# Revision of the Genus *Afroleius* Mahunka, 1984 (Acari: Oribatida)

By

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# Declaration

I declare that this thesis, hereby handed in for the qualification Doctor of Philosophy in Zoology in the Faculty of Natural and Agricultural Sciences at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at another university/faculty.

Louise Coetzee

Date

"You are never too old to set another goal or to dream a new dream"

C.S. Lewis

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# Introduction

## Background

The invention of the microscope in the late seventeenth century opened up a whole new world for mankind. For the first time, inquisitive biologists could see organisms too small to be seen by the naked eye. In his historic book *Micrographia*, published in September 1665, Robert Hooke detailed his observations through a microscope. Hooke's spectacular images of the micro-world inspired a wide interest in the new science of microscopy.

With the advancement of technology and improved optics, the study of micro-organisms became much more detailed and available to a large scientific community.

Ancient Greeks were aware of *akari* and understood some of the effects these small creatures had on nature, but it was only from the late 1700s that they became more recognised.

Linnaeus listed 29 species of *Acarus* in the 10th edition of his *Systema Naturae* (see Krantz & Walter 2009; Walter & Proctor 1999), four of them referable to Oribatida (Schatz 2002).

Acarology—the study of mites—includes all taxa in the arachnid subclass Acari. Mites are among the



From: *Micrographia* Robert Hooke 1665. "I observ'd in Oxford several of these little pretty creatures to wander to and fro ... " oldest of arthropod groups, with fossil evidence from the Devonian (380–400 mya) (Walter & Proctor 1999), when terrestrial vegetation began to flourish (Norton 1985). The earliest known oribatid mite fossils derive from the Middle Devonian Gilboa mudstones in New York State (Norton *et al.* 1988). Early in their evolutionary history oribatid mites became established in soil habitats and played an important role in soil ecology (Labandeira *et al.* 1997; Norton 1985; Schaefer *et al.* 2010). Mites, in the broad sense, have evolved to occupy almost all possible habitats—aquatic, arboreal, terrestrial and parasitic—and have developed a wide range of lifestyles (Walter & Proctor 1999).

The Acari is divided into two superorders, namely Parasitiformes (including ticks) and Acariformes. The superorder Acariformes comprises those mites which Walter & Proctor (1999) call the "mite-like mites" and includes two orders, namely Trombidiformes (mainly plant and predatory mites) and Sarcoptiformes. The suborder Oribatida form the largest group within Sarcoptiformes (Krantz & Walter 2009). The suborder Oribatida includes the cohort Astigmatina, previously regarded as a suborder of Sarcoptiformes (as Astigmata). Norton (1994, 1998, 2007) explored the strong morphological evidence for the hypothesis that Astigmata originated from within Oribatida and this is supported by the molecular analysis of Dabert *et al.* (2010). However, the contradicting results of Domes *et al.* (2007) do not support the origin of Astigmata within Oribatida.

Oribatid mites (excluding Astigmatina) are mostly organic decomposers. The vast majority inhabit the soil-litter system, while some are arboreal and a few are aquatic. Oribatid mites are small, ranging from  $300-700 \mu m$  (with extremes from  $150-2 \ 000 \mu m$ ), and while they lack in size, they make up in numbers. In organic soils they are often the dominant arthropod group (Behan *et al.* 1978; Nel & Ryke 1970; Norton & Behan-Pelletier 2009). Abundance and diversity of oribatids vary greatly according to soil conditions. As many as 200 000 individuals per m<sup>2</sup> are commonly reported from Canadian soils, with extremes as high as 1 million (Behan-Pelletier 1993) and diversities of 100–150 species per m<sup>2</sup> (Norton & Behan-

Pelletier 2009). South African soils do not support such high abundance or diversity. Hugo-Coetzee & Avenant (2011) recorded 49 species in Free State grasslands over a period of three years, with an average abundance of 2 600 individuals per m<sup>2</sup> (litter and 5 cm-deep soil). Nel & Ryke (1970) investigated the abundance and diversity of soil fauna in two separate habitats (indigenous forest and sugarcane plantations) in KwaZulu-Natal (Indian Ocean Coastal Belt, see Mucina & Rutherford 2006); densities of on average 70 730 individuals per m<sup>2</sup> in the forest and 13 800 individuals per m<sup>2</sup> in sugarcane plantations were recorded, comprising at least 90 species of which 84% were restricted to the forest and 16% to sugarcane plantations. Other examples are numbers calculated per m<sup>2</sup> from samples (litter and topsoil): grasslands with about 1 500 individuals and up to 20 species, coastal scrub vegetation with about 20 000 individuals and up to 40 species, coastal forests with about 46 000 individuals and up to 50 species, and savanna from the Indian Ocean Coastal Belt with about 100 000 individuals and up to 140 species per m<sup>2</sup> (own data, unpublished).

Soil mites are mostly particle-feeding saprophages and mycophages (Norton & Behan-Pelletier 2009) and play an important role in decomposition of organic material, soil fertility, nutrient cycling and soil formation (Behan-Pelletier 1993; González & Seastedt 2001; Schneider & Maraun 2005; Seastedt 1984).

Early acarologists made important contributions to the study of Oribatida, sometimes under difficult conditions and with primitive equipment. One of the first major contributors was Carl Ludwig Koch (1778–1857) who lived and worked in Germany (Alberti 2004). He described many oribatid species, but mistakenly based some species and even genera on juveniles of other species or genera (Norton & Ermilov 2014). The Swiss-born Hercule Nicolet (1801–1872) was a talented lithographer and naturalist who worked in France (Cuevas 2006). He noticed these oversights of Koch, and also studied the development and morphological transformation of mites (Norton & Ermilov 2014). The British lawyer Albert Davidson Michael (1836–1927) retired at the age of 50 to devote himself to acarology and microscopy. He was

an educated, well-travelled gentleman and became a highly respected scientist. Biology and life-histories of mites were of special interest to him (Baker & Colloff 2006). A.C. Oudemans (1858–1943) was a Dutch biology teacher who had an interest in the historical development of zoology and acarology in particular. In 1943 his private library contained about 10 000 publications on mites! Oudemans published 322 papers on acarological subjects, and described 194 new genera and 731 new species (Eyndhoven 1965).

A champion of acarology during the late 1800s to early 1900s was Antonio Berlese (1863– 1927). Berlese was regarded as one of the foremost acarologists and entomologists of his time. He spent most of his working life in Florence, Italy, where he amassed a collection of about 12 750 determined, labeled and catalogued slides, as well as several hundred samples preserved in alcohol. He was also the inventor of the first version of the extraction apparatus (Berlese-Tullgren funnel), still used today (Ragusa 1982).

From Italy the baton was passed to France—to François Grandjean (1882–1975). Grandjean was a professor at the prestigious *Ecole des Mines* in St Etienne and later in Paris, where he taught paleontology, geology and mineralogy. His research focused on liquid crystals. He had a passion for nature and was interested in arthropods from an early age. He published his first paper on oribatid mites in 1928, the first of 241 papers. Grandjean retired in 1941, aged 59, and focused his attention entirely on acarology (Travé & Vachon 1975).

Grandjean defined the morphology of oribatids in great detail. He characterised the various parts and structures of oribatid mites (e.g. Grandjean 1952) thereby laying the modern foundation for their study. From early on he paid particular attention to juveniles, recognising the importance of ontogeny in natural classification (see Grandjean 1954). He also unraveled questions of segmentation and setation during ontogeny (see Grandjean 1933, 1934a, 1934b, 1939, 1949, 1961; Norton & Ermilov 2014). His work on tactile organs of legs (setae and solenidia) is of great importance for explaining their development during

ontogeny (see Grandjean 1935, 1940, 1946, 1964) and also for comparisons between taxa (see Grandjean 1941, 1942). These works were made more accessible by the interpretation of Norton (1977) since Grandjean's entire body of work is published in French. Grandjean's work is also characterised by the outstanding quality and detail of his figures.

The decades after the Second World War saw a remarkable increase in the number of oribatid species described, and the number of acarological papers published increased rapidly. Schatz (2002) analysed the oribatid literature and species described from 1758 until 2001. He found that more than 80% of studies focusing on Oribatida were published during the period 1951 to 2001. More than half of these papers dealt with classification, descriptions and distribution. During this golden age 7 579 oribatid species were described, with half of all known species described between 1975 and 2001 (Schatz 2002).

However, since the turn of the century there has been a marked decline in the number of acarological papers, comprising all groups of Acari (Walter & Proctor 2010; Zhang 2014). A checklist of the Oribatida of the world published by Subías (2004) documents approximately 9 000 known species and subspecies. In the 2014 electronic update of this work,10 498 species and subspecies are listed, an increase of almost 1 500 species in 10 years. This slowing down of species descriptions could be attributed to the decrease in oribatid systematists, an alarming result of the current trend of lack of research opportunities in taxonomy due to mainly financial restrictions worldwide.

Apart from the collective works of Grandjean, important publications to aid oribatid research include the generic key of Balogh & Balogh (1992), which although outdated by now, may be useful to point the researcher towards a certain group. A much cited work which includes a description of oribatid morphology and a key to families based on the concepts of Grandjean, is the chapter on Oribatida by Norton & Behan-Pelletier (2009) in the third edition of the *Manual of Acarology* (Krantz & Walter 2009). Herein the authors explain the

terminology used in oribatid morphology and summarise the main characteristics of each family. The species catalogue of Subías (2004), maintained and electronically updated each year, provides a checklist of superfamilies, families, genera and species. Although some entries are not in accordance with the views of many oribatologists, it is useful in the sense of providing a list of species and their distribution ranges. The importance of the morphology of juvenile instars had been emphasised by Grandjean (1954), and descriptions of immatures are scattered through the literature. A recent catalogue and historical overview of juvenile instars of oribatid mites by Norton & Ermilov (2014) will undoubtedly prove to be of much value.

Advances in molecular techniques made the use of molecular data in phylogenetic systematics (e.g. Dabert *et al.* 2010; Domes *et al.* 2007; Heethoff *et al.* 2006; Maraun *et al.* 2003, 2004) and DNA barcoding (Young *et al.* 2012) more accessible and these approaches are being increasingly applied. Molecular data is also combined with morphological, morphometrical and chemical data (Heethoff *et al.* 2011; Pfingstl *et al.* 2010). DNA sequences can be made publicly available by uploading onto open access databases for example GenBank and at present there are 5 477 Oribatida nucleotide sequences and 4 973 protein sequences available (NCBI).

