum on 17–24. *Papillae:* Paired minute swellings, on 10–29,30,31,32.

Internal: *Septa:* 4/5 5/6, 7/8 8/9 thickened moderately, 6/7 and 9/10 thickened slightly, other septa thin. *Gizzard:* In 7. *Calciferous glands:* Paired, in 10, separated ventrally and dorsally. *Intestine:* Originates in 13. *Typhlosole:* Commence with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, coiled, elongate tubules, with J-shaped bladders. *Male funnels:* Holandric arrangement, iridescent. *Seminal vesicles:* Two pairs of sacs, commencing posteriorly in segments 11 and 12. Anterior pair confined to segment 11, posterior pair to 12, extending sometimes to 13. *Spermathecae:* In segments 12–16, near to septa 12/13–16/17. Multiple tiny globular bladders, of various sizes, 1–8 at each side. *Ovaries:* Not observed. *Genital glands:* In 10–30.

Material examined: Western Cape: *Holotype:* 18 km SE Porterville (33°00'S:19°00'E), from ditch near road R 44, 13 August 1997, clitellate, NMSA/Olig.02595; Paratypes collected together with holotype: 6 clitellates, 7 juveniles, NMSA/Olig.02654. All material collected by JDP & TL.

Remarks: Similar to *pentus* in number of segments with multiple spermathecae and calciferous glands but differs in the shape and position of the clitellum.

Kazimierzus occidualis (Plisko, 1998)

Microchaetus occidualis: Plisko 1998: 277; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Whitish-grey. *Body dimension:* Holotype: 178 mm, 6 mm wide at tubercula pubertatis; paratype 142 mm long, 9 mm wide at tubercula pubertatis. *Segment number:* Holotype 302, paratypes 204–241. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1 and 2 fused with irregular longitudinal grooves. Segment 3 simple, 4–6 with 2 simple ringlets similar in size and appearance, 7–9 with 2 irregularly annulated ringlets, 9 with second shorter than first, 10 and postclitellar segments randomly annulated. *Setae:* Closely paired, minute, first pairs of *ab* on 4, $ab = cd$, $aa < bc < dd$. *Nephridial pores:* Obvious on clitellar segments, small on other parts of the body, first pair on anterior part of segment 4, close to intersegmental furrow 3/4 much below *cd* setal lines. *Female pores:* On segment 14, near clitellum. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows 13/14–15/16, multiple per furrow. *Clitellum:* Saddle-shaped, segmented, on 12,13–22. *Tubercula pubertatis:* broad rectangles, segmented, on 17–20, very close to clitellum. *Papillae:* Paired, various sizes, on 10–29, well developed on 22–29.

Internal: *Septa:* 4/5 5/6, 7/8 8/9 9/10 slightly thickened, similar in size and appearance, 6/7 thickened less than the anterior, other septa thin. *Gizzard:* Large in 7. *Calciferous glands:* Paired, large, in 10–11 with septum 10/11 over middle of glands, separated ventrally and dorsally. *Intestine:* Originates in 13. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, coiled tubules with J-shaped bladders. *Male funnels:* Holandric, large, iridescent funnels in segments 10 and 11 respectively. *Seminal vesicles:* Two pairs commencing at septa 10/11 and 11/12, anterior pair smaller than posterior pair and confined to segment 11. Posterior pair extended

through segments 12 and 13. *Spermathecae*: Tiny, globular ampullae with necks embedded in body wall, 3–7 at each side, close to septa 13/14–15/16. *Ovaries*: Not observed. *Genital glands*: Corresponds with papillae, in 12–27.

Material examined (OLD): Western Cape: *Holotype*: 21 km S of Clanwilliam (33°10'S:18°55'E), swamp near road N 7, 14 August 1997, clitellate, NMSA/Olig.02608; Paratypes collected together with holotype: 1 clitellate in 2 pieces, NMSA/Olig.02607; 31 juveniles, NMSA/Olig.02609; 1 clitellate abscised, NMSA/Olig.02656. All material collected by JDP & TL.

(NEW): Western Cape: Clanwilliam (32.13593S: 18.99529E) at ca 605 m, riparian area in dense sedge stand, 06 September 2011, 2 clitellate, 7 juveniles + 4 variably abscised post-ends, NMSA/Olig.04787; Clanwilliam (32.21516S: 18.91328E) at ca 120 m, Old Cape Road above reservoir, 06 September 2011, 1 clitellate, NMSA/Olig.04791; 3 juveniles, NMSA/Olig.04962 a-c; Clanwilliam (32.13194S: 18.90865E) at ca 253 m, along R364, open area in damp spot, 06 September 2011, 9 juveniles, NMSA/Olig.04958 a-i.

Remarks: Similar to *occiduus* by having three segments with spermathecae but differs in the thickness of septum 9/10 which is thicker in *occidualis* than in *occiduus*.

Kazimierzus occiduus (Plisko, 1998)

Microchaetus occiduus: Plisko 1998: 279; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General*: Body cylindrical. *Colour*: Violetish-grey dorsally, ventrally whitish-grey. *Body dimension*: Holotype: 102 mm, 4 mm wide at tubercula pubertatis; paratypes 90–125 mm long, 4–5 mm wide at tubercula pubertatis. *Segment number*: Holotype 260, paratypes 198–296. *Prostomium*: Prolobous. *Segmentation*: Preclitellar segments with secondary annulations. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–6 with 2 simple ringlets similar in size and appearance, 7–9 with 2 irregularly annulated

ringlets, second shorter than first, 10 and postclitellar segments with irregular annulation. *Setae*: Closely paired, minute, first pairs of *ab* on 4, $ab = cd$, postclitellarly $aa < bc > dd$. *Nephridial pores*: On clitellar segments, less noticeable on other parts of body. *Female pores*: On segment 14, near *a* setal line. *Male pores*: Not observed. *Spermathecal pores*: In intersegmental furrows: 13/14 14/15 15/16. *Clitellum*: Saddle-shaped, dorsally and laterally segmented, clearly bordered anteriorly and posteriorly, on segments 12,13–23. *Tubercula pubertatis*: Flat glandular bands, segmented, on 17–20, dorsal edges below nephridial pores. *Papillae*: Paired swellings on 10–23.

Internal: *Septa*: 4/5 5/6 6/7 thickened moderately, in some paratypes septa 7/8 and 8/9 were also slightly thickened, other septa thin. *Gizzard*: In 7. *Calciferous glands*: In 10, separated ventrally and dorsally. *Intestine*: Originates in 13. *Typhlosome*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia, one pair per segment, tightly coiled tubules with J-shape bladders, postclitellar nephridia much larger. *Male funnels*: Holandric, large funnels, iridescent on some specimens. *Seminal vesicles*: Two pairs commencing at septa 10/11 and 11/12, posterior pair commencing as 2 small pouches at septum 11/12, developing into pair of lobulated, pouched sacs, and extending backward, the extension, size and shape vary in different individuals, extends up to 23,24. *Spermathecae*: Large, different shapes near septa 12/13 13/14 14/15, multiple, 2–4 at each side. *Ovaries*: Not observed. *Genital glands*: Corresponds with papillae, in 10–23.

Material examined: Western Cape: *Holotype*: 34 km NE Ceres (33°21'S:19°19'E), near road R 301, from sandy soil under short indigenous bushes, 12 August 1997, clitellate, NMSA/Olig.02653; Paratypes: 10 clitellate, 11 juveniles, NMSA/Olig.02581, collected together with holotype; 17 km N Ceres, near road R 303, from rocky, sandy soil on hillside with Proteas, 13 August 1997, 1 clitellate, 4 juveniles, NMSA/Olig.02584; 19 km N Ceres, near road R46, from rocky soil on hillside, 13 August 1997, 1 with absorbed clitellum, 7 juveniles, NMSA/Olig.02589; 9 km N Tulbagh (33°19'S:19°08'E), near road R44, from dry soil under indigenous bush, 4 clitellate, 11 juveniles, NMSA/Olig.02594; Other material: 17 km NE Vanrhynsdorp (31°37'S:18°44'E), De Kom, on bank of flowing river, from sandy soil, 16 August 1997, 3 specimens with tubercula pubertatis, NMSA/Olig.02617; 30 juveniles, NMSA/Olig.02618. All material collected by JDP & TL.

Remarks: Similar to *occidualis* by having three segments with spermathecae but differs in the thickened septum 9/10, which is thicker in *occidualis* than *occiduus*. Some paratypes are highly annulated with two or three annuli.

Kazimierzus pauli (Plisko, 1998)

Microchaetus pauli: Plisko 1998: 283; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Dorsally dark grey, ventrally whitish-grey. *Body dimension:* Holotype: 160 mm long, 11 mm wide at tubercula pubertatis; paratypes 136–182 mm long, 5–7 mm wide at tubercula pubertatis. *Segment number:* Holotype 423, paratypes 292–406. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1 and 2 fused, with no clear separation, with irregular longitudinal grooves. Segment 3 simple, 4–7 with 2 simple ringlets similar in size and appearance, 8–9 with 2 ringlets, second shorter than first, 10 and postclitellar segments simple. *Setae:* Closely paired, minute, $ab = cd$, postclitellarly $aa < bc < dd$, first pairs of ab on 4. *Nephridial pores:* Small, first pair in intersegmental furrow 3/4, near cd setal lines. *Female pores:* On segment 14, above b setal lines. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows: 11/12–16/17, multiple. *Clitellum:* Saddle-shaped, dorsally segmented, with segments much longer than preclitellar and postclitellar segments, clearly bordered anteriorly and posteriorly, on 12–28. *Tubercula pubertatis:* Segmented, flat, on 17–23,24, dorsally below clitellar edges. *Papillae:* Paired tiny swellings, associated with ab setae, on 10–28.

Internal: *Septa:* 4/5 5/6, 7/8 8/9 thickened, muscular, other septa thin. *Gizzard:* In 7. *Calciferous glands:* In 10, separated dorsally and ventrally. *Intestine:* Originates in 12. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, tightly coiled tubules with J-shaped bladders. *Male funnels:* Holandric arrangement, iridescent. *Seminal vesicles:* Single pair of dorsolateral sacs commencing at septum 10/11. In holotype, sacs are differently lobulated on

left and right side and the extension is not the same on both sides, right side to segment 17 and left side to segment 15. *Spermathecae*: In segments 12–16, near septa 11/12–16/17, multiple, tiny round ampullae, deeply embedded in body tissue, the number varies in the rows, in holotype 1–4 ampullae at each side, in paratypes 1–8. *Ovaries*: Not observed. *Genital glands*: In segments 10–33, small.

Material examined: Western Cape: *Holotype*: 20 km W Vanrhynsdorp (31°37'S:18°44'E), on top of the hill De Kom, from moist sandy soil, 16 August 1997, clitellate, NMSA/Olig.02619; paratypes collected together with holotype, 2 clitellate, 1 abscised, NMSA/Olig.02620. All material collected by JDP & TL.

Remarks: Similar to *imitatus* by having multiple small spermathecae in six rows. These species differ in the position of the clitellum (12,13–26 in *imitatus*, 12–28 in *pauli*) and the origin of intestine.

***Kazimierzus pearsonianus* (Pickford, 1975)**

Microchaetus pearsonianus: Pickford 1975: 31; Reynolds & Cook 1976: 153; Plisko 1992: 339; 1993b: 235; 1998: 285; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General*: Body cylindrical. *Colour*: Somewhat brown throughout the body. *Body dimension*: Paratype: > 80 mm long, 10 mm wide at tubercula pubertatis. *Segment number*: Unknown, specimen in poor condition. *Prostomium*: Prolobous. *Segmentation*: Segments are highly annulated. Segment 1–3 simple, 4–6 have four ringlets, and segment 7 has 10 ringlets. *Setae*: Closely paired, minute, $aa = bc$, $aa > bc < dd$, visible on tubercula pubertatis. *Nephridial pores*: Not observed. *Female pores*: Not observed. *Male pores*: Not observed. *Spermathecal pores*: Not observed. *Clitellum*: Saddle-shaped, dorsally segmented, not clearly bordered anteriorly and posteriorly, on 11,12–28. *Tubercula pubertatis*: On 17,18–25,26, dorsally below clitellar edges, narrowly separated from clitellum. *Papillae*: Slight swellings, on 12–28.

Internal: *Septa:* 4/5 5/6 6/7 muscular, other septa thin. *Gizzard:* In 7, large. *Calciferous glands:* In 10, fused dorsally and ventrally. *Intestine:* Originates in 14. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, tubules with J-shaped bladders. *Male funnels:* Holandric, iridescent. *Seminal vesicles:* A single pair commencing in 11/12 extending backwards to 15, in paratype the organ is no longer fully attached so the number of segments they are extended into is estimated from original description, in other material the posterior pair does not extend. *Spermathecae:* Near septa 13/14–15/16, multiple, deeply embedded in body tissue. *Ovaries:* Not observed. *Genital glands:* In segments 12–28, size varies.

Material examined (OLD): Western Cape: Paratype: Cape Peninsula, National Botanic Gardens, Kirstenbosch (34°00'S: 18°25'E), in soggy sandy soil at depth of 18 inches to 2 feet below lawn, 29 October 1925, 1 clitellate, SAM 21544; other material: Cape Peninsula, Paradise Estate, near Kirstenbosch, open grassy slope by stream, below Lady Anne Barnard's ruined cottage, very wet and boggy soil raised in mounds, 16 June 1926, 1 specimen well preserved, not fully mature, SAM 21545. All material collected by GE Pickford.

(NEW): Western Cape: Constantia (34.06226S: 18.41207E) at ca 146 m, Tokai Arboretum/plantation in small swamp uphill from tea house, 29 August 2011, 4 clitellate, NMSA/Olig.04806; 5 clitellate, 6 juveniles, NMSA/Olig.04984a-k; 4 clitellate, 4 juveniles, NMSA/Olig.04985. All material collected by JDP & S James.

Remarks: Paratype is in poor condition, partly decomposed and abscised. Tubercula pubertatis could not be traced on the paratype but Pickford (1975) reported it on 18–23; on new material it is longer, occupying segments 17,18–25,26. The seminal vesicles in paratype are no longer fully attached and the backward extension was estimated according to the length of the vesicles. In other material the anterior and posterior sacs are confined to segment 11 and 12 respectively. This species is similar to *guntheri* by having multiple spermathecae in three segments but *pearsoniunus* has reduced seminal vesicles with only one pair which extends backwards. However, *guntheri* has both pairs but confined to 11 and 12 respectively.

Kazimierzus pentus (Plisko, 1998)

Microchaetus pentus: Plisko 1998: 286; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Whitish-grey. *Body dimension:* Holotype: 103 mm, 4 mm wide at tubercula pubertatis; paratypes 103–118 mm long, 4–5 mm at tubercula pubertatis. *Segment number:* Holotype 324, paratypes 301. *Prostomium:* Epilobous. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–7 with 2 simple ringlets similar in size and appearance, 8–9 with 2 ringlets, second shorter than first, clitellar segments ventrally with irregular annulation. *Setae:* Closely paired, minute, $ab = cd$, postclitellarly $aa < bc$, first pairs of ab on 4. *Nephridial pores:* Small, first pair in intersegmental furrow $3/4$ much below cd setal lines. *Female pores:* Not observed. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows 11/12–15/16, not clearly visible but traced from inside. *Clitellum:* Saddle-shaped, segmented, on 12–26. *Tubercula pubertatis:* Flat bands, segmented, overlapping clitellum, on 17–23. *Papillae:* Paired, small, on 10–29.

Internal: *Septa:* 4/5 5/6, 7/8 8/9 thickened moderately, similar in size and appearance, 6/7 and 9/10 slightly thickened, much thinner than anterior, other septa thin. *Gizzard:* In 7. *Calciferous glands:* In 10, small, separated ventrally and dorsally. *Intestine:* Originates in 13. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, one pair per segment, tightly coiled tubules with J-shape bladders. *Male funnels:* Holandric, iridescent. *Seminal vesicles:* Two pairs of sacs commence at septum 10/11 and 11/12, anterior pair confined to segment 11, posterior pair gradually enlarging and extending to segments 12–14,15. *Spermathecae:* In segments 12–16, multiple, near septa 11/12–15/16, tiny, round, various sizes with thin necks embedded in body tissue, number of spermathecae per row varies from 1–4 on each side. *Ovaries:* Not observed. *Genital glands:* On 10–29, various sizes.

Material examined: Western Cape: *Holotype*: 17 km N Citrusdal (32°36'S:19°01'E), near road N7, open veld, 14 August 1997, clitellate, NMSA/Olig.02605; paratypes collected together with holotype, 4 clitellate, 2 juveniles, NMSNOlig.02672. All material collected by JDP & TL.

Remarks: Segment number for the holotype is different from original description, I found it to be 324 not 329. This species is similar to *alipentus*, as the names suggest, they have spermathecae in five segments, but differs in that *alipentus* has a reduced pair of seminal vesicles while *pentus* has both pairs well developed.

Kazimierzus peringueyi (Michaelsen, 1913)

Microchaetus peringueyi: Michaelsen 1913*ba*: 535; 1913*b*: 60; Pickford 1975: 23; Reynolds & Cook 1976: 155; Plisko 1992: 340; 1998: 294; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General*: Cylindrical. *Body dimension*: 330 mm, width 6–10 mm. *Segment number*: 445. *Prostomium*: Prolobous. *Segmentation*: Preclitellar segments with secondary annulations. Segments 4–9 divided into two ringlets. *Setae*: Laterally visible behind the clitellum, ventrally visible from segment 9, strictly paired, behind the clitellum *aa:bc:dd*=4:6:8, at the hinder end of the body *aa:bc:dd*=4:5:6. *Nephridial pores*: Beneath *c* setal line. *Spermathecal pores*: In groups of 2–6 at each side of the intesegmental furrows 12/13–16/17, in the lines of the nephridial pores, and dorsally from them. *Clitellum*: Saddle-shaped on 12,13–25 (= 13,14). *Tubercula pubertatis*: On 17–20, broad copulatory cushion. *Papillae*: On 25–27 are oval and glandular, similar but more indistinct papillae observed in fore-part of the clitellar region.

Internal: *Septa*: 4/5 5/6 6/7 strongly thickened, 7/8 8/9 moderately thickened, 9/10 slightly thicker, other septa thin. *Gizzard*: In 7. *Calciferous glands*: In 10. *Male funnels*: Holandric, in 10 and 11. *Seminal vesicles*: In 11 and 12. *Spermathecae*: Pear-shaped, shortly and narrowly stalked. *Genital glands*: In 12–17, have a shape of slightly bent thick sausage.

Remarks: No material was available for anatomical study, Michaelsen (1913) reported that the specimen was in South African Museum, however, the material is not in the SAM collection. Therefore the original description from Michaelsen (1913b) is presented. This species is only known from its type locality in Nieuwoudtville in the Bokkeveld Mountains, Calvinia Division. This species is similar to *nieuwoudtvillensis* in appearance but differs in spermathecae which are in five segments in *peringueyi* but in four in *nieuwoudtvillensis*. Michaelsen (1913) highlighted that the clitellum in *peringueyi* starts partly on 13, in *nieuwoudtvillensis* the clitellum is on 12–25.

Kazimierzus phumlani Nxele & Plisko, 2017

Kazimierzus phumlani: Nxele et al. 2017: 191.

Description

External: *General:* Body cylindrical. *Colour:* Whitish grey. *Body dimension:* Holotype: > 60 mm, abscised, width 6 mm at tubercula. *Segment number:* > 105. *Prostomium:* Prolobous, small. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1–2 simple and fused, appearing as one segment. Segment 3 simple, segments 4–7 with two annuli, 8–9 with second annuli appearing smaller. *Setae:* $aa < bc$; $ab > cd$, closely paired, minute. *Nephridial pore:* Not observed. *Female pores:* Not observed but probably in 14. *Male pore:* Not observed. *Spermathecal pores:* Not observed but traced from internal to 13/14–15/16. *Clitellum:* Saddle-shaped on 12–22, segmented, dorsal borders well-marked. *Tubercula pubertatis:* On 17–20, band in clitellar tissue. *Papillae:* On 13–27.

Internal: *Septa:* 4/5 5/6 thickened, strong, 6/7 7/8 8/9 9/10 thickened but less so. Other septa thin. *Gizzard:* Very small in 7. *Calciferous glands:* In 10, separated dorsally and ventrally. *Intestine:* Originates in 13. *Typhlosome:* Commences with the intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Holoic, one pair per segment with J-shaped bladders. *Male funnels:* Holandric, male funnels in separate sacs, both pairs iridescent in clitellate holotype. *Seminal vesicles:* In 11 and 12, one pair per segment, pair in 12 larger.