#### **Oribatid research in South Africa**

Taxonomic research on Oribatida in South Africa commenced in the early 1960s with Prof. Rocco van Pletzen, head of the Zoology Department at the University of the Free State, Bloemfontein. Van Pletzen and some of his post-graduate students published revisions of various superfamilies and families, including species descriptions, based on South African material such as Galumnoidea (Engelbrecht 1969, 1972a, 1972e, 1972f, 1972g, 1972i, 1973); Microzetoidea (Engelbrecht 1972b, 1972c, 1972d, 1972h, 1972j) Oribatuloidea (Oripodoidea) (Coetzer 1968), Oppioidea (Kok 1967) and Scheloribatidae (Van Pletzen 1963, 1965). Towards the end of the decade the research focus at the university changed and the collections of material and literature were donated to the National Museum, where research continued.

#### Aim of this study

The genus *Afroleius* (Acari, Oribatida) was instituted by Mahunka (1984) on the basis of three species collected by Dr S. Endrödi from Nature's Valley in the Western Cape. Mahunka placed the genus in the family Haplozetidae Grandjean, 1936. He remarked that the genus resembles *Magyaria* Balogh, 1963 (in Haplozetidae), but that the latter has only four pairs of genital setae and one claw, whereas *Afroleius* has six pairs of genital setae and three claws.

The relationship of *Afroleius* to other genera has been uncertain. Balogh & Balogh (1992) placed it in the family Haplozetidae (Oripodoidea), as well as under "ceratozetoid genera" (Ceratozetoidea). Subías (2004) placed it in Haplozetidae, but in the most recent electronic update (Subías 2014), the genus was placed in the family Humerobatidae (Ceratozetoidea) despite arguments presented by Coetzee & Tiedt (2013) for its relationship to Mycobatidae. Subías (2014) also recombined *Africoribates undulatus* Balogh, 1959 in *Afroleius*. No reasons were given for the transfer of *Afroleius* to Humerobatidae.

Earlier work on *Afroleius* includes a re-description of the three known species (Coetzee 2007) and a multivariate morphometric study of *A. simplex* Mahunka, 1984 (Coetzee 2010). At the time the genus was regarded as a member of Haplozetidae (Oripodoidea).

*Afroleius* is widely distributed in South Africa. Representatives of this genus are litter inhabitants (apart from *A. caudatus* Coetzee, 2014 which may be arboreal), relatively habitat specific and, in terms of number of species and abundance, are found mainly in the southern and eastern parts of the country where they prefer well-vegetated habitats such as forests and coastal vegetation. A number of species occupy the savanna, grasslands and montane

grasslands of the eastern regions. Only one species (*A. lucidus* Coetzee, In Press) is found in the dry western part of the country. The collection at the National Museum contains many specimens of this genus. The aim of this study is therefore firstly to resolve the "identity crisis" of *Afroleius*, and secondly to describe the new species.

#### Layout of the thesis

This thesis consists of published and as yet unpublished papers on the relationship of the genus, descriptions of new species, recombinations of species described in other genera, and a discussion of the phylogeny of the genus.

Material and Methods and References are treated separately in each chapter, relevant to the species described. The format of presentation (e.g. journal citation of References, grammatical conventions of British English versus American English and citation of species authors) may differ in the following chapters, depending on the requirements of the journals in which the papers were published.

#### Introduction

In this section a historical background to Oribatida research, including a short biography of early contributors, and an overview of the advancement of the science is presented. A history of oribatid research in South Africa is also given. The aim of the study and layout of the thesis is described.

## **Chapter 1**

Coetzee, L. & Tiedt, L.R. 2013. Overview of the genus *Afroleius* Mahunka, 1984 (Acari, Oribatida). In: Schausberger, P. (Ed.). *Acari in a changing world: Proceedings of the 7<sup>th</sup> symposium of EURAAC, Vienna, 2012. Acarologia*, 53(2): 163–173.

This paper is co-authored by Dr Louwrens Tiedt (North-West University, Potchefstroom) for his contribution of SEM images. It is a requirement according to the policies of the North West University to recognise contributions of its staff by at least one co-authorship. The research was presented at the 7<sup>th</sup> symposium of the European Association of Acarologists (EURAAC) held in Vienna, 2012, and published in the proceedings of the symposium the following year.

In this paper the relationship of the genus is explored on the basis of hitherto neglected or unrecognised traits. Arguments for the placement of *Afroleius* in the family Punctoribatidae (= Mycobatidae) (Ceratozetoidea) and a comprehensive diagnosis of the genus are presented.

#### Chapter 2

Coetzee, L. 2013. New species of the genus *Afroleius* Mahunka, 1984 (Acari, Oribatida, Mycobatidae) from South Africa. *Acta Zoologica Academiae Scientiarum Hungaricae*, 59(4): 307–319.

This paper contains the description of three species of *Afroleius*, namely *A. crassus* Coetzee, 2013, *A. decurvatus* Coetzee, 2013 and *A. deformatoides* Coetzee, 2013. It was published in *Acta Zoologica Academiae Scientiarum Hungaricae*, in a memorial issue in honour of Dr Sándor Mahunka who passed away in December 2012.

## Chapter 3

Coetzee, L. 2014. Rare new species of the genus *Afroleius* Mahunka, 1984 (Acari, Oribatida, Mycobatidae) from South Africa. *Navorsinge van die Nasionale Museum*, *Bloemfontein*, 30(5): 71–85.

In this paper two species, which are regarded as rare on the basis of their limited distribution, are described, namely *A. natalensis* Coetzee, 2014 and *A. caudatus* Coetzee, 2014.

#### Chapter 4

Coetzee, L. 2014. *Afroleius floridus* (Mahunka, 1985) comb. nov. and three new *Afroleius* Mahunka, 1984 species (Acari, Oribatida, Mycobatidae) from South Africa. *Zootaxa*, 3889(4): 553–573.

*Magyaria florida* Mahunka, 1985 is recombined as *Afroleius floridus* and three new species are described, namely *A. amieae* Coetzee 2014, *A. inae* Coetzee, 2014 and *A. valerieae* Coetzee, 2014. The first juveniles of this genus, those of *A. floridus*, are also described.

#### Chapter 5

Coetzee, L. In Press. Key to the species of *Afroleius* Mahunka, 1984 (Acari, Oribatida, Punctoribatidae), recombination of *A. polygonatus* (Mahunka, 1985), description of *A. lucidus* sp. nov. and discussion of *A. undulatus* (Balogh, 1959). *Systematic and Applied Acarology.* 

This paper contains the re-combination of *Kilimabates polygonatus* Mahunka, 1985 in *Afroleius*, a re-description based on type material and additional material from the collection of the National Museum, and description of its juveniles. A new species *A. lucidus* is described and *A. undulatus* (Balogh, 1959) is discussed. The latter species was described by Balogh (1959) in the genus *Africoribates* and recombined in *Afroleius* by Subías (2014). *Afroleius undulatus*, described from the foothills of Mt Kilimanjaro in Tanzania, is the only species of *Afroleius* known from outside South Africa. An updated genus diagnosis is presented as more traits came to light during the course of the study, and earlier oversights are corrected. The name of the family is changed to Punctoribatidae, as this name has priority over the name Mycobatidae. A key to all the species of *Afroleius* is presented.

#### Chapter 6

Molecular phylogeny of Afroleius

This chapter can be seen as experimental, in that tentative results of a molecular analysis of the 28S ribosomal DNA gene are presented. The molecular "wet work" (DNA extraction, PCR and sequencing) was performed by a commercial laboratory (Inqaba Biotec, Pretoria) from specimens in the collection of the Museum. The results were not optimal, as few sequences were successfully retrieved. Sequences of representatives of Ceratozetoidea and outgroups were selected from GenBank.

#### Discussion

This chapter summarises the work presented in this thesis and discusses the state and future of traditional taxonomy and the application of molecular data.

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# Abstract

The genus *Afroleius* Mahunka, 1984 was proposed for three species (*A. deformis*, *A. minor* and *A. simplex*) and is so far known only from South Africa. New species (not yet described) have been identified from the Oribatida collection of the National Museum in Bloemfontein, South Africa. An overview of the genus, including an analysis of character states on genus level and an investigation of its relationships, is presented. Habitat preferences and distribution are also discussed.

#### OVERVIEW OF THE GENUS AFROLEIUS MAHUNKA, 1984 (ACARI, ORIBATIDA)

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ABSTRACT — The genus *Afroleius* Mahunka, 1984 was proposed for three species (*A. deformis, A. minor* and *A. simplex*) and is so far known only from South Africa. New species (not yet described) have been identified from the Oribatida collection of the National Museum in Bloemfontein, South Africa. An overview of the genus, including an analysis of character states on genus level and an investigation of its relationships, is presented. Habitat preferences and distribution are also discussed.

KEYWORDS — Acari; Oribatida; Systematics; Afroleius; Mycobatidae

#### INTRODUCTION

The genus Afroleius was described by Mahunka in 1984 from material collected at Nature's Valley, Western Cape, South Africa. At the same time he described three species namely A. deformis (type species), A. minor and A. simplex. Mahunka placed the genus in the family Haplozetidae (Oripodoidea) and remarked that it resembles Magyaria Balogh (also Haplozetidae) but differs from the latter genus by the number of genital setae (four pairs in Magyaria; six pairs in Afroleius) and number of claws (one claw in Magyaria; three claws in Afroleius). The character states in Mahunka's diagnosis (1984) are: darkly sclerotized, sculptured body; wide, marginal lamella; weak translamella; movable pteromorph; ten pairs of minute notogastral setae; four pairs of sacculi; epimeral and ano-adanal surfaces ornamented; six pairs of genital setae, 0 (?) or one pair of aggenital setae, two pairs of anal and three pairs of adanal setae present; all legs tridactyle.

http://www1.montpellier.inra.fr/CBGP/acarologia/ ISSN 0044-586-X (print). ISSN 2107-7207 (electronic) In their key to oribatid mite genera of the world, Balogh and Balogh (1992) treated this genus under Haplozetidae (p. 133) as well as under "ceratozetoid" genera (p. 147) and listed the genus under Ceratozetidae (p. 222). Subías (2004, 2012) and Coetzee (2007) followed the classification as originally suggested by Mahunka (1984) with placement in the family Haplozetidae.