Spermathecae: Between *ab* and *cd* setal lines, multiple per segment in 13/14–15/16, 1 or 2 at each side, round-shaped. *Genital glands*: Large on 13–27.

Material examined (NEW): Western Cape: Holotype: Doringbos (31.77182S: 19.29114E) at ca 734 m, 25 km N of Doringbos on R364, 05 September 2011, clitellate, NMSA/Olig.04951/2e; additional material collected together with holotype, 6 juveniles, NMSA/Olig.04951/2b, c. All material collected by JDP, S James, T Nxele & P Madonda.

Remarks: This species is similar to *occidualis*, Both species have clitellum on 12–22, spermathecae in segments 13/14–15/16. However, *occidualis* has calciferous glands in 10–11 with septum 10/11 in the middle of glands whilst *phumlani* has calciferous glands confined to segment 10. In *occidualis* the anterior pair of seminal vesicles is confined to segment 11, the posterior pair may extend through 12 and 13, while in *phumlani* the anterior pair is confined to segment 11 and the posterior pair to 12.

Kazimierzus rosai (Michaelsen, 1908)

Microchaetus rosai: Michaelsen 1908: 38.

Microchaetus rosae [*lapsus*]: Pickford 1975: 23.

Microchaetus rosai: Michaelsen 1918: 311; Reynolds & Cook 1976: 164; Hartwig & Kiliyas 1989: 283; Plisko 1992: 339; 1993*b*: 222; 1995*b*: 47; 1998: 293; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General*: Front body thick, medium and rear body proportionally slim and thin. *Colour*: Grey, without pigment. *Body dimension*: 105 mm long, 2–3 mm wide. *Segment number*: ca 425. *Prostomium*: Prolobous. *Segmentation*: Preclitellar segments with secondary annulations. Segment 1 and 2 not well separated, 4–9 with 2 ringlets, the anterior of which always carry the setae and nephridial pores. The two ringlets of segments 4–7 are equal, 8 a little bit shorter, 9 with significantly shorter posterior segment. *Setae*: Closely paired, visible

from 3, ventral median distance significantly smaller than the medial lateral ($aa=5/7bc$), dorsal median distance larger than the middle lateral, about one third of the circumference of the body ($bc=4/5dd=ca\ 1/3\ u$, $aa:bc:dd=5:7:9$). *Nephridial pores*: Between *b* and *c* setal line. *Male pores*: Not visible on the outside (possibly on intersegmental furrow 19/20). *Spermathecal pores*: 4 pairs, on intersegmental furrows 12/13–15/16 close to the nephridial pores. *Clitellum*: On 15–24. *Tubercula pubertatis*: On 17–20, wide, between *b* and *c* setal lines. *Papillae*: On 10–23.

Internal: *Septa*: 4/5 5/6 6/7 very strongly thickened, other septa thin. *Gizzard*: In 7. *Calciferous glands*: In 10–11, spherical. *Intestine*: Originates in 13. *Typhlosole*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia. *Male funnels*: Holandric, two pairs in segments 10 and 11 respectively. *Seminal vesicles*: Two pairs vesicles, commencing posterior to septa 10/11 and 11/12, anterior pair small, confined to 11, the posterior pair extends through many segments backwards. *Spermathecae*: Near septa 12/13–15/16, pear-shaped ampullae with long neck. *Genital glands*: 10–23.

Remarks: Plisko (1995b) found segment number to be 223 not 425 contrary to Michaelsen (1908). This species is similar to *davidi* in having four segments with spermathecae but differs in that *davidi* has multiple spermathecae per segment while *rosai* has one pair per segment. In addition *davidi* has seminal vesicles confined to segments 11 and 12 and not extending backwards as in *rosai*.

***Kazimierzus senarius* (Plisko, 1998)**

Microchaetus senarius: Plisko 1998: 289; 2003: 281; 2006: 51; Nxele et al. 2016: 112.

Description

External: *General*: Body cylindrical. *Colour*: Whitish-grey. *Body dimension*: Holotype: 102 mm long, 5 mm wide at tubercula pubertatis; paratypes 89–100 mm long, 4–7 mm at tubercula pubertatis. *Segment number*: Holotype 252, paratypes 181–295. *Prostomium*: Prolobous. *Segmentation*: Preclitellar segments with secondary annulations. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–7 with 2 simple ringlets

similar in size and appearance, 8–9 with 2 ringlets, second shorter than first. *Setae*: Closely paired, minute, $ab = cd$, postclitellarly $aa < bc < dd$, first pairs of ab on 4. *Nephridial pores*: Not observed. *Female pores*: Not observed. *Male pores*: Not observed. *Spermathecal pores*: In intersegmental furrows 11/12–16/17, multiple. *Clitellum*: Saddle-shaped, not clearly marked, on 12–26, separated from tubercula pubertatis. *Tubercula pubertatis*: Segmented, on 17–24, dorsally below clitellar edges, ventral borders just above ab setae. *Papillae*: Paired swellings, associated with ab setae, on 10–31.

Internal: *Septa*: 4/5 5/6 thickened moderately, 7/8 8/9 more thicker, other septa thin. *Gizzard*: In 7. *Calciferous glands*: In 10, separated dorsally and ventrally. *Intestine*: Originates in 12. *Typhlosole*: Commences with intestine. *Dorsal blood vessel*: Simple throughout the body. *Nephridia*: Meganephridia, one pair per segment. Coiled, elongate loops, with elongated J-shaped bladders. *Male funnels*: Holandric arrangement, iridescent funnels in segments 10 and 11. *Seminal vesicles*: Two pairs vesicles, commencing posterior to septa 10/11 and 11/12, anterior pair confined to segment 11, posterior pair extends bulging through segments 12–14,15. *Spermathecae*: In segments 12–16, near septa 11/12–16/17, multiple, small globular ampullae, with thin necks, deeply embedded in body tissue. Number of ampullae varies from 1–7 at each side, and differs in different individuals. *Ovaries*: Not observed. *Genital glands*: Correspond with papillae, in segments 9–31.

Material examined: Western Cape: *Holotype*: Porterville (33°00'S: 19°00'E), 18 km SE, from ditch near road R44, 13 August 1997, clitellate, NMSA/Olig.02667. *Paratypes*: collected with holotype, 2 not fully mature specimens, NMSA/Olig.02671; 17 km NW Porterville (33°00'S: 19°00'E), from ditch near road R44, 13 August 1997, 1 clitellate, NMSA/Olig.02598. All material collected by JDP & TL.

Remarks: Similar to *pauli* by having six segments with multi spermathecae, however, *pauli* has reduction in seminal vesicles but *senarius* has both seminal vesicles.

Kazimierzus sirgeli (Plisko, 1996)

Proandricus sirgeli: Plisko 1996b: 305; 2002: 184; 2003: 293; 2006: 52; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Whitish grey. *Body dimension:* Holotype: 145 mm long, 11 mm wide at tubercula pubertatis. *Segment number:* 277. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segment 1 and 2 simple, short, with irregular longitudinal grooves. Segment 3–5 with 2 simple ringlets, similar in size and appearance, 6–8 with 2 ringlets, each with 2 short annuli, 9 with 2 annuli, clitellar segments much larger, ventrally irregularly annulated, postcutellar segments randomly annulated. *Setae:* Minute, closely paired, on first ringlet, first pairs on 3. *Nephridial pores:* Below *c* setal lines, first pair in 2/3 intersegmental furrow. *Female pores:* On 14, small near *a* setal line. *Male pores:* Not observed. *Spermathecal pores:* Traced on intersegmental furrows 12/13–15/16. *Clitellum:* Saddle-shaped, segmented, on 12–24. *Tubercula pubertatis:* On 17–20, segmented, below clitellar edges, flat, glandular bands. *Papillae:* Small swellings, paired, on 10–23.

Internal: *Septa:* 4/5 5/6 thickened slightly, similar in thickness and appearance, 7/8 8/9 thickened moderately, other septa thin. *Gizzard:* Large, in 7. *Calciferous glands:* In 9, fused. *Intestine:* Originates in 13. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* One pair of meganephridia per segment, coiled, elongate loops, with elongated J-shaped bladders. *Male funnels:* One pair in 10, small funnels enclosed in one sac. *Seminal vesicles:* One pair in 11, large sacs. *Spermathecae:* In four segments, one pair per segment, round, with very short, thin neck, close to septa 12/13–15/16. *Ovaries:* Not observed. *Genital glands:* In 10–22, finger-like shape.

Material examined: Western Cape: *Holotype:* Hottentots Holland Nature Reserve, Hottentots Holland hiking trail, opposite Landdroskloof (34°02'S:18°59'E) at *ca* 1350 m, on eastern

slope of mountain, on walking path, on rainy day, 7 September 1994, clitellate, NMSA/Olig.02001. Collected by WF Sirgel.

Remarks: This species is proandric, an uncommon condition in *Kazimierzus*.

Kazimierzus sophieae (Plisko, 2002)

Microchaetus sophieae: Plisko 2002: 211; 2003: 281; 2006: 52; Nxele et al. 2016: 112.

Description

External: *General:* Body cylindrical. *Colour:* Dark grey. *Body dimension:* Holotype: 112 mm, 4 mm wide at tubercula pubertatis; paratypes 100–130 mm long, 4–5 mm wide at tubercula pubertatis. *Segment number:* 300–342. *Prostomium:* Prolobous. *Segmentation:* Preclitellar segments with secondary annulations. Segments 1 and 2 fused, with irregular longitudinal grooves. Segment 3 simple, 4–9 with 2 ringlets, first longer than second, 10 and 11 short, simple, irregularly annulated, postclitellar randomly annulated. *Setae:* Minute, closely paired, postclitellarly $ab = cd$, $aa < bc > dd$, first pairs on segment 3. *Nephridial pores:* Much ventral to cd setal lines, more visible on clitellar segments. *Female pores:* In 14 between bc setae. *Male pores:* Not observed. *Spermathecal pores:* In intersegmental furrows 13/14–15/16 close to nephridial pores. *Clitellum:* On 12,13–22,23 saddle-shaped, with simple, segments, clearly bordered anteriorly and posteriorly, with ventral edges above ab setal lines. *Tubercula pubertatis:* Flat pads, overlapping clitellum on 17–20, dorsal edges below nephridial pores. *Papillae:* Paired, minute swellings, shapes and sizes vary, on segments 10–23.