More specimens of this genus have been identified from the Acarology collection of the National Museum in Bloemfontein, South Africa, including eight new species which will be described in the near future, and three species which are to be transferred from other genera namely from *Magyaria* Balogh (one species), *Africoribates* Evans (one species) and *Kilimabates* Mahunka (one species); *Kilimabates* has been synonymized with *Africoribates* by Balogh and Balogh (1992) and with *Anellozetes* Hammer by Subías (2004). So far, immatures of *Afroleius* are unknown. A detailed study of adults

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FIGURE 1: Afroleius minor. Dorsal view.



FIGURE 2: a – *Afroleius minor*. Anterior view, with costular ridge (arrow) between lamellar apices; b – *Afroleius* sp. Anterior view, costular ridge absent.

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FIGURE 3: a – Afroleius sp. Notogaster, porose area Aa; b – Afroleius minor. Notogaster, sacculus Sa.

revealed new information which led to changes to the interpretation of certain character states, and hence to the classification.

#### MATERIALS AND METHODS

The material examined is housed in the Acarology collection of the National Museum. It consists of Berlese-funnel extractions of litter samples collected from 1960, and represents a wide range of localities and habitats in South Africa. Specimens are stored in 70 % ethanol + 5 % glycerol. For light microscope studies selected specimens were cleared in lactic acid at 50°C for 24 hours and then mounted temporarily in cavity slides with glycerol. A Nikon Eclipse 50i light microscope equipped with a DS-Fi1 digital camera and NIS-Elements imaging software were used for light microscope images. The following procedure was followed for Scanning Electron Microscope micrographs: Samples were fixed in 70 % ethanol for a minimum of 24 hours, dehydrated in a graded ethanol series of 80 %, 90 % and 2X 100 % for 30 minutes each. The samples were critical point dried in liquid carbon dioxide, mounted with double sided carbon tape on SEM-stubs and coated with 15 nm gold/palladium in a sputter coater, and viewed under a FEI Quanta 250 FEG SEM at 5 kV under high vacuum mode.

#### RESULTS

Revised diagnosis — *Afroleius* Mahunka, 1984 (Figures 1 – 9)

Integument — Darkly sclerotized, foveate or reticulate (Figure1)

Size — Medium-sized ranging between 250 – 420  $\mu\mathrm{m}$ 

Dorsal side (Figures 1, 2, 3, 4 and 8) — Lamella marginal, very short lamellar cusp present in some species, costular connection between lamellar apices sometimes present; rostral seta of medium length; lamellar seta of medium length; interlamellar seta minute; bothridium cup-shaped with lateral slit of which the lobes may overlap or not; narrow anterior notogastral tectum present, no medial process; pteromorph fully hinged; octotaxic system consists of four pairs of sacculi or porose areas; ten pairs of notogastral setae present, all setae minute ( $\sim 10 \ \mu$ m); lyrifissures of medium length, lyrifissures *ia* situated anteriorly on pteromorph, lyrifissures *im*, *ih*, *ips* and *ip* in usual positions; undivided posterior notogastral tectum present.

Ventral side (Figures 4, 5, 6 and 7) — Genal notch and broad genal tooth present (see discussion); axillary saccule of subcapitulum present at base of palp; subcapitulum without mental tectum; epimeral surface foveate or partly punctate; apodemes II, sj and III short, parallel; epimeral seta 1*c* present or absent; epimeral setae 3*c* and 4*c* absent (number of setae on Coetzee L. and Tiedt L.R.



FIGURE 4: Afroleius simplex. Ventral view. Note posterior notogastral tectum (arrow).

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FIGURE 5: Afroleius sp. Subcapitulum. Note genal notch and genal tooth (arrows).

epimeres from anterior to posterior: 2/3-1-2-2); custodium absent; discidium large, triangular; darkly sclerotized band running from acetabulum IV on one side anterior of genital plates to acetabulum IV on other side; ventral plate foveate; genital plates with six pairs of setae, integument faintly striate or foveate; one pair of aggenital setae present (except *A. deformis*, aggenital seta absent); anal plates with two pairs of setae, integument foveate; lyrifissure *iad* anterolaterally of anal plates; three pairs of adanal setae present, inserted close to each other on posterolateral border of anal plates (except *A. deformis*); pre-anal organ with narrow stem; post anal porose area present (see discussion).

Lateral view (Figure 8) — Tutorium consisting of dorsal ridge with deep incurvation in basal part; prodorsal integument at incurvation covered with granular cerotegument; pedotectum I broad, covering acetabulum I, dorsally reaching exobothridial seta; pteromorph large, distal edge rounded, with complete hinge; two slightly curved carinae present dorsally of acetabulum IV.

Legs (Figure 9) — All legs heterotridactylous; dorsal integument of tibiae and tarsi of legs I, II and IV thickened; tarsi I and II with dorsal dens (varying from small point to large well-developed spur) proximally to tectal setae; genu I with dorsal and ventral distal cusps, genu II with sharp cusp situated ventrally or laterally; tarsus IV in some species proximo-dorsally with flattened ridge (? tectum) running from seta ft'' to proximo-ventral end of tarsus; femur IV with ventral projection forming ridge on abaxial side; femora I-IV and trochantera III-IV with porose areas. Coetzee L. and Tiedt L.R.



FIGURE 6: Afroleius minor. Subcapitulum. Note the axillary saccules (arrows).



FIGURE 7: Afroleius simplex. Ventral view. Note the short apodemes II, sj and III, sclerotized band anterior of genital plates (arrow), narrow pre-anal organ and the absence of custodium.

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FIGURE 8: *Afroleius minor*. Lateral view (pteromorph removed). Note the shape of the tutorium (tu), lateral slit in bothridium (bo), shape of pedotectum I (pdI) and the lateral carinae dorsal of acetabulum IV (arrows).



FIGURE 9: a – *Afroleius* sp. A. Genu I with anterior cusps (arrows); b – *Afroleius* sp. B. Genu II with lateral cusp (arrow); c – *Afroleius* sp. B. Tarsus II with dorsal dens (arrow).

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Setal formula (number of setae per digit from trochanter to tarsus, famulus included): Leg I 1-5-3-4-20; Leg II 1-4-3-4-15; Leg III 2-2-1-3-15; Leg IV 1-2-2-3-12. Solenidial formula (number of solenidia per digit from genu to tarsus): Leg I 1-2-2; Leg II 1-1-2; Leg III 1-1-0; Leg IV 0-1-0.

#### DISCUSSION

The axillary saccule is a porose sacculus extending internally from the base of the palp. It was first described by Grandjean (1936) as a brachytrachea in *Pelops acromios* Hermann. Norton and Behan-Pelletier (1986) brought this structure to the attention again fifty years later when they investigated the systematic relationships of *Propelops* Jacot. They determined the presence of this saccule in various poronotic brachypyline families, and it was found to be present in all Ceratozetoidea and some other superfamilies such as Oribatelloidea (certain families) and Galumnoidea, but no trace of such a structure was found in any of the Oripodoidea (Norton and Behan-Pelletier 1986; Norton *et al.* 1997; Norton and Behan-Pelletier 2009).

Another important character in Ceratozetoidea is the genal notch and genal tooth (Norton and Behan-Pelletier 2009). This is an indentation in the genal border, forming a tooth (dens) which can be long and narrow as in the majority of Ceratozetidae, to short and broad as in Zetomimidae (Behan-Pelletier 1986; Behan-Pelletier 1996). The genal tooth in *Afroleius* is short and broad, but may also be absent or fused to the rostrum. Niemi and Behan-Pelletier (2004) noted that a fused or absent genal tooth is rare in Ceratozetoidea, as is the case in *Nuhivabates* (Mycobatidae) and some species of *Melanozetes* (Ceratozetidae).

The third key character of Ceratozetoidea is the shape of Pedotectum I. A large, broad pedotectum which completely covers acetabulum I and stretches dorsally almost to the base of the bothridium is present in Ceratozetoidea as well as Oribatelloidea (Norton and Behan-Pelletier 2009). In *Afroleius* pedotectum I reaches the insertion of the exobothridial seta, with the distal margin straight to very slightly convex.

The tutorium of *Afroleius* has a peculiar shape, with a thickened ridge dorsally and the basal part with a deep incurvation. The integument beneath the incurvation is covered with granular cerotegument. The lamellae are situated marginally, sometimes with a very short cusp. Whether the ridge in some species between the lamellar apices can be called a translamella is debatable - in lateral view it appears to be a steep decline in the prodorsal profile. This ridge is variously expressed, from a costular ridge to (mostly) absent.

On the lateral side beneath the pteromorph, two carinae are present above acetabulum IV. Similar carinae are also present in the mycobatid genera *Mycobates* Hull, *Punctoribates* Berlese and *Pelopsis* Hall (Behan-Pelletier and Eamer 2003). These carinae are difficult to observe under light microscope despite having the pteromorph and leg IV removed.

The cup-shaped bothridium has no scales (extensions of the anterior border) as in the majority of Ceratozetoidea (Behan-Pelletier 1986), but has a lateral slit of which the lobes may overlap or not. The bothridium is often hidden beneath the pteromorph, and sometimes directed ventrally.

The octotaxic system is expressed as sacculi or porose areas, often surrounded by thick cuticle. The sacculi can be round or tubular. In the past much emphasis has been placed on whether the octotaxic system is expressed as sacculi or porose areas, but the systematic importance of this trait has been shown to be of value only on species level (Norton and Alberti 1997, Norton and Behan-Pelletier 2009, Weigmann 2009a, Weigmann 2009b).