Internal: *Septa:* 4/5–8/9 somewhat thickened, 4/5 slightly, 5/6 7/8 8/9 thickened moderately, 6/7 thinner than 4/5, other septa thin. Variation in thickness of septa observed. *Gizzard:* In 7. *Calciferous glands:* In 10, paired, small glands, separated dorsally and ventrally. *Intestine:* Originates in 12. *Typhlosole:* Commences with intestine. *Dorsal blood vessel:* Simple throughout the body. *Nephridia:* Meganephridia, coiled loops with J-shaped bladders. *Male funnels:* Holandric, in 10 and 11, large, iridescent. *Seminal vesicles:* Two pairs of sacs commencing at posterior parts of septa 10/11 and 11/12, each pair different in size and

appearance, the anterior pair much smaller than the posterior pair and confined to 11, posterior pair commencing at septa 11/12 and forming bulged, dissimilar pouches extending backwards differently on left and right side, in holotype the left vesicle extends to segment 16, the right vesicle to segment 18 and in new material up to 24. *Spermathecae*: Paired, globular, large ampullae with long, slender ducts entering body wall at intersegmental furrows 13/14–15/16. *Ovaries*: Not observed. *Genital glands*: Single or paired, small to minute oval bladders, in segments 10–23.

Material examined (OLD): Northern Cape: Holotype: Nieuwoudtville (31°22'S: 19°06'E) from arable field under fungicide experiment, 17 July 1998, clitellate, NMSA/Olig.03548; Paratypes collected with holotype, 3 clitellate and 1 semi dry specimen, NMSA/Olig.03549. All specimens collected by M Maboeta.

(NEW): Western Cape, Northern Cape: Van Rhyn's Pass (31.37131S: 19.04739E) at *ca* 803 m, flats above Van Rhyn's pass, Nieuwoudtville side, 05 September 2011, 3 clitellate, 4 juveniles, NMSA/Olig.04794; 1 clitellate, 1 juvenile, NMSA/Olig.04956a,d; Van Rhyn's Pass (31.37474S: 19.01782E) at *ca* 750 m, soil among boulders on west side of pass, 05 September 2011, 1 semi adult, 15 juveniles, NMSA/Olig.04793; 1 clitellate, 28 juveniles, NMSA/Olig.04949; 1 clitellate, 2 juveniles, NMSA/Olig.04950a-c; Clanwilliam (32.22321S: 18.9217E) at *ca* 124 m, a few meters above level of reservoir, 06 September 2011, 1 clitellate, 2 juveniles, NMSA/Olig.04963a-c. All material collected by JDP, S James, T Nxele & P Madonda.

Remarks: Variation in shapes, sizes and extension of posterior pairs of seminal vesicles was noted. *Kazimierzus sophieae* is similar to *metandrus* by having paired spermathecae in three segments; however, in *sophieae* male funnels are in 10 and 11 but only in 11 in *metandrus*.

5.5 Discussion

Morphological characters in earthworms often show intraspecific variability (Decaëns et al. 2013; Loongyai et al. 2011; Briones et al. 2009) which may cause ambiguity in traditional

morphology-based earthworm taxonomy. In the phylogeny (Figure 4), even though clades have support, the tree is not resolved. *Kazimierzus alipentus*, *imitatus* and *pauli* are in one clade which is well supported, these species share: extending seminal vesicles, a reduction in one pair of seminal vesicles (anterior or posterior), multiple spermathecae in each segment (but in different segments). The clade with *kleinoodi*, *nietvoorbiji* and *sirgeli* is also well supported, these species share fused calciferous glands (in 9, 11 and 9), the spermathecae are paired in different segments and seminal vesicles do not extend backwards. This clade also share a recent common ancestor with *crousi* and the remaining species have a distant common ancestor that is not shared with *kleinoodi*, *nietvoorbiji* and *sirgeli*.

The seminal vesicles are very interesting in *Kazimierzus*. There are two groups, one that has seminal vesicles extending backwards and the other in which seminal vesicles do not extend. Seminal vesicles do not extend in ten species (*circulatus*, *crousi*, *davidi*, *franciscus*, *guntheri*, *kleinoodi*, *nietvoorbiji*, *peringueyi*, *phumlani* and *sirgeli*), these species have fused calciferous glands, except *davidi* and *phumlani*. Fifteen species (*alipentus*, *hamerae*, *imitatus*, *ljungstroemi*, *metandrus*, *nieuwoudtvillensis*, *obscurus*, *occidualis*, *occiduus*, *pauli*, *pearsonianus*, *pentus*, *rosai*, *senarius* and *sophieae*) have seminal vesicles extending backwards, extended segments differs between and within species. These species have paired calciferous glands with the exception of two species (*nieuwoudtvillensis* and *pearsonianus*). The two groups, with seminal vesicles extending and seminal vesicles not extending, could belong to two genera.

Reduction in one pair of seminal vesicles was observed in six species: *alipentus*, *imitatus*, *metandrus*, *pauli*, *pearsonianus* and *sirgeli*. Four of these species (*alipentus*, *imitatus*, *pauli* and *pearsonianus*) are holandric with well-developed multi spermathecae; the remaining two species have paired spermathecae, *metandrus* is metandric whilst *sirgeli* is proandric. Iridescence in male funnels or spermathecae was observed in majority of specimens indicating the presence of sperm which means parthenogenesis is eliminated as an explanation for the reduction in seminal vesicles.

The intersegmental furrow 13/14 is associated with spermathecal pores in all species (100 % occurrence). Plisko (1998) reported that the occurrence of spermathecal pores in 13/14 intersegmental furrow is typical of holandric species but in *Kazimierzus* it occurs in all holandric species plus the metandric *metandrus*, as well as, the proandric *sirgeli*.

Spermathecae are paired in nine species while multiple spermathecae occur in 16 species (Table 2). The number of segments occupied by spermathecae and their position differ, for example species may have spermathecae in 3 segments, in one species it could be 12–14 or 14–16 in another.

The nephridia in *K. metandrus* are V-shaped not J-shaped as in other species. The male funnels are in segment 11 only and this species is the only one with a metandric condition in this genus. However, the specimens have: fused first two segments, simple dorsal blood vessel and extending seminal vesicles, which are unique to Kazimierzidae. Given that the characteristics of *K. metandrus* do not fully fit in *Kazimierzus*, the suggestion is to accommodate it separately. Metandry condition seems to be a derived trait as no other species from closely related members share this character. *Kazimierzus sirgeli* is proandric with one pair of male funnels in 10; this condition was observed only once in this genus. Out of 25 known species, 23 are holandric and only the two above mentioned species differ, one metandric and one proandric. This may indicate mutation or may suggest species exclusion from the genus.

Endemism in soil fauna has been observed in groups such as millipedes (Hamer & Slotow 2002; Vohland & Hamer 2013). This makes them vulnerable to extinction (Hamer & Slotow 2000) because they have poor dispersal ability over long distances. The transformation of habitats by humans may reduce species richness and diversity, the resulting patchiness increases the possibility of losing endemic species (Suarez et al. 1998). *Kazimierzus* species show some level of endemism; habitat transformation poses a big threat to them since some species are known only from their type localities. This localised endemism may increase the potential for allopatric or parapatric speciation, which has been observed in flightless beetles (Bell et al. 2004).

Table 2. Comparison of *Kazimierzus* species using selected morphological characters.

Species	Calciferous glands	Spermathecae	Seminal vesicles
<i>K. alipentus</i>	in 10, paired	multiple, 11/12-15/16: 5 segments	Extending

<i>K. circulatus</i>	in 10, fused	paired, 12/13–15/16: 4 segments	Not extending
<i>K. crousi</i>	in 10–11, fused	multiple, 13/14–16/17: 4 segments	Not extending
<i>K. davidi</i>	in 10, paired	multiple, 12/13–15/16: 4 segments	Not extending
<i>K. franciscus</i>	in 11, fused	multiple, 13/14–15/16: 3 segments	Not extending
<i>K. guntheri</i>	10, fused	multiple, 13/14–15/16: 3 segments	Not extending
<i>K. hamerae</i>	in 10, paired	paired, 12/13–15/16: 4 segments	Extending
<i>K. imitatus</i>	in 10, paired	multiple, 11/12–16/17: 6 segments	Extending
<i>K. kleinoodi</i>	in 9, fused	paired, 12/13–16/17: 5 segments	Not extending
<i>K. ljungstroemi</i>	in 10, paired	paired, 13/14–15/16: 3 segments	Extending
<i>K. metandrus</i>	in 10, paired	paired, 13/14–15/16: 3 segments	Extending
<i>K. nietvoorbij</i>	in 11, fused	paired, 10/11–15/16: 6 segments	Not extending
<i>K. nieuwoudtvillensis</i>	in 10–11, fused	multiple, 12/13–15/16: 4 segments	Extending
<i>K. obscurus</i>	in 10, paired	multiple, 12/13–16/17: 5 segments	Extending
<i>K. occidualis</i>	in 10–11, paired	multiple, 13/14–15/16: 3 segments	Extending
<i>K. occiduus</i>	in 10, paired	multiple, 12/13–14/15: 3 segments	Extending
<i>K. pauli</i>	in 10, paired	multiple, 11/12–16/17: 6 segments	Extending
<i>K. pearsonianus</i>	in 10, fused	multiple, 13/14–15/16: 3 segments	Extending
<i>K. pentus</i>	in 10, paired	multiple, 11/12–15/16: 5 segments	Extending
<i>K. peringueyi</i>	in 10, fused	multiple, 12/13–16/17: 5 segments	Not extending
<i>K. phumlani</i>	in 10, paired	multiple, 13/14–15/16: 3 segments	Not extending
<i>K. rosai</i>	10–11, paired	paired, 12/13–15/16: 4 segments	Extending

<i>K. senarius</i>	in 10, paired	multiple, 11/12–16/17: 6 segments	Extending
<i>K. sirgeli</i>	in 9, fused	paired, 12/13–15/16: 4 segments	Not extending
<i>K. sophieae</i>	in 10, paired	paired, 13/14–15/16: 3 segments	Extending

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CHAPTER 6

(This study was submitted in 2018 to the journal *African Invertebrates* and is still under review.)