An undivided posterior notogastral tectum is present as well as a post anal porose area, often covered by the tectum. Of the possible 14 species, the post anal porose area is absent in four species, in which it is considered a secondary loss. The presence of a post anal porose area is "almost universal" in Ceratozetoidea (Norton and Behan-Pelletier 1986). The presence or absence of a tectum along the posterior border of the notogaster is of importance in poronotic families, distinguishing for example between Ceratozetidae and Mycobatidae (Behan-Pelletier and Eamer 2008; Grandjean 1954). In *Afroleius* the custodium is absent, as is the case in Schausberger, P. (ed.) Acari in a Changing World: Proceedings of the 7<sup>th</sup> Symposium of EURAAC, Vienna, 2012 Acarologia 53(2): 163–173 (2013)



1. High rainfall & forests. 2. Savanna & montane grassland. 3. Grassland. 4. Arid

FIGURE 10: Distribution of *Afroleius*. Map of South Africa with biomes and rainfall (smoothed) indicated. The majority of species occur in the eastern high rainfall area.

some other mycobatid genera such as *Punctoribates*, where it may vary between species, and *Nuhivabates* (Behan-Pelletier and Eamer 2008; Niemi and Behan-Pelletier 2004).

The legs of *Afroleius* have a number of character states which are also found in some other mycobatid genera, such as the thickened dorsal integument of tarsi and tibiae I, II and IV, the dens on tarsus II (in *Afroleius* also present on tarsus I), and the ventral projections on femur IV. These character states are also seen in some species of *Pelopsis* and *Punctoribates* (Behan-Pelletier and Eamer 2008). The dens in *Afroleius* varies in size from a large, welldeveloped spur to a small point. Genu I of *Afroleius* has dorsal and ventral cusps on the distal end, while genu II has a ventral (or sometimes lateral) cusp similar to that found in *Nuhivabates* (Niemi and Behan-Pelletier 2004).

#### **Distribution and habitat (Figure 10)**

So far, *Afroleius* is known only from South Africa apart from one species from Tanzania, described by Balogh in 1959 as *Africoribates undulatus*. The oribatid faunas in the countries lying between Tanzania and South Africa, namely Malawi, Mozambique and Zimbabwe are unknown. *Afroleius* is mainly a species of the temperate forests and thickets of the eastern and southern regions of South Africa, which is a high rainfall area with annual precipitation from 700 mm. The brown soil of temperate forests is typiCoetzee L. and Tiedt L.R.

cally extremely fertile with abundant leaf litter (Van As *et al.* 2012). Of the possible 14 species, eight occur only in this biome. A further four species occur in the temperate forests as well as the savanna and temperate grassland biomes, with one species restricted to montane grassland. Only one species occurs predominantly in the grassland and arid regions. Only litter samples have been investigated.

#### Relationship

The three key characters of adult Ceratozetoidea namely the axillary saccule, the genal notch and the large pedotectum I (Norton and Behan-Pelletier 2009) are present in *Afroleius*. Therefore, this genus is firmly excluded from Oripodoidea and hence from Haplozetidae. Norton and Behan-Pelletier (2009) include nine families in Ceratozetoidea. The presence of a posterior notogastral tectum in *Afroleius* excludes Ceratozetidae, Euzetidae and Zetomimidae; the absence of a custodium excludes Ceratokalummidae, Chamobatidae, Humerobatidae and Onychobatidae and the presence of a pteromorphal hinge excludes Maudheimiidae.

Mycobatidae is a family with a rather chequered set of character states with the presence of the posterior notogastral tectum (complete or divided) the definitive character state in adults of this family (Behan-Pelletier and Eamer 2008; Behan-Pelletier and Ryabinin 1991; Grandjean 1954). The presence of the pteromorphal hinge (complete or partial; also referred to as line of desclerotization) used to carry much weight (Grandjean 1954), but this character state has since been shown not to be constant in Mycobatidae (e.g. absent in Ceresella Pavlitshenko and Cyrtozetes Behan-Pelletier) (Behan-Pelletier and Eamer 2008). The phylogenetic importance and polarity of certain character states on family level such as the sculptured integument, marginal lamellae and the peculiar shape of the tutorium needs to be established, while immature forms will undoubtedly provide valuable clues to the relationship of this genus. However, until the abovementioned questions have been resolved, this genus should be regarded as a member of Mycobatidae.

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# Abstract

Three new species in the genus *Afroleius* Mahunka,1984 are described from South Africa. *A. crassus* sp. n. can be recognised by the wedge-shaped lateral profile and undulated lateropostero notogastral margin; *A. decurvatus* sp. n. is recognised by the slender, sharply decurved sensillar stalk and thick-walled tubular sacculi, and *A. deformatoides* sp. n. is similar to the type species *A. deformis* but differs by the octotaxic system consisting of porose areas and the presence of foveae in the central parts of the notogaster.
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# NEW SPECIES OF THE GENUS *AFROLEIUS* MAHUNKA, 1984 (ACARI, ORIBATIDA, MYCOBATIDAE) FROM SOUTH AFRICA

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Three new species in the genus *Afroleius* Mahunka,1984 are described from South Africa. *A. crassus* sp. n. can be recognised by the wedge-shaped lateral profile and undulated lateropostero notogastral margin; *A. decurvatus* sp. n. is recognised by the slender, sharply decurved sensillar stalk and thick-walled tubular sacculi, and *A. deformatoides* sp. n. is similar to the type species *A. deformis* but differs by the octotaxic system consisting of porose areas and the presence of foveae in the central parts of the notogaster.

Key words: Oribatida, Mycobatidae, Afroleius, new species, South Africa.

# INTRODUCTION

The genus *Afroleius* has been described by MAHUNKA (1984) from material collected in the southern parts of the Western Cape Province of South Africa. At the time Mahunka described three species namely *A. deformis* (type species), *A. minor* and *A. simplex*. The relationship of this genus has been uncertain. Mahunka placed the genus in the family Haplozetidae (MAHUNKA 1984) while BALOGH and BALOGH (1992) treated it under Haplozetidae as well as under "ceratozetoid" genera. SUBÍAS (2004, 2012) placed the genus in Haplozetidae.

More new species have been discovered in South Africa, of which the first three new species *A. crassus* sp. n., *A. decurvatus* sp. n. and *A. deformatoides* sp. n. are described in this paper. COETZEE and TIEDT (2013) presented arguments for the inclusion of the genus in the family Mycobatidae. A much neglected structure of certain groups of oribatid mites is the axillary saccule - a porose saccule extending internally from the base of the palp. GRANDJEAN (1936) first noted this saccule in *Pelops acromios* Hermann. In brachypyline families, the axillary saccule is present in all Ceratozetoidea, certain families of Oribatelloidea and Galumnoidea, but it is absent in Oripodoidea (NORTON & BEHAN-PELLETIER 1986, NORTON *et al.* 1997, NORTON & BEHAN-PELLETIER 2009). The axillary saccule is present in *Afroleius*, which excludes it from Oripodoidea. It must be emphasized that this study was based on adult characters only, as immatures are still unknown.

Hungarian Natural History Museum, Budapest

#### Diagnosis of Afroleius Mahunka, 1984

Integument darkly sclerotized, foveate or reticulate; lamella marginal, rostral seta short to medium length; lamellar seta medium to long; interlamellar seta minute; pteromorph large, distal edge rounded, fully hinged; lyrifissure *ia* situated paraxially on pteromporph; octotaxic system consisting of four pairs of sacculi or porose areas; ten pairs of minute notogastral setae present; undivided posterior notogastral tectum present; genal notch and broad genal tooth usually present; axillary saccule of subcapitulum present; number of setae on epimeres I–IV 2/3–1–2–2, epimeral seta 1c present or absent; epimeral setae 3c and 4c absent; custodium absent; darkly sclerotized band running from acetabulum IV on one side anterior of genital plates to acetabulum IV on other side; genital plates with six pairs of setae, adanal setae inserted close to each other on posterolateral border of anal plates, ad, inserted posterior to iad (exceptions: A. deformis Mahunka, 1984 and A. deformatoides sp. n.); post anal porose area usually present; tutorium consisting of dorsal ridge with deep incurvation in basal part; pedotectum I broad, covering acetabulum I; all legs heterotridactylous; dorsal integument of tibiae and tarsi of legs I, II, and IV thick; tarsi I and II with dorsal dens proximal to tectal setae, varying from small point to large well-developed spur (exception: dens on tarsus I of A. deformis and A. deformatoides absent); distal end of genua I and II antiaxially with prominent cusps; femur IV with ventral projection forming ridge on abaxial side; porose areas present on femora I–IV, and trochantera III and IV.

#### MATERIAL AND METHODS

All studied material derives from South Africa. Soil fauna was extracted by Berlese-Tullgren funnels. Specimens were temporarily mounted on cavity slides with glycerol for study purposes and thereafter stored in 70% alcohol. All material, including holotypes and paratypes, are deposited in the Acarology Collection of the National Museum, Bloemfontein, South Africa.

# RESULTS

# Afroleius crassus sp. n. (Figs 1–7)

Diagnosis – Notogastral surface reticulate, prodorsal and ventral surfaces foveate; lateral and posterior margins of notogaster undulate; octotaxic system consisting of saccules with wide openings; bothridial seta clavate, stalk short; rostral seta very short; lamellar seta long, roughened, curving medially; interlamellar seta minute; notogastral setae minute; lateral notogastral profile

wedge-shaped; prominent dorsal dens on tarsi I and II; distal end of genu I antiaxially with prominent dorsal and ventral cusps; distal end of genu II antiaxially with prominent lateral cusp.

Dimensions – Holotype (female): Length 330  $\mu$ m, width 230  $\mu$ m. Paratypes: Females (n = 2): Length 324  $\mu$ m (311–338  $\mu$ m), width 222  $\mu$ m (213–236  $\mu$ m). Males (n = 7): Length 299  $\mu$ m (284–316  $\mu$ m), width 206  $\mu$ m (196–218  $\mu$ m).

Prodorsum (Figs 1 & 2) – Rostrum rounded in dorsal view, rostral profile deeply indented in lateral view; prodorsal surface foveate, foveae becoming smaller and fractioned towards posterior border of prodorsum; lamella (*L*) wide, extending over lateral margin of prodorsum; rostral seta (*ro*) (ventrally visible) short, (~ 16 µm) roughened, inserted at anterior apex of tutorium; lamellar seta (*le*) long (~ 32 µm), roughened, curved medially; interlamellar seta (*in*) minute (~ 7 µm); bothridium small, opening directed ventrally; head of bothridial seta (*bo*) clavate, granular, surface roughened, length of bothridial stalk more or less as long as bothridial head.