Molecular phylogeny of *Kazimierzus* Plisko, 2006 (Clitellata, Kazimierzidae) from the Western and Northern Cape Province inferred from mitochondrial DNA sequences

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6.1 Abstract

Species identification of earthworms using morphology can be challenging and inconclusive as homoplasy in many characters is high. The use of molecular DNA technology such as the use of conserved regions in mtDNA and nuclear DNA has unravelled the phylogenetic background of several earthworm species. The current study utilised the cytochrome c oxidase subunit I (COI) mitochondrial marker to reconstruct the phylogeny of *Kazimierzus* Plisko, 2006 species from the Western and Northern Cape provinces of South Africa. Phylogenetic reconstructions were implemented using Bayesian inference as well as maximum likelihood. Both tree building methods adhered to the monophyly of the majority of the taxa. Results showed that species fell into two clades and validated eleven currently

known *Kazimierzus* species (*K. circulatus* (Plisko, 1998), *K. franciscus* (Pickford, 1975), *K. guntheri* (Pickford, 1975), *K. hamerae* (Plisko, 1998), *K. kleinoodi* Nxele and Plisko, 2017, *K. nietvoorbiji* Nxele and Plisko, 2017, *K. nieuwoudtvillensis* Nxele and Plisko, 2017, *K. occidualis* (Plisko, 1998), *K. pearsonianus* (Pickford, 1975), *K. phumlani* Nxele and Plisko, 2017, *K. sophieae* (Plisko, 2002)). Cryptic diversity is recovered in *K. occidualis* with genetic divergence greater than 12 % among populations. *Kazimierzus franciscus* and *K. ljunstroemi* (Pickford, 1975) have a low genetic variability suggesting close relatedness or probable conspecificity. A group of specimens from Clanwilliam are morphologically identical to *K. sophieae*, but are genetically distinct and may belong to undescribed species. This study demonstrates the importance of integrative taxonomy in earthworms in order to present reliable taxonomic and biogeographic data.

Keywords: DNA, COI, mtDNA, taxonomy, earthworms, cryptic diversity.

6.2 Introduction

Earthworms constitute a large component of soil invertebrates and are regarded as soil engineers (Jouquet et al. 2006). They alter soil properties and enhance nutrient cycling (Lavelle 1988) which determines plant community composition. Regardless of their importance, African earthworm fauna have not received much attention and their evolutionary relationships are subject to debate. Species identification is possible by investigating their anatomy (Chang et al. 2007, Csuzdi 2010, Csuzdi and Zicsi 2003, Plisko and Zicsi 1991), but the structural simplicity of their body plan and existence of cryptic species may hinder taxonomic classification (Domínguez et al. 2015). Traditional morphology-based identification also requires substantial taxonomic expertise in this group because it involves observation of minute morphological characters (Richard et al. 2010). As such, the use of molecular DNA technology in species delineation is an important complement to morphological classification of earthworms.

The use of DNA sequences has increased in the recent past because it is less subjective than morphological characters, allows for the analysis of several characters (Scotland et al. 2003) and is applicable at all developmental stages (Decaëns et al. 2013; Chang and James 2011). The molecular studies of earthworms that have used the mitochondrial cytochrome c oxidase

subunit I (COI) gene in integrative taxonomy have shown good results (Blakemore 2013a,b; Blakemore et al 2010; Bantaowong et al. 2011; Huang et al. 2007; Richard et al. 2010; King et al. 2008; Rougerie et al. 2009; Chang et al. 2009; James et al. 2010). This marker is able to distinguish the intra and interspecific genetic variation and groups conspecifics together because COI sequences are variable enough to differentiate between taxa, but are less variable in conspecifics (Rougerie et al 2009; Stoeckle and Hebert 2008; Valentini et al. 2008).

According to Nxele (2012), knowledge of the taxonomic diversity of the indigenous megadriles in South Africa is incomplete. Therefore, integration of taxonomic methods is vital to improve the knowledge and understanding of the megadrile fauna. Furthermore, Plisko (2013) stressed that a molecular study on indigenous South African megadriles is essential in order to reveal the evolutionary relationships among them. Against this background, the phylogenetic relationships in *Kazimierzus*, a genus occurring in the Western and Northern Cape provinces of South Africa was investigated.

6.3 Materials and methods

6.3.1 Sampling

In order to obtain species of *Kazimierzus*, qualitative sampling was carried out in 2011 and 2015 during the rainy season (July–September) in the Western and Northern Cape, South Africa. Besides the focus on type localities, potential sites other than the type localities were also sampled. Earthworms were collected by digging and hand sorting. Collected specimens were anesthetized in 20 % ethanol solution, fixed in 4 % formalin solution and preserved in 75 % ethanol for taxonomic purposes. A sub sample was preserved in absolute ethanol for molecular analysis. All specimens were examined using a Wild Heerbrugg stereo-microscope and were identified according to the descriptions in Plisko (1996, 1998, 2002), Pickford (1975), Michaelsen (1913) and Nxele et al. (2017). All specimens are in KwaZulu-Natal Museum.

6.3.2 Genomic DNA extraction, amplification and sequencing

Tissue from the posterior section of the earthworm was used. All DNA extractions were performed using the ZR Genomic DNA™ Tissue MicroPrep kit, following the manufacturer's standard protocol. The concentration of DNA in each sample was estimated using the NanoDrop 2000 (Thermo Scientific). A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA 3') primers (Folmer et al., 1994). Polymerase chain reactions (PCR) were performed in a final volume of 25µl using the BIO RAD T100 Thermal Cycler and contained: 2µl of DNA template (approximately 35ng/ul), 12.5µl One Taq Quick-Load 2X Master Mix with standard buffer, 0.5µl of 10uM forward and reverse primers and sterile water. The thermocycler conditions were as follows: 95° C for 2.30 min for initial denaturation followed by 35 cycles at 95° C for 30 sec denaturation, 50° C to 52° C for 45 sec annealing and 72° C for 75 sec extension. A final extension step at 72° C for 10 min completed the reactions.

Sequencing of the 675 bp fragment of the COI mtDNA was conducted at Inqaba Biotechnical Industries (Pty) Ltd.

6.3.3 Sequence alignment and phylogenetic analysis

Identity of sequences was verified by the Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology (NCBI). Sequences of *Amyntas minimus* (Horst, 1893) and *Amyntas corticis* (Kinberg, 1867) were included as outgroup taxa. The sequences for outgroup taxa were obtained from GenBank (Accession nos: AB542509.1, AB542469.1) and current sequences are added as supplementary data. All specimens are at the KwaZulu-Natal Museum. The sequences were aligned using CLUSTAL X 2.1 (Larkin et al. 2007). These alignments were then manually edited using BIOEDIT 3.3.19.0 (Hall 1998). Unreliable nucleotides (low signal strength) as well as primers sequences were trimmed off at both the 5' and 3' ends. The program JMODELTEST v.0.1.1 (Darriba et al. 2012) was used to select the best-fit evolutionary model using the AKAIKE information criterion (AIC; Akaike 1973). Phylogenetic analyses were based on two approaches, Bayesian inference was performed using MRBAYES 3.2 (Huelsenbeck & Ronquist 2001) and maximum likelihood (ML)

analysis was performed using GARLI (Zwickl 2011). In each case the best-fit evolutionary model selected by JMODELTEST was specified.

Clade support was evaluated by 1000 bootstrap replicates for the maximum likelihood analysis and posterior probability values for the Bayesian analysis. For Bayesian analyses, all MRBAYES analyses were run for 5000 000 generations with a sampling frequency of 1000. The deviation of split frequencies was less than 0.01 at the conclusion of all analyses which confirmed that the MCMC chains had converged. The program TRACER v1.5 (Drummond & Rambaut 2007) was used to check that the Effective Sampling Size >200 and that posterior distribution for all parameters was unimodal. Consensus trees were generated using PHYLIP 3.69 (Felsenstein 2005) and viewed in FIG TREE v1.3.1 (Rambaut 2009). Uncorrected *p* genetic distances were obtained for the sequenced specimens using MEGA 6 (Tamura et al. 2013). Each species is represented by one specimen except where the species appeared in more than one clade on the phylogenetic tree.

6.4 Results

6.4.1 Phylogenetic analyses

The sequences were 675 bp. Variable sites were 431 bp and conserved sites 233 bp showing great differentiation amongst taxa.

Most species pair comparisons showed a genetic distance above 13 % except for *K. ljunstroemi* and *K. franciscus* who have one percent genetic distance between them (Table 1).

The maximum likelihood and Bayesian Inference trees were congruent therefore, support values were annotated onto the branches of the most likely trees generated for each of the data sets analysed (ML run with no bootstrap, rooted using outgroup species (*Amyntas minimus* and *Amyntas corticis*)).

Table 2. Pairwise *p* genetic distances (%) between the investigated *Kazimierzus* species/lineages.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. <i>K. nietvoorbij</i>																
2. <i>K. phumlani</i>	21.1															
3. <i>K. hamerae</i>	22.3	20.1														
4. <i>K. sophieae</i>	25.4	25.5	16.9													
5. <i>K. sp</i>	20.3	18.3	17.5	23.0												
6. <i>K. sophieae</i>	23.1	20.1	21.5	25.5	19.0											
7. <i>K. nieuwoudtvillensis</i>	18.8	20.7	19.9	25.5	17.4	22.6										
8. <i>K. circulates</i>	19.0	17.5	18.5	23.8	17.9	19.5	16.6									
9. <i>K. occidualis</i>	18.7	15.5	20.9	25.2	18.2	19.6	17.7	15.8								
10. <i>K. occidualis</i>	19.0	16.9	22.2	26.3	19.5	20.9	19.0	17.2	3.0							
11. <i>K. occidualis</i>	21.5	21.9	23.1	28.5	21.5	24.2	20.4	19.8	15.5	16.1						
12. <i>K. franciscus</i>	19.9	19.9	22.0	26.8	19.3	21.7	19.1	19.9	18.7	19.8	23.6					
13. <i>K. ljungstroemi</i>	19.9	20.4	22.2	27.3	18.8	21.9	19.3	19.8	18.7	19.6	23.4	1.1				
14. <i>K. guntheri</i>	13.6	21.1	21.1	26.6	20.7	23.1	19.3	20.4	19.5	18.7	19.3	21.4	21.5			
15. <i>K. kleinoodi</i>	13.2	21.4	23.9	28.5	21.9	23.8	20.9	22.5	19.5	20.7	22.6	22.6	22.8	15.0		
16. <i>K. pearsonianus</i>	14.0	22.6	23.8	27.3	23.4	25.4	22.2	22.0	21.7	22.3	23.6	22.2	22.0	15.0	15.8	

Bootstrap values and posterior probabilities below 50 % and 0.50 were not shown on the trees. Two clades, A and B, are distinct, clade A separates further to clade C and D whilst clade B separates to clade E and F (Fig. 1). Both tree building methods support monophyly of majority of taxa (Fig. 1). *Kazimierzus sophieae* is paraphyletic, found in two distinct clades; one shared with *K. sp* and the second clade with *Kazimierzus hamerae* (clade D). The two species in clade C, *K. circulatus* and *K. nieuwoudtvillensis*, are sister taxa. *Kazimierzus phumlani* and *K. occidualis* are sister taxa comprising clade E. Clade F has six species. *Kazimierzus franciscus* and *K. ljungstroemi* form a strongly supported sub-clade in clade F. The other species (*K. guntheri*, *pearsonianus*, *kleinoodi* and *nietvoorbiji*) form the other sub-clade.