Notogaster (Figs 1 & 2) – Surface reticulate; medially slightly elevated, marginally depressed, postero-lateral margin deeply undulate; octotaxic system consisting of saccules, openings of saccules surrounded by thick integument; ten pairs of minute (~ 7  $\mu$ m) notogastral setae present; lyrifissure *im* clearly visible; opisthosomal gland *gla* small, situated close to saccule *S*<sub>1</sub>; in lateral view posterior part of notogaster much higher than anterior part.

Podosoma and gnathosoma (Figs 2 & 3) – Surface of mentum foveate, foveae fading posteriorly; genal notch (gn) present; genal tooth very broad, short; axillary saccule short; epimeral setae minute; epimeral surface foveate; epimeral seta 1c absent; tutorium (tu) narrow, anterior surface below tu finely granulate; pedotectum I (pdI) large, wide, surface foveate; pedotectum II (pdI) small.

Ventral plate (Fig. 3) – Surface of genital and anal plates faintly foveate, ventral plate medially with large foveae, marginally with smaller foveae; region posterior to anal plates granulate; *iad* situated laterally of anal plates;  $ad_{1-2}$  inserted posteriorly to anal plate,  $ad_3$  inserted medio-laterally to anal plate, posterior to *iad*; post anal porose area small, round; preanal organ of medium length.

Legs (Figs 4–7) – Setal formula (trochanter to tarsus, famulus included): Leg I 1–5–3– 4–20; Leg II 1–5–3–4–15; Leg III 2–2–1–3–15; Leg IV 1–2–2–3–12. Solenidial formula (genu to tarsus): Leg I 1–2–2; Leg II 1–1–2; Leg III 1–1–0; Leg IV 0–1–0.

Legs relatively short; dorsal integument of tarsi I, II and IV and tibiae I, II and IV thick; porose areas on femora I-IV narrow, situated postero-dorsally; porose areas on trochanters III and IV situated paraxially. Leg I: Sharply pointed curved dens present on dorsal side of tarsus proximal to tectal setae; antiaxial fastigial seta (ft'') very short and thin; famulus ( $\varepsilon$ ) minute; distal end of genu antiaxially with dorsal and ventral cusps (indicated by arrows). Leg II: Tarsus short, broad; sharply pointed curved dens present on dorsal side of tarsus proximal to tectal setae; distal end of genu antiaxially with ventral cusp (indicated by arrow). Leg III: All setae smooth. Leg IV: Dorsal surface of tarsus and tibia uneven; tarsus antiaxially with diagonal tectum running from insertion of ft'' to proximo-ventral base of segment; femur ventrally with wide flange; dorsal seta (d) on femur thick, roughened, all other setae smooth.

Material examined: Holotype (female) (NMB 2099.32.1) (Fig. 18) and nine paratypes (two females, seven males) (NMB 2099.32.2) – Winklespruit 30°07′S 30°50′E, 7 March 1982 (C. M. Engelbrecht), soil and plant debris from urban environment.

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Additional material: Indian Ocean coastal belt – Soil and litter samples from temperate forests, riverine forests, thickets and urban environments collected at Mtunzini (Waterloo Estate) (28°59'S 31°44'E), Vernon Crookes Nature Reserve (30°16'S 30°35'E), Mapelane (28°22'S 32°23'E), St. Lucia (Mission Rocks) (28°15'S 32°30'E), Cape Vidal (28°6'S 32°33'E), Dukuduku (28°20'S 32°18'E), Charter's Creek (28°22'S 32°24'E), Oribi Gorge (30°41'S 30°16'E)



**Figs 1–3.** *Afroleius crassus* sp. n.: 1 = dorsal aspect, 2 = lateral aspect, 3 = ventral aspect. Scale bar 100 μm.

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Scottburgh (30°16′S 30°44′E), Umkomaas (30°14′S 30°46′E), Margate (30°2′S 30°22′E), Palm Beach (31°03′S 30°14′E). Northern mistbelt forests – Lydenburg (25°08′S 30°32′E).

**Figs 4–7.** *Afroleius crassus* sp. n.: 4 = leg I, right side, antiaxial aspect, 5 = leg II, right side, antiaxial aspect, 6 = leg III, left side, antiaxial aspect, 7 = leg IV, right side, antiaxial aspect. Diagonal tectum indicated. Scale bar 50  $\mu$ m.

Etymology – The species name refers to the coarse appearance of the notogastral integument.

Remarks – The uneven outline of the notogaster in dorsal view, the different patterns of the integument of the notogaster (reticulate) and the ventral plate (large foveae), and the wedge-shaped lateral profile distinguishes this species from its congeners. Although this species seems to be widely distributed in the eastern parts of the country, it is not abundant - on average about two *A. crassus* sp. n. specimens per sample. This species is restricted to the eastern, well vegetated regions of South Africa, where high rainfall occurs.

# Afroleius decurvatus sp. n. (Fig 8–14)

Diagnosis – Dorsal and ventral surfaces reticulate; bothridial seta clavate, finely barbed, stalk sharply decurved proximally at about 1/3 of length of stalk, directed posteriorly; rostral seta short, finely barbed; lamellar seta long, smooth, curved medially, apices meeting in front of rostrum; interlamellar seta very short; notogastral setae minute; four pairs of thick-walled, tubular sacculi present; tarsi I and II each with large, curved dorsal dens; genu I and II each with large antiaxial distal cusp.

Dimensions – Holotype (female): Length 314  $\mu$ m, width 239  $\mu$ m. Paratypes: Females (n = 8) Length 312  $\mu$ m (range 306–322  $\mu$ m); width 222  $\mu$ m (range 211–230  $\mu$ m). Males (n = 4) Length 293  $\mu$ m (range 282–304  $\mu$ m); width 206  $\mu$ m (range 196–213  $\mu$ m).

Prodorsum (Figs 8 & 9) – Rostrum rounded in dorsal view; prodorsal profile in lateral view with slight indentation at level of lamellar apices; prodorsal surface reticulate; band of small tubercles present anterior of dorsosejugal furrow, up to level of interlamellar setae; lamella wide, extending slightly over lateral margin of prodorsum; rostral seta short (~ 19 µm), barbed, slightly phyliform, visible ventrally and laterally, inserted below apex of tutorium; lamellar seta long (~ 53 µm), smooth, curving medially, apices overlapping slightly in front of rostrum; interlamellar seta very short (~ 13 µm), smooth, curving medially; head of bothridial seta clavate, finely barbed; stalk long, sharply decurved proximally at about 1/3 of length of stalk, directed posteriorly, total length from where stalk emerges from bothridium to tip of bothridial head ~ 52 µm; bothridium ventrally with overlapping slit.

Notogaster (Figs 8 & 9) – Notogastral setae minute (~ 5  $\mu$ m), all setae more or less the same length, smooth; surface reticulate, forming large irregular circles; octotaxic saccules tubular, thick-walled, openings wide; opisthosomal gland very small, difficult to detect, situated close to S1; pteromorphae large, distal edge without reticulation but surface irregular; posterior notogastral tectum wide.

Podosoma and gnathosoma (Figs 9 & 10) – Surface of mentum faintly patterened; axillary saccule short; genal tooth short, broad; epimeral setae minute, seta 1*c* absent; epimeral surface reticulate; longitudinal line present on lateral sides of epimeres stretching more or less from 1*b* to 4*b*; tutorium narrow, anterior surface below *tu* with small tubercles;

rostral seta inserted below apex of tutorium; pedotectum I large, wide, surface reticulate; pedotectum II small.

Ventral plate (Fig. 10) – Surface of genital plate faintly patterned, surface of anal plate faintly reticulate; ventral plate reticulate; *iad* situated laterally of anal plates;  $ad_{1-2}$  inserted close to each other posteriorly to anal plate,  $ad_3$  inserted medio-laterally to anal plate, posterior to *iad*; post anal porose area absent; preanal organ short.



**Figs 8–10.** *Afroleius decurvatus* sp. n.: 8 = dorsal aspect, 9 = lateral aspect, 10 = ventral aspect. Scale bar 100 μm.



**Figs 11–14.** *Afroleius decurvatus* sp. n.: 11 = leg I, right side, paraxial aspect, 12 = leg II, right side, paraxial aspect, 13 = left side, paraxial aspect, 14 = left side, paraxial aspect. Diagonal tectum indicated. Scale bar 50 μm.

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Legs (Figs 11–14) – Setal formula (trochanter to tarsus, famulus included): Leg I 1–5– 3–4–20; Leg II 1–5–3–4–15; Leg III 2–2–1–3–15; Leg IV 1–2–2–3–12; Solenidial formula (genu to tarsus): Leg I 1–2–2; Leg II 1–1–2; Leg III 1–1–0; Leg IV 0–1–0.

Legs relatively short; dorsal integument of tarsi I, II and IV and tibiae I, II and IV thick; porose areas on femora I and II large; porose areas on femora III and IV narrow, situated dorsally; porose areas on trochanters III and IV large. Leg I: Large, curved dens present on dorsal side of tarsus, situated proximal to tectal setae; antiaxial fastigial seta (ft'') short, thin; famulus ( $\varepsilon$ ) minute; distal end of genu antiaxially with ventral cusp (indicated by arrow). Leg II: Tarsus short, broad; large, curved dens present on dorsal side of tarsus, situated proximal to tectal setae; distal end of genu antiaxially with large cusp (indicated by arrow). Leg III: All setae (except (p) and (u)) barbed. Leg IV: Dorsal surface of tarsus and tibia uneven; diagonal tectum antiaxially present on tarsus running dorsally from insertion of ft'' to proximo-ventral base of segment; femur ventrally with wide flange; dorsal seta (d) on femur thick, roughened.

Material examined – Holotype (female) (NMB 1735.10.1) (Fig. 18) and 12 paratypes (four males, eight females) (NMB 1735.10.2) – Frankfort, eastern Free State 27°17′S 28°30′E, 18 January 1982 (C. M. Engelbrecht), soil and plant debris associated with shrubs.

Additional material: Grassland biome – soil and litter samples from natural grassland, soil and litter associated with shrubs and urban environments collected at Golden Gate (28°29'S 28°38'E), Fouriesburg (28°37'S 28°12'E), Kroonstad (27°39'S 27°14'E), Potchefstroom (26°42'S 27°05'E), Northam (24°57'S 27°16'E), Rosendal (28°30'S 27°55'E), Reitz (27°48'S 28°25'E), Heilbron (27°17'S 27°58'E), Clarens (28°31'S 28°25'E), Weenen (28°51'S 30°03'E). Northern Mistbelt Forests – forest litter collected at Montrose (25°27'S 30°42'E), Sabie (25°04'S 30°47'E). Kalahari Thornvled - litter under *Rhus lancea* Warrenton (28°06'S 24°50'E). Indian Ocean coastal belt – litter from coastal dune forests – Cape Vidal (28°07'S 32°33'E), St Lucia (28°07'S 32°33'E).

Etymology - The species name refers to the sharply decurved sensillar stalk.

Remarks – This species is superficially similar to *Afoleius simplex* Mahunka, 1984, but the thick-walled, tubular sacculi and sharply decurved sensillar stalk are characteristic of this species and distinguish it from congeners. The absence of the post anal porose area is probably a secondary loss. This species seems to be ecologically tolerant as it has been collected from diverse habitats such as Kalahari Thornveld in the arid western part of the country, to coastal dune forests in the moist eastern part of the country.

# Afroleius deformatoides sp. n. (Figs 15–17)

Diagnosis – Dorsal surface and ventral plate foveate; epimeral surface partly foveate; octotaxic system consisting of porose areas; in lateral view notogaster indented, ventral plate convex; bothridial seta clavate, stalk short; bothridial opening wide, directed anteriorly; rostral seta minute; lamellar seta

long, smooth, curving medially; interlamellar seta minute; notogastral setae very short; tarsus I without dorsal dens; dorsal dens on tarsus II reduced to small point.

Dimensions – Holotype (female): Length 374  $\mu$ m, width 279  $\mu$ m. Paratypes: Females (n = 2): Length 363  $\mu$ m (362–364  $\mu$ m), width 281  $\mu$ m (279–282  $\mu$ m). Males (n = 2): Length 331  $\mu$ m (325–338  $\mu$ m), width 247  $\mu$ m (238–256  $\mu$ m).

Prodorsum (Figs 15 & 16) – Rostrum rounded in dorsal view; prodorsal surface foveate; lamella wide, extending over lateral margin of prodorsum, surface finely punctate; rostral seta (ventrally visible) minute (~ 5  $\mu$ m), inserted at anterior apex of tutorium; lamellar seta long (~ 66  $\mu$ m), smooth, curved medially; interlamellar seta minute (~ 8  $\mu$ m); bothridium large, opening wide, directed anteriorly; head of bothridial seta clavate, granular, surface smooth; prodorsal profile in lateral view declining sharply from lamellar apex towards rostrum, with narrow ridges from lamellar apex towards rostral seta (see remarks).

Notogaster (Figs 15 & 16) – Ten pairs of minute notogastral setae (~ 8  $\mu$ m) present; entire surface foveate; in dorsal view anteriorly with high elevation, medially slightly elevated, laterally with deep depression in region of *im*; postero-lateral margin slightly undulate; four pairs of porose areas, canals often clearly visible; porose areas A1 and A2 situated close together, near seta h<sub>3</sub>; opisthosomal gland *gla* small.

Podosoma and gnathosoma (Figs 16 & 17) – Surface of mentum with fine ridges and punctae; genal notch absent but small ridge present extending from genal border towards anterior end of tutorium; axillary saccule of medium length, epimeral setae minute; epimeral surface anteriorly foveate, posteriorly finely punctate; epimeral setae *1c*, *3c* and *4c* absent; tutorium narrow; anterior surface below tutorium finely granulate; pedotectum I large, wide, surface foveate, in ventral view with deep notch at point of fusion with epimere I; pedotectum II small.

Ventral plate (Fig. 17) – Ventral plate highly convex; surface of genital plates striated, anal plates faintly foveate, ventral plate with large foveae; aggenital setae present; preanal organ long, narrow; post anal porose area absent.

Legs – Setal formula (trochanter to tarsus, famulus included): Leg I 1–5–3–4–20; Leg II 1–4–3–4–15; Leg III 2–2–1–3–15; Leg IV 0–2–2–3–12; Solenidial formula (genu to tarsus): Leg I 1–2–2; Leg II 1–1–2; Leg III 1–1–0; Leg IV 0–0–0.

Legs similar to *A. deformis* Mahunka, 1984 (see COETZEE 2007); dorsal integument of tarsi I, II and IV and tibiae I, II and IV thick. Leg I: Antiaxial fastigial seta short and thin; famulus very short; distal end of genu antiaxially with dorsal and ventral cusps. Leg II: Tarsus with small point on dorsal side proximal to tectal setae; distal end of genu antiaxially with dorsal and ventral cusps. Leg III: Less sclerotized than other legs. Leg IV: Dorsal surface of tarsus and tibia slightly uneven; tarsus antiaxially with diagonal tectum running from ft'' to proximo-ventral base of segment; femur ventrally with wide flange; Solenidion  $\phi$  on tibia IV absent, solenidial canal present.

The limited number of specimens available for study prohibited dissection of material for detailed sketches of legs.

Material examined – The holotype (female) (NMB 2905.4) (Fig. 18) and four paratypes (two females, two males) (NMB 2905.4, NMB 2904. 4, 2902.5) – Royal Natal National Park 28°40'S 28°55'E, 14 December 1982 (C. M. Engelbrecht), soil and litter in forested gorges on eastern slopes of the northern Drakensberg mountain range.

Etymology – The suffix –oides meaning "like" or "similar to" refers to the superficial resemblance of the new species to *A. deformis*.

Remarks – This species is very similar to the type species of the genus, *Afroleius deformis* Mahunka, 1984 in the unusual indented shape of the notogaster and convex shape of the ventral plate. It differs from *A. deformis* in having porose areas instead of the unusually shaped sacculi present in *A.* 



**Figs 15–17.** *Afroleius deformatoides* sp. n.: 15 = dorsal aspect, 16 = lateral aspect, 17 = ventral aspect. Scale bar μm.

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*deformis*, central part of the notogaster foveate (smooth in *A. deformis*) and the presence of aggenital setae (absent in *A. deformis*). The ridges extending from the lamellar apices towards the rostral seta are in my view not homologous to prolamellae, but may be the result of the unusual shape of the prodorsum. These ridges are also present in *A. deformis*. The new species is also smaller than *A. deformis*. The average length and width of *A. deformis* is 392 µm × 323 µm, while in the new species it is 353 µm × 271 µm. *A. deformis* is an inhabitant of lowlying forested areas in the south and east of the country, while *A. deformatoides* sp. n. has so far only been found in the high altitude forested gorges of the Drakensberg mountain range.

#### DISCUSSION

These are the first additional species in the genus *Afroleius*. COETZEE (2007) redescribed the three species of MAHUNKA (1984), but the presence of the axillary saccule and notogastral tectum was not recorded at the time.



**Fig. 18.** Known distribution of *A. crassus* sp. n. (circle); *A. decurvatus* sp. n. (triangle) and *A. deformatoides* sp. n. (cross) in South Africa.

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Therefore, the family relationship was not questioned. Since immatures are still unknown, their discovery in future may bring further clarity.

\*

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# **Chapter 3**

Coetzee, L. 2014. Rare new species of the genus *Afroleius* Mahunka, 1984 (Acari, Oribatida, Mycobatidae) from South Africa. *Navorsinge van die Nasionale Museum*, *Bloemfontein*, 30(5): 71–85.

# Abstract

Two rare new species in the genus *Afroleius* Mahunka,1984 are described from South Africa. *Afroleius natalensis* spec. nov. can be recognised by long, smooth lamellar setae with overlapping apices and rostral setae of medium length, thick and coarsely barbed. *Afroleius caudatus* spec. nov. is recognised by a caudal protuberance of the notogaster and long, ribbon-shaped lamellar setae. The known distribution of *A. natalensis* spec. nov. is restricted to a small area in the KwaZulu-Natal Province, while *A. caudatus* spec. nov. is represented by a few specimens from forests along the southern and eastern coastal regions and is probably arboreal.



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# Rare new species of the genus *Afroleius* Mahunka, 1984 (Acari, Oribatida, Mycobatidae) from South Africa

by

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#### ABSTRACT

**Coetzee, L. 2014. Rare new species of the genus** *Afroleius* **Mahunka, 1984 (Acari, Oribatida, Mycobatidae) from South Africa**. *Navors. nas. Mus., Bloemfontein* **30(5): 71-85.** Two rare new species in the genus *Afroleius* Mahunka,1984 are described from South Africa. *Afroleius natalensis* spec. nov. can be recognised by long, smooth lamellar setae with overlapping apices and rostral setae of medium length, thick and coarsely barbed. *Afroleius caudatus* spec. nov. is recognised by a caudal protuberance of the notogaster and long, ribbon-shaped lamellar setae. The known distribution of *A. natalensis* spec. nov. is restricted to a small area in the KwaZulu-Natal Province, while *A. caudatus* spec. nov. is represented by a few specimens from forests along the southern and eastern coastal regions and is probably arboreal.

(Oribatida, Mycobatidae, Afroleius, new species, South Africa)

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#### **INTRODUCTION**

Since the description of the genus *Afroleius* by Mahunka (1984) from material collected in the southern parts of South Africa, three new species have been added to the genus by Coetzee (2013) with the description of *A. crassus, A. decurvatus* and *A. deformatoides*. Coetzee & Tiedt (2013) presented a discussion of the genus with the description of additional characters, for example the presence of axillary saccules (Grandjean 1936, Norton *et al.* 1997, Norton & Behan-Pelletier 1986, Norton & Behan-Pelletier 2009) and the posterior notogastral tectum (Norton & Behan-Pelletier 2009). These characters have implications for the relationship of the genus. Coetzee & Tiedt (2013) presented arguments for the inclusion of the genus in the family Mycobatidae based on adult characters. No immatures have so far been found.