6.5 Discussion

Morphological examination revealed eleven currently known species (*circulatus*, *franciscus*, *guntheri*, *hamerae*, *kleinoodi*, *nietvoorbiji*, *nieuwoudtvillensis*, *occidualis*, *pearsonianus*, *phumlani* and *sophieae*); however the current phylogenetic analysis resulted in additional lineages that suggest cryptic diversity (Fig. 1) and a new species, *K. sp.* Most lineages had strong support but weak branch support was noted in deeper nodes with some clades having bootstrap values less than 50 % hence not annotated on the tree. Genetic distance also support the relationships observed in the phylogenetic tree. Genetic distance between species was greater than 13 %, for example *K. circulatus* and *nieuwoudtvillensis* have 16.6 % distance between them (following recommendations by Hebert et al. 2003, Decaëns et al. 2013 and Huang et al. 2007 for separation of species by genetic distance).

Kazimierzus hamerae is similar in appearance to *sophieae* but molecular data confirmed that they are separate species. The phylogenetic tree (Fig. 1), however, highlights, unexpectedly, that *K. sophieae*, includes two clades consisting of specimens that were collected from two localities, one in Clanwilliam and the other in van Rhyn's Pass. The two clades may be because *sophieae* is polyphyletic or there is no morphological divergence of these taxa that are different species genetically. The type locality of *sophieae* is Nieuwoudtville, which is close to van Rhyn's Pass. It is likely that the specimens of *sophieae* from van Rhyn's Pass that are in the same clade as *hamerae* are probably more related to *sophieae*. The specimens of *sophieae* from Clanwilliam that are in the same clade as *K. sp.* may possibly be undescribed species however a comparison of sequences of *sophieae* specimens and those from the type locality, Nieuwoudtville is necessary. It seems that morphological evolution is not rapid in this complex of taxa and there is presence of cryptic diversity. Clade D has poor maximum likelihood support (52 %) but bayesian inference support is high (0.99). The low maximum likelihood support may suggest that the present data is not sufficient to resolve this polytomy fully.

Cryptic diversity was observed in *occidualis*. Finding cryptic diversity is common in earthworms, Novo et al. (2010) reported five cryptic species within the complex of *Hormogaster elisae*, Richard et al. (2010) reported cryptic species in *Lumbricus terrestris*, and Pérez-Losada et al. (2009) found cryptic diversity within *Aporrectodea caligininosa*

species complex. The observations support the view that diagnosis based on morphology only underestimates taxonomic diversity.

The specimens of *Kazimierzus franciscus* and *Kazimierzus ljungstroemi* were collected in two neighbouring forests, Duiwelbos and Koloniesbos in Marloth Nature Reserve, Swellendam. Duiwelbos is a type locality of *franciscus* whilst the type locality for *ljungstroemi* is Great Winterhoek, Tulbagh District. Therefore it is possible that all specimens belong to *K. franciscus*.

Although analysis of other conserved genomic regions in both mtDNA and nuclear DNA in the future would benefit the study of *Kazimierzus* species, the phylogenetic analysis of COI recovered several well supported phylogenetic relationships, some of which are congruent with existing classification.

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CHAPTER 7

Predicting the distribution patterns of *Kazimierzus hamerae* (Plisko, 1998) based on large scale variables

7.1 Abstract

Knowledge of geographical distribution of species as well as environmental changes that affect them is useful for the conservation and management of biodiversity. Environmental parameters as well as vegetation contribute to the distribution and abundance of species in space and time. Physical and chemical properties of the soil also contribute to species distribution. Species distribution models are useful in predicting earthworm distribution patterns and abundance at different scales. The current study used Maxent to model the distribution of *Kazimierzus hamerae*. The predicted suitable localities were different from the actual locations where the species were collected; the most interesting is the possibility of occurrence in Namibia. The occurrence of *K. hamerae* was tested in Namibia, along the coast in Oranjemund, Rosh Pinah and Aus. Unfortunately no targeted species were found during sampling. The sampling took place during the time southern Africa was experiencing drought. The results also demonstrated that precipitation in the driest month is the most relevant predictor in spatial distribution patterns of *K. hamerae*.

Keywords: Western Cape, Northern Cape, Namibia, *Kazimierzus*, habitat prediction, Maxent, distribution, drought.

7.2 Introduction

Soil research often depends on biological knowledge however, there is a paucity of data (Janion-Scheepers et al. 2016) because, in general, soils are poorly studied (Decaëns 2010, Janion-Scheepers et al 2016). Below ground taxa have not attracted significant interest within the scientific community, hence; there is a dearth in literature on the biogeography, taxonomy and systematics (Decaëns et al. 2006). Nevertheless, the recognition of the importance of soil fauna is increasing and more studies on the taxonomic diversity and species distribution are being conducted.

Earthworms are important organisms in soil ecology because they are responsible for decomposition or nutrient availability through soil engineering and they also promote soil biodiversity and soil health (Lavelle et al. 1997, 2006). Earthworms are directly related to soil ecosystem function so populations depict spatial and temporal heterogeneity in patches and their population structure can therefore be influenced by changes in soil and above ground characteristics such as vegetation, as well as, biotic and abiotic interactions (Margerie et al. 2001; Whalen 2004; Fey 2010; Valckx et al. 2011; Ismail and Murthy 1985).

Species distribution models are useful in predicting species distribution patterns and abundance (Schröder & Seppelt 2006). According to Palm et al. (2013) species distribution models can be used to identify changes associated with land use or climate change. Small and large-scale soil variables such as climate and land use have significant effect on the distribution of earthworms (Palm et al. 2013).

Kazimierzidae earthworms are not easy to collect due to their restricted distribution (Nxele et al. 2016). Finding distribution models that enable the selection of suitable sampling sites could provide more information on their poorly understood distribution. *Kazimierzus hamerae* (Plisko, 1998) has been collected in both the Western and Northern Cape. In the present study I used *K. hamerae* as a model for the inference of suitable habitats because the habitat type for *K. hamerae* is similar to that of other *Kazimierzus* species.

7.3 Material and methods

Occurrence data for *Kazimierzus hamerae* were compiled from the Oligochaeta database at the KwaZulu-Natal Museum. Anatomical examinations were conducted under a stereomicroscope subsequent to dorsal dissection of specimens in order to expose the internal organs, after which specimens were identified using Plisko (1996, 1998, 2002) and Pickford (1975). I used Maxent (version 3.3.3 by Phillips & Dudík, 2008) to assess the possible distribution pattern of this species in South Africa.

Marchán et al. (2015) showed that Maxent can be used as a sampling site selection tool using presence absence data. They also showed that there is a relationship between the predictor variables and soil variables, that is, they proved that the model has the ability to predict soil variables from environmental ones. Here, Maxent was selected because of its ability to use

presence data and because it has good predictive ability even when datasets used are not big (Phillips & Dudik, 2008). The model was constructed based on six bioclimatic variables, reflecting the influence of water availability and temperature variation, from WorldClim (<http://www.worldclim.org>). These variables were: annual mean temperature (bio1), mean diurnal range (bio2), mean temperature of the coldest quarter (bio11), precipitation in the wettest (bio13) and driest (bio14) months and precipitation of coldest quarter (bio19). Maxent models were run using standard default settings. The performance of models was evaluated with the area under the curve (AUC) of the receiver operating characteristics and fivefold cross-validation procedures.

7.4 Results

The models obtained demonstrated good average AUC values. The value for training data AUC was 0.970 showing best fit of models. Random prediction value was 0.5. The average MaxEnt model indicated that the variable ‘precipitation of driest month’ was important in describing the realised climatic distribution of *K. hamerae* (Table1)

Table 1. Relative contribution of each variable to the prediction model.

Variable	Percentage contribution	Permutation importance
Precipitation of driest month(bio14)	59.1	88.1
Precipitation of coldest quarter (bio19)	21.4	4.9
Mean temperature of coldest quarter (bio11)	13.8	1.5
Mean diurnal range (bio2)	4.9	5.5
Annual mean temperature (bio1)	0.9	0
Precipitation of wettest month (bio13)	0	0

The environmental variable with highest gain when used in isolation is the precipitation in driest month (bio14), which has most useful information by itself. Precipitation of the coldest quarter (bio19) when omitted decreases the gain the most, it also appears to have the most information that is not present in the other variables (Fig.1).

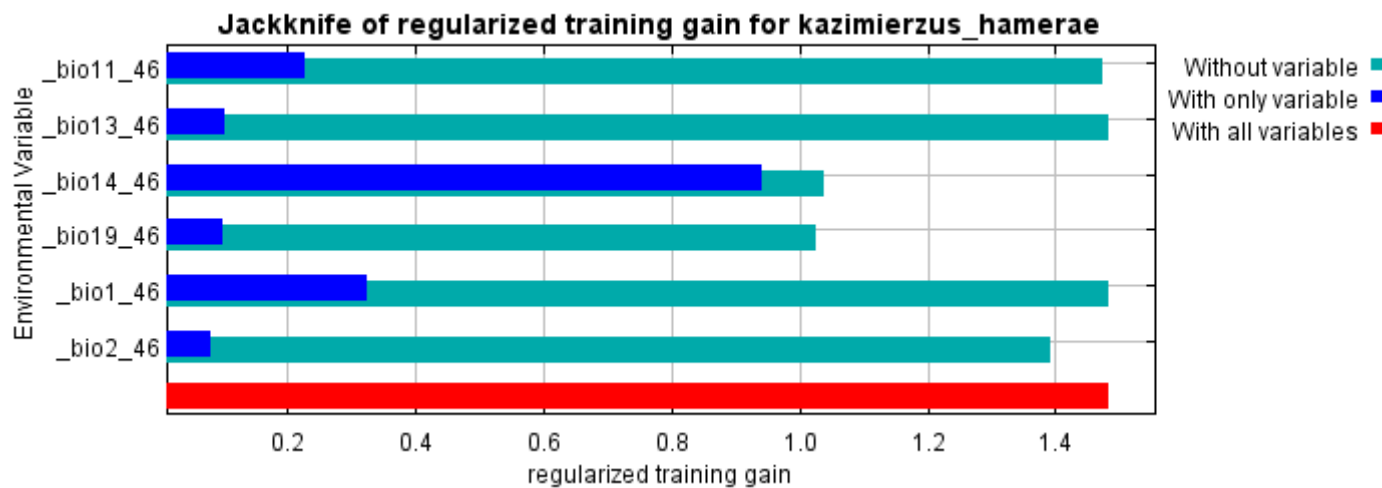


Figure 1. The jackknife test of variable importance showing the environmental variables with highest and lowest gains in the model.

The predicted distribution falls in the already known provinces of South Africa (Fig. 2).

7.5 Discussion

The measure of predictive performance was good (training data AUC = 0.970) showing high predictive power of the model. The mean value of 0.5 for *K. hamerae* current locations was obtained, this is accepted threshold by MaxEnt. Similar success was obtained by other researchers that have used few species for niche modelling (Tarrant & Armstrong 2013, Marchán et al. 2015, Mwabvu & Schoeman 2016, Latif et al. 2017). The predicted suitable localities were different from the localities where the species was present.

The suitable habitat range is narrow and is associated with the Atlantic Ocean. The climatic conditions, soil and vegetation type along the Atlantic Ocean is different from those inland, this may be the reason why this species is limited to this area, however, this hypothesis is yet

to be tested (Nxele et al. 2016). The major finding was that the predicted distribution includes the Atlantic coast of Namibia which was long suspected (Nxele et al. 2016). This is not surprising as the climatic conditions along the Atlantic Ocean should be similar. The occurrence of *K. hamerae* was tested in Namibia, along the coast in Oranjemund, Rosh Pinah and Aus. Unfortunately no targeted species were found during sampling. The sampling took place in 2017, during this time southern Africa was experiencing drought which may be the reason for not finding any target earthworms. In Aus it hadn't rained for the past three years (pers. comm. with a store manager in Aus). Resampling will be conducted when the climatic conditions are better.

The result that precipitation of driest month is the most relevant predictor for spatial distribution patterns (Fig. 1) may be due to the fact that precipitation reflect the availability of water in the soil throughout the year, this is an essential requirement for earthworms (Latif et al. 2017).

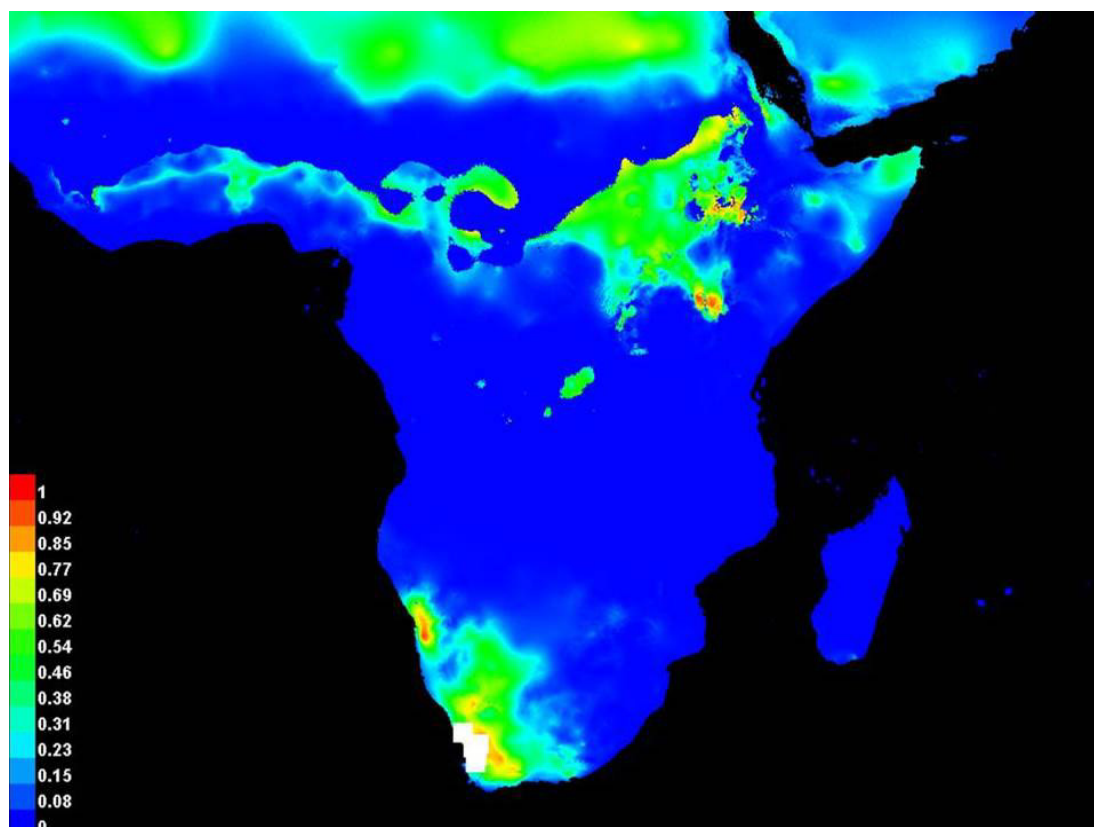


Figure 2. Potential predicted habitats with white patches representing present locations. Colour represent potential, blue is the least whilst red is the most suitable.

7.6 References

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CHAPTER 8

Common names for the *Kazimierzus* (Clitellata, Kazimierzidae) earthworm species

8.1 Abstract

South Africa has different climates because of the influence of the Atlantic and Indian Oceans. The Western and Northern Cape provinces receive winter rainfall because of the effect of the Atlantic Ocean. Earthworm species found in this area are unique. There is increasing awareness of the importance of earthworms in South Africa, which increases the need to give indigenous earthworms common names. We present here a list of common names, as well as, scientific names for earthworm species belonging to the genus *Kazimierzus*, which occur in the Western and Northern Cape provinces of South Africa.

Keywords: Earthworms, English names, conservation, agriculture, South Africa, *Kazimierzus*, farmers, awareness.

8.2 Introduction

In biodiversity studies, species are the important basic biological units (Schmelz et al. 2017). Therefore, species identity and distribution form a very important base for conservation and monitoring of biodiversity. In South Africa, terrestrial fauna are more prevalent in grasslands and forest (Plisko 1997, 2012). More studies are therefore necessary because they are an opportunity to contribute to understanding evolutionary processes and provide information for conservation and agriculture. *Kazimierzus* species occur in the Western and Northern Cape provinces (Fig. 1) of South Africa in undisturbed natural habitats (Fig. 2), most species were collected in sandy soils (Fig. 3). Currently there are 25 described *Kazimierzus* species but none of them have been included in any conservation plans. Earthworms are rarely included in environmental monitoring or conservation programmes (Nxele et al. 2015) despite the fact that their contribution in soil processes is immeasurable. According to Schmelz et al. (2017), identifying species correctly is important in linking species to data associated with the species. In the present paper I provide a list of English common names for earthworms

belonging to *Kazimierzus*. Nevertheless, it must be conceded that common names are not more precise than scientific names but they are more convenient to use by non-specialists.

This is the second paper listing English names for indigenous earthworm species in South Africa (see Armstrong and Nxele 2017). More people are interested in knowing the earthworms that they have in their gardens and farmers have also shown interest in knowing more about earthworm species in their soils (No-till conference 2017). Indigenous earthworms have been recorded in farms (see Nxele 2015) and this increases the necessity to give indigenous earthworms common names.

8.3 Material and methods

The method of compilation of names followed that in Armstrong & Nxele (2017). The *Kazimierzus* species occurring in the Western and Northern Cape and the scientific nomenclature were obtained from relevant publications (Pickford 1975; Plisko 1998, 1996, 2002, 2006, 2012; Michaelsen 1908, 1913 and Nxele et al. 2017).

8.4 Results

The scientific names and proposed English names of the species of earthworms belonging to the genus *Kazimierzus* are given in Table 1.

Table 1. English names for *Kazimierzus* species from the Western and Northern Cape.

Scientific Name	English Name
<i>K. alipentus</i> (Plisko, 1998)	Rocky soil worm
<i>K. circulatus</i> (Plisko, 1998)	Circle-gland worm
<i>K. crousi</i> (Pickford, 1975)	Crous's worm

<i>K. davidi</i> (Plisko, 1998)	David's worm
<i>K. franciscus</i> (Pickford, 1975)	Francis's worm
<i>K. guntheri</i> (Pickford, 1975)	Gunther's worm
<i>K. hamerae</i> (Plisko, 1998)	Hamer's worm
<i>K. imitatus</i> (Plisko, 1998)	Imitating worm
<i>K. kleinoodi</i> Nxele & Plisko, 2017	Kleinood worm
<i>K. ljungstroemi</i> (Pickford, 1975)	Ljungström's worm
<i>K. metandrus</i> (Plisko, 1998)	Hillside worm
<i>K. nietvoorbij</i> Nxele & Plisko, 2017	Nietvoorbij worm
<i>K. nieuwoudtvillensis</i> Nxele & Plisko, 2017	Nieuwoudtville worm
<i>K. obscurus</i> (Plisko, 1998)	Indistinct-clitellum worm
<i>K. occidualis</i> (Plisko, 1998)	Swamp worm
<i>K. occiduus</i> (Plisko, 1998)	Violet worm
<i>K. pauli</i> (Plisko, 1998)	Paul's worm
<i>K. pearsonianus</i> (Pickford, 1975)	Pearson's worm
<i>K. pentus</i> (Plisko, 1998)	Violet-dark grey worm
<i>K. peringueyi</i> (Michaelsen, 1913)	Peringuey's worm
<i>K. phumlani</i> Nxele & Plisko, 2017	Phumlani's worm
<i>K. rosai</i> (Michaelsen, 1908)	Rosa's worm
<i>K. senarius</i> (Plisko, 1998)	Yellowish-grey clitellum worm
<i>K. sirgeli</i> (Plisko, 1996)	Sirgel's worm
<i>K. sophieae</i> (Plisko, 2002)	Sophie's worm

8.5 Discussion

Natural habitat destruction is a serious threat to biodiversity (Costello et al. 2013). Amongst many reasons for habitat destruction is that people do not understand the importance of taxa and the role that they play in the environment. Earthworms have received little attention in South Africa although they are threatened by land transformation (Plisko 1995, 2000). Recently, there has been increasing awareness of the importance of earthworms in South Africa after the first earthworms were given common names (Armstrong & Nxele 2017). Consequently, I discerned a need to continue to assigning English common names to more earthworm species. We hope that by giving common names communities will start to appreciate the role that earthworms play in soils and that it will inspire more earthworm-based research in the agricultural sector. The use of common names will also facilitate communication among scientists and communities/citizen scientists.