Two new species are described in this paper, nl. *A. natalensis* spec. nov. and *A. caudatus* spec. nov., both regarded as rare. Oribatid mites are generally abundant in litter from the regions where these species derive from. A total of 4 430 specimens (in 54 genera) were collected from 12 samples containing litter and soil (about 500 ml each) in an area roughly 15 minutes latitude by 15 minutes longitude (approximately 690 km<sup>2</sup>), while only seven *A. natalensis* spec. nov. specimens were found. Furthermore, this species was not found in any other samples currently in the Acarology collection of the National Museum. In the case of *A. caudatus* spec. nov. only five specimens are present in the Museum's Acarology Collection and were collected from three localities, between 400 and 600 km apart.

This work is part of a comprehensive study of the genus *Afroleius* and additional new species are in the process of being described. A key to species in the genus will be presented in a later paper.

#### Diagnosis of Afroleius Mahunka, 1984

For a comprehensive description see Coetzee & Tiedt (2013).

Integument foveate or reticulate; lamella marginal; rostral seta short to medium length; lamellar seta medium to long; interlamellar seta minute; pteromorph large, fully hinged; octotaxic system consisting of four pairs of saccules or porose areas; ten pairs of minute notogastral setae present; undivided posterior notogastral tectum present; genal notch and broad genal tooth usually present; axillary saccule of subcapitulum present; epimeral setae *3c* and *4c* absent; custodium absent; epimeral border 4 heavily sclerotized; pedotectum I

large, broad; genital plates with six pairs of setae, adanal setae (three pairs) inserted close to each other on postero-lateral border of anal plates; preanal organ of medium length, stem broadening towards base; postanal porose area usually present; tutorium consisting of dorsal ridge with deep incurvation in basal part; all legs heterotridactylous; tarsi I and II with dorsal dens proximal to tectal setae, varying from small point to large well-developed spur; distal end of genua I and II antiaxially with prominent cusps.

#### MATERIAL AND METHODS

All studied material was collected in South Africa. Soil fauna was extracted by Berlese-Tullgren funnels. Specimens were temporarily mounted on cavity slides with glycerol for study purposes and thereafter stored in 70% alcohol. A Nikon Eclipse 50i light microscope equipped with a DS-Fi1 digital camera and NIS-Elements imaging software were used for light microscope images. All material, including holotypes and paratypes, is deposited in the Acarology Collection of the National Museum, Bloemfontein, South Africa.

# **DESCRIPTION OF SPECIES**

#### Afroleius natalensis spec. nov.

(Figures 1-5, 11; Plates 1 A-D, 2 A)

#### Species diagnosis

Dorsal surface foveate, granular integument present anterior of dorsosejugal scissure; lateral epimeral surface granulate; octotaxic system consisting of saccules with wide openings; head of bothridial seta small, clavate, sparsely barbed, stalk long, thin; rostral seta of medium length, thick, coarsely barbed; lamellar seta long, smooth, thin, curving medially; interlamellar seta minute; notogastral setae minute; postanal porose area absent; very small dorsal dens on tarsi I and II; distal end of genu I antiaxially with dorsal and ventral cusps; distal end of genu II antiaxially with lateral cusp.

#### Dimensions

**Holotype** (male): Length 315  $\mu$ m, width 230  $\mu$ m. Paratypes: Females (n = 4): Length 323  $\mu$ m (312 – 333  $\mu$ m), width 240  $\mu$ m (229 – 254  $\mu$ m). Males (n = 2): Length 304  $\mu$ m (301 – 306  $\mu$ m), width 224  $\mu$ m (210 – 237  $\mu$ m).

#### **Prodorsum** (Figs 1 & 2)

Rostral margin uneven in dorsal view; prodorsal surface foveate with granular integument anterior of dorsosejugal scissure; narrow band of granular cerotegument present between lamellar apices; lamella (*L*) wide, extending over lateral margin of prodorsum; rostral seta (*ro*) of medium length, (~ 19  $\mu$ m), stout, thick at base tapering towards apex, coarsely barbed, inserted at anterior apex of tutorium; lamellar seta (*le*) long (~ 55  $\mu$ m), smooth, thin, curving medially, apices overlapping; interlamellar seta (*in*) minute (~ 5  $\mu$ m); bothridial opening directed laterally, with overlapping slit in ventral bothridial wall; head of bothridial seta small, clavate, sparsely barbed, stalk long, thin.



Figures 1-3: Afroleius natalensis spec. nov. 1 - Dorsal aspect; 2 - Lateral aspect; 3 - Ventral aspect. Scale bar 100  $\mu m$ 



Figures 4-5: Afroleius natalensis spec. nov. 4 - Leg I, antiaxial aspect (small dorsal dens indicated by arrow); 5 - Tarsus II, antiaxial aspect. Scale bar 50 μm

#### Notogaster (Figs 1 & 2; Pl. 1 C)

2014

Surface foveate; octotaxic system consisting of saccules, openings of saccules wide, surrounded by thickened integument; ten pairs of minute (~ 7  $\mu$ m) notogastral setae present; lyrifissure *im* clearly visible; orifice of opisthosomal gland (*gla*) very small, situated medially of  $h_3$ .

#### Podosoma and Gnathosoma (Figs 2 & 3; Pl. 1 D)

Surface of mentum anteriorly faintly foveate, fading posteriorly; genal notch (gn) present; genal tooth broad, short; axillary saccule short; epimeral setae minute; epimeral surface antero-medially foveate, laterally granulate; epimeral seta lc absent; tutorium (tu) narrow.

#### Ventral plate (Fig. 3; Pl. 1 A)

Surface of genital plate without clear foveae or striae; surface of anal plate foveate; ventral plate with large foveae; broad band of granular integument along border of ventral plate; *iad* situated laterally of anal plate;  $ad_{I-3}$  inserted postero-laterally to anal plate, posterior to *iad*; postanal porose area absent.



Plate 1 A-D: Afroleius natalensis spec. nov. A - Lateral aspect, showing granular border of ventral plate;
B - Leg I. Dorsal dens on tarsus (a) and cusps on genu (b) indicated; C - Fragment of notogaster. Pteromporph (*pter*), setae *la*, *c*<sub>2</sub> and saccule *Sa* indicated; D - Epimeral region. Pedotectum II (*pdII*) and discidium (*disc*) indicated.



Plate 2 A: Afroleius natalensis spec. nov. A - Leg IV. Thick integument (a) and tectum (b) on tarsus and tectum (c) on femur indicated. Plate 2 B-D: Afroleius caudatus spec. nov. B - Dorsal aspect; C – Ventral aspect; D - Leg II. Solenidion on tibia (a) and dorsal dens on tarsus (b) indicated.

Legs (Figs 4 & 5, Pls 1 B & 2 A)

**Setal formula** (trochanter to tarsus, famulus included): Leg I 1-5-3-4-20; Leg II 1-5-3-4-15; Leg III 2-2-1-3-15; Leg IV 1-2-2-3-12. **Solenidial formula** (genu to tarsus): Leg I 1-2-2; Leg II 1-1-2; Leg III 1-1-0; Leg IV 0-1-0.

Legs relatively short; dorsal integument of tarsi I, II and IV and tibiae I, II and IV thick; medial claw of all legs strong, lateral claws thin. Leg I: Very small dens present on dorsal side of tarsus proximal to tectal setae; antiaxial fastigial seta (ft") very short and thin; famulus ( $\varepsilon$ ) short; distal end of genu antiaxially with dorsal and ventral cusps. Leg II: Tarsus short, broad; small dens present on dorsal side of tarsus proximal to tectal setae; distal end of genu antiaxially with ventral cusp. Leg III: Relatively short. Leg IV: Dorsal surface of tarsus and tibia uneven, integument thick; tarsus antiaxially with diagonal tectum running from insertion of ft" towards proximo-lateral base of segment; femur ventrally with wide tectum; dorsal seta (d) on femur thick, roughened.

**Material examined** (Fig. 11): Holotype (male) (NMB 1949.16.1) and four paratypes (three females, one male) collected between Ixopo and Bulwer 30°03'S 29°55'E, KwaZulu-Natal, 9 February 1982 (C.M. Engelbrecht), moist litter underneath stands of large trees. Additional material collected between Richmond and Ixopo 30°02'S 30°13'E, KwaZulu-Natal, 9 February 1982 (C.M. Engelbrecht), litter and soil underneath Aloe.

#### Etymology

The species name refers to the province KwaZulu-Natal, the only province where it has so far been located.

#### Remarks

This species can readily be distinguished from other *Afroleius* species by the long, smooth, overlapping lamellar setae and short, thick, coarsely barbed rostral setae. The dorsal dens on tarsi I and II is much reduced. The bothridium has a slit in the ventral wall of which the sides overlap, similar to *A. minor* Mahunka, 1984 (Coetzee & Tiedt 2013, Fig. 8). This is a rare species, where only a few specimens were collected from otherwise rich samples, as discussed in the Introduction. The Acarology collection of the National Museum is reasonably representative of the South African oribatid fauna, and the so far known distribution of *A. natalensis* spec. nov. is restricted to a small area (see Fig. 11).

*Afroleius caudatus* spec. nov. (Figures 6-10, 11; Plates 2 B-D)

#### **Species diagnosis**

Outline of notogaster and rostrum uneven; posteriorly notogaster with clearly defined caudal protuberance; dorsal and ventral surfaces foveate; octotaxic system consisting of porose areas; head of bothridial seta large, more or less globular, surface coarsely punctate, stalk thin, short; rostral seta of medium length, densely barbed; lamellar seta long, smooth, ribbon-shaped with defined central core, apex pointed; interlamellar seta minute; notogastral setae minute; postanal porose area present; dorsal dens on tarsi I and II of medium size; distal end of genua I and II antiaxially with lateral cusp; solenidion  $\sigma$  on genua I and II and  $\varphi$  on tibia II hook-shaped.