8.6 References

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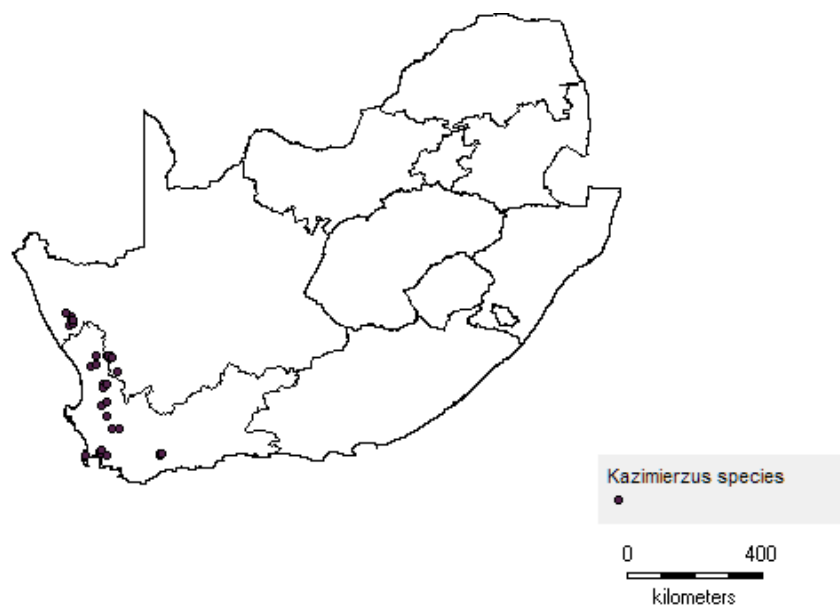


Figure 1. Distribution of *Kazimierzus* species in the Western and Northern Cape.



Figure 2. The general habitat for *Kazimierzus* species in the Western and Northern Cape.



Figure 3. *Kazimierzus* species in sandy soil and their various excrements

CHAPTER 9

Conclusion

During the presented study, all objectives outlined in the introduction were met. The following tasks were completed: 1. described species were validated, a morphological cladistics phylogeny was developed and the family revised and a key for identification of all species was provided; 2. a molecular phylogeny was developed and 3. an assessment of species distribution in southern Africa is discussed.

Results presented here showed that *Kazimierzus* species does not belong to Microchaetidae; hence, a new family was erected for them. In the molecular phylogeny, although most taxa are monophyletic, some revealed cryptic diversity that was unknown before the study. The use of genetic data demonstrated the power of using both morphology and molecular data in taxonomy. However, more markers should be used to increase the resolution of the phylogenetic tree of *Kazimierzus*. In South Africa, earthworms in agroecosystems are not well documented. However, more farmers are adopting the no-till conservation agriculture thus there is a need to study earthworms in these soils so that adequate information becomes available to farmers. The main issue for farmers is that scientific names are difficult to pronounce and remember. So with earthworms being given English common names there is hope that future studies in agroecosystems, conservation planning and environmental impact assessments will communicate clearly to the intended beneficiaries.

The hypothesis that the distribution of *Kazimierzus* includes the coastal regions of Namibia is yet to be tested. As such, more sampling in Namibia is needed when conditions are favourable.

Future research

The following are some of the gaps that have been identified in the study of earthworms, in general.

1. There is a need to construct a phylogeny of all indigenous earthworm families using more markers. This is necessary to get the position of all families on the earthworm

tree of life. It is also important to establish the relationships between the families because this will make species data more reliable and facilitate identification of unique genotypes.

2. Collaboration between botanist and soil scientists is necessary in order to unravel the complexities in the interactions between earthworms and among biota. To understand the levels of endemism shown by most indigenous species, it is important to understand the soil, as well as, vegetation that make the habitats unique.
3. More sampling in agroecosystems and working with farmers has to be enhanced. Earthworms are very important in the soil and this need to be communicated with the very people that are responsible for food security. There is a huge gap of knowledge between farmers and the life in their soils in South Africa. Earthworms in agroecosystems are not well documented hence working with farmers in the near future is recommended.

APENDIX

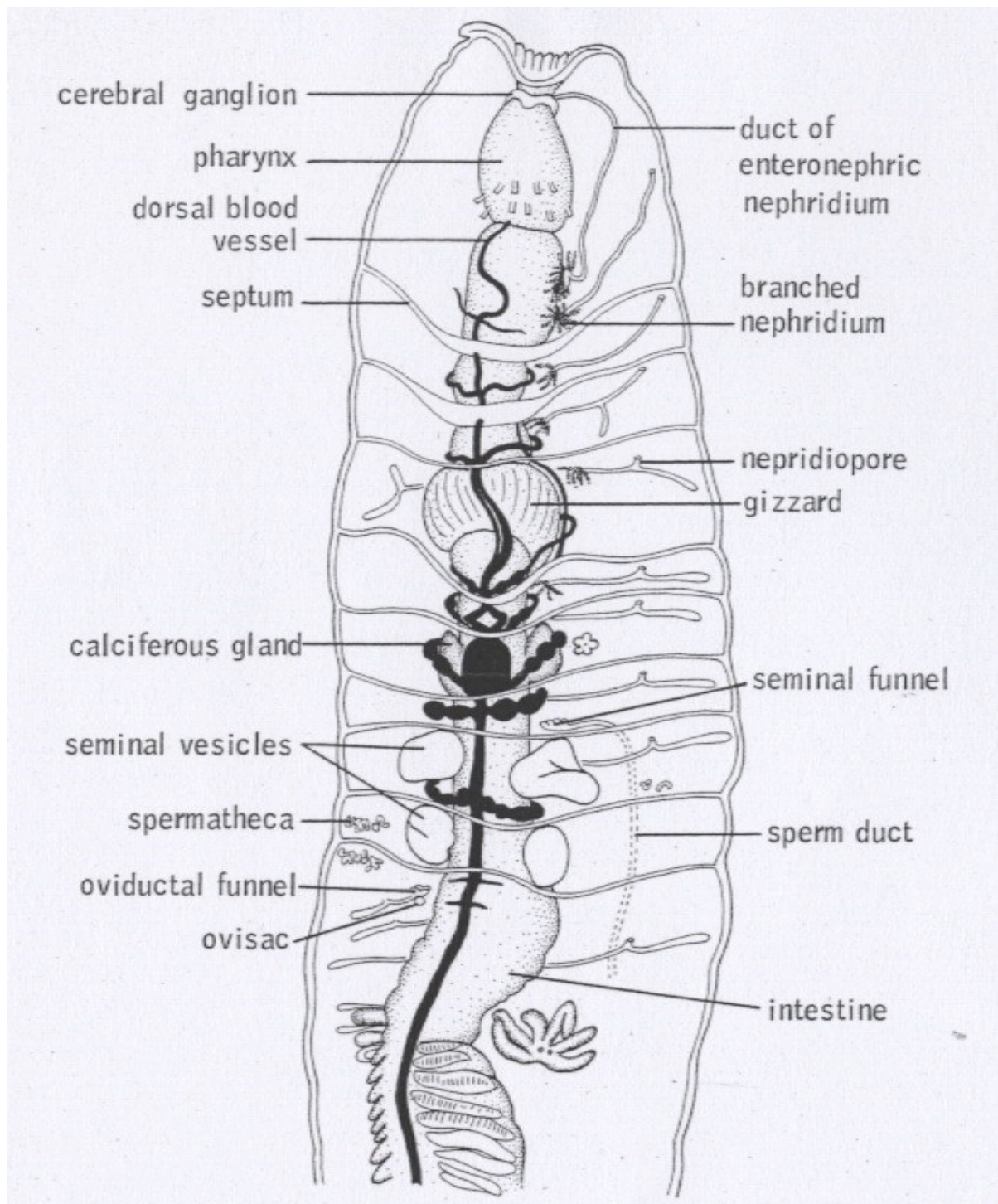


Figure A1. Dorsal view of earthworm, *Microchaetus* showing internal characters (After Barnes 1974).

Table A1. Data matrix of morphology characters used in the morphology analyses of *Kazimierzus* species

	1	2	3	4	5	6	7	8
<i>Kazimierzus alipentus</i>	0	1	0	0	1	1	0	1
<i>Kazimierzus circulatus</i>	0	0	1	1	1	0	0	0
<i>Kazimierzus crousi</i>	1	0	1	1	1	1	0	1
<i>Kazimierzus davidi</i>	0	1	1	1	1	1	0	0
<i>Kazimierzus franciscus</i>	1	0	1	1	0	1	0	1
<i>Kazimierzus guntheri</i>	0	0	1	1	0	1	0	1
<i>Kazimierzus</i>	0	1	0	1	1	0	0	0
<i>Kazimierzus imitatus</i>	0	1	0	0	1	1	0	1
<i>Kazimierzus kleinoodi</i>	1	0	1	1	1	0	0	1
<i>Kazimierzus ljungstroemi</i>	0	1	0	1	0	0	0	0
<i>Kazimierzus metandrus</i>	0	1	0	0	0	0	1	0
<i>Kazimierzus nietvoorbiji</i>	1	0	1	1	1	0	0	1
<i>Kazimierzus nieuwooudtvillensis</i>	1	0	0	1	1	1	0	0
<i>Kazimierzus obscurus</i>	0	1	0	1	1	1	0	0
<i>Kazimierzus occidualis</i>	1	1	0	1	0	1	0	0
<i>Kazimierzus occiduus</i>	0	1	0	1	0	1	0	1
<i>Kazimierzus pauli</i>	0	1	0	0	1	1	0	1
<i>Kazimierzus pearsonianus</i>	0	0	0	0	0	1	0	1
<i>Kazimierzus pentus</i>	0	1	0	1	1	1	0	0
<i>Kazimierzus peringueyi</i>	0	0	1	1	1	1	0	1
<i>Kazimierzus phumlani</i>	0	1	1	1	0	1	0	0
<i>Kazimierzus rosai</i>	1	1	0	1	1	0	0	1