Figures 6-8: *Afroleius caudatus* spec. nov. 6 - Dorsal aspect; 7 - Lateral aspect; 8 - Ventral aspect (NB: note that the postanal porose area (*pa*) is a structure of the ventral plate). Scale bar 100 µm



Figures 9-10: *Afroleius caudatus* spec. nov. 9 – Leg I, antiaxial aspect; 10 - Tarsus, Tibia and Genu II, antiaxial aspect. Scale bar 50 μm

#### Dimensions

**Holotype** (female): Length 337  $\mu$ m, width 215  $\mu$ m. Paratypes: Females (n = 1): Length 329  $\mu$ m, width 213  $\mu$ m. Males (n = 3): Length 307  $\mu$ m (300 – 320  $\mu$ m), width 194  $\mu$ m (191 – 198  $\mu$ m).

#### Prodorsum (Figs 6 & 7, Pl. 2 B)

Rostral margin uneven in dorsal view; prodorsal surface with large foveae; lamella (*L*) wide, extending over lateral margin of prodorsum; prodorsum sharply sloped between lamellar apices, creating the impression of a translamella in dorsal view; rostral seta (*ro*) of medium length (~ 24  $\mu$ m), thick, densely barbed, inserted at anterior apex of tutorium; lamellar seta (*le*) long (~ 50  $\mu$ m), ribbon-shaped with defined central core, smooth, curved medially, apices pointed; interlamellar seta (*in*) minute (~ 6  $\mu$ m); bothridium small, mostly

hidden below pteromorph, opening directed ventrally; head of bothridial seta large, more or less globular, surface coarsely punctate, stalk thin, short.

#### Notogaster (Figs 6 & 7, Pl. 2 B)

Notogastral outline uneven; posteriorly notogaster with clearly defined caudal protuberance; surface with large foveae; octotaxic system consisting of large porose areas, position of porose areas the same in males and females; ten pairs of minute (~ 6  $\mu$ m) notogastral setae present; lyrifissure *im* clearly visible; orifice of opisthosomal gland (*gla*) very small, situated medially of  $h_3$ .

#### Podosoma and Gnathosoma (Figs 7 & 8)

Surface of mentum foveate; genal notch (gn) and genal tooth indistinct; axillary saccule short; epimeral surface foveate; epimeral setae minute; epimeral seta lc absent; apodemata short; tutorium (tu) narrow.

#### Ventral plate (Fig. 8, Plate 2 C)

Surface of genital and anal plates with small foveae; surface of ventral plate with large foveae; *iad* situated laterally of anal plate;  $ad_{1-3}$  inserted postero-laterally to anal plate, posterior to *iad*; postanal porose area (*pa*) large.

Legs (Figs 9 &10, Pl. 2 D)

#### Setal formula similar to A. natalensis spec. nov.

Legs relativley short; dorsal integument of tarsi I, II and IV and tibiae I, II and IV thick; medial claw of all legs strong, lateral claws thin. Leg I: Medium-sized dens present on dorsal side of tarsus proximal to tectal setae; antiaxial fastigial seta (ft") short and thin; famulus ( $\varepsilon$ ) of medium length; distal end of genu antiaxially with ventral cusp; solenidion ( $\sigma$ ) on genu short, hook-shaped, curving antiaxially. Leg II: Tarsus short, broad; medium dens present on dorsal side of tarsus proximal to tectal setae; distal end of genu antiaxially with ventral cusp; apices of solenidia on tarsus ( $\omega_1$  and  $\omega_2$ ) slightly bent; solenidion ( $\phi$ ) on tibia short, hook-shaped, curving antiaxially; solenidion ( $\sigma$ ) on genu very short, hookshaped, curving antiaxially. Leg III: Relatively short. Leg IV: Dorsal surface of tarsus and tibia uneven, integument thick; tarsus antiaxially with diagonal tectum running from insertion of ft" towards proximo-lateral base of segment; femur ventrally with wide tectum; dorsal seta (d) on femur thick, roughened.

**Material examined** (Fig. 11): Holotype (female) (NMB 3676.2.1) and two paratypes (males) collected at Dwesa forest 32° 15' 17" S, 28° 52' 39" E, Wild Coast, Eastern Cape Province, January 1990 (F. Brusse) feeding on understorey leaves of *Buxus natalensis*. Additional material: Tuin van Eden (between Knysna and Plettenberg Bay) 34° 01' 35" S, 23° 11' 37"E, Western Cape Province, 28 January 1963 (R. van Pletzen) litter from indigenous forest; between Highflats and Scottburgh 30° 16' 50" S, 30° 26' 16" E, KwaZulu-Natal, 2 September 1982 (C.M. Engelbrecht) litter.

#### Etymology

The species name refers to the caudal protuberance on the posterior margin of the notogaster.

#### Remarks

*Afroleius caudatus* spec. nov. is easily distinguished from other *Afroleius* species by the caudal protuberance as well as long, broad, ribbon-shaped lamellar setae. This species is almost certainly arboreal as the holotype and two paratypes from Dwesa forest have been collected directly from understorey leaves, noted as "feeding on leaves". The almost globular shape of the head of the bothridial seta, short bothridial stalk (the stalk is noticeably shorter than that of other *Afroleius* species) and large porose areas also indicate an arboreal habitat (Aoki 1973; Behan-Pelletier & Walter 2000; Karasawa & Hijii 2004). The only other specimens encountered so far (one from each locality) have been collected from litter, but may have landed in the litter accidentally. This species is regarded as rare since very few specimens have been encountered so far, collected from three well-separated localities. However, arboreal surveys may indicate a wider distribution.



Figure 11: Known distribution of Afroleius natalensis spec. nov. and A. caudatus spec. nov.

#### DISCUSSION

These two new species bring the number of *Afroleius* species to eight, together with the three original species *A. deformis*, *A. minor* and *A. simplex* described by Mahunka (1984) (redescribed by Coetzee (2007)) and three new species *A. crassus*, *A. decurvatus* and *A. deformatoides* described by Coetzee (2013). This genus is widely distributed along the southern and eastern coastal regions of South Africa with generally more forested areas and a much higher rainfall than the central and western parts. Further studies will undoubtedly produce more new species.

#### **OPSOMMING**

Twee skaars spesies in die genus *Afroleius* Mahunka, 1984 van Suid-Afrika word beskryf. *Afroleius natalensis* spec. nov. word herken aan lang, gladde lamellaarsetas waarvan die punte oor mekaar vou en medium-lang, dik, growwe rostraalsetas. *Afroleius caudatus* spec. nov. word uitgeken aan 'n gepunte uitsteeksel aan die posterior rand van die notogaster en lang, lintvormige lamellaarsetas. Die tot dusver bekende verspreiding van *A. natalensis* spec. nov. is beperk tot 'n klein gebied in die KwaZulu-Natal-provinsie, terwyl *A. caudatus* spec. nov. deur slegs 'n paar eksemplare van die suidelike en oostelike kusstreke verteenwoordig word en heel moontlik boomlewend is.

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# Abstract

Three new species of the genus *Afroleius* Mahunka, 1984 are described: *A. amieae* **sp. nov.** with round notogastral foveae and long rostral and lamellar setae, *A. inae* **sp. nov.** with long lamellae and rostral and lamellar setae of similar length, and *A. valerieae* **sp. nov.** with ventrally directed bothridium and octotaxic system consisting of porose areas. *Magyaria florida* Mahunka, 1985 is recombined in the genus *Afroleius* as *A. floridus* **comb. nov.** Juvenile instars of *A. floridus* **comb. nov.** are described.



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# *Afroleius floridus* (Mahunka, 1985) comb. nov. and three new *Afroleius* Mahunka, 1984 species (Acari: Oribatida: Mycobatidae) from South Africa

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# Abstract

Three new species of the genus *Afroleius* Mahunka, 1984 are described: *A. amieae* **sp. nov.** with round notogastral foveae and long rostral and lamellar setae, *A. inae* **sp. nov.** with long lamellae and rostral and lamellar setae of similar length, and *A. valerieae* **sp. nov.** with ventrally directed bothridium and octotaxic system consisting of porose areas. *Magyaria florida* Mahunka, 1985 is recombined in the genus *Afroleius* as *A. floridus* **comb. nov.** Juvenile instars of *A. floridus* **comb. nov.** are described.

Key words: Acari, Oribatida, Mycobatidae, Magyaria, Afroleius, Juvenile instars, South Africa

# Introduction

During the 1980s Dr. Sándor Mahunka (Hungarian Natural History Museum, Budapest) described a number of oribatid species from South Africa which he obtained from material collected by Dr. S. Endrődi and Dr. S. Endrődy-Younga (Mahunka 1984, Mahunka 1985a, Mahunka 1985b, Mahunka 1986a, Mahunka 1986b). The majority of samples were collected in the Western Cape (Nature's Valley and the environs of Cape Town) in the Fynbos Biome of South Africa (Mucina & Rutherford 2006, Van As *et al.* 2012). Nature's Valley lies in the heart of pockets of Southern Afrotemperate forests (Mucina & Rutherford 2006), remnants of which stretch along the southern coastline of South Africa. The area around Cape Town is devoid of forests and the vegetation is characterized by fine-leaved, evergreen shrubs (Van As *et al.* 2012).

From these Nature's Valley Oribatida he described *Afroleius* Mahunka, 1984, with three species. Mahunka (1984) placed the genus in the family Haplozetidae and remarked that it resembles *Magyaria* Balogh, 1963. Differences between *Magyaria* and *Afroleius* as pointed out by Mahunka (1984) were the presence of four pairs of genital setae and the monodactylous state of the ambulacra in *Magyaria*, while *Afroleius* has six pairs of genital setae and the ambulacra are tridactylous. Five new species have since been added to the genus (Coetzee 2013, Coetzee 2014), and its relationship has provisionally been changed to the family Mycobatidae (Coetzee & Tiedt 2013). No juveniles were known at the time.

Mahunka (1985b) also described *Magyaria florida* from the Tsitsikamma Mountains. A reexamination of this species indicated its closer relationship to the genus *Afroleius* and it is recombined here to the genus as *A. floridus* **comb. nov.** This species occurs commonly in the southern regions of the Western and Eastern Cape provinces of South Africa, particularly in forests and along forest edges.

Species of *Afroleius* are widespread in South Africa, particularly in the eastern regions, and more new species have been discovered. Juveniles of *A. floridus* **com. nov.** have also been found. In this paper *A. floridus* **comb. nov.** is redescribed based on adults and juvenile instars, and three new species, *A. amieae* **sp. nov.**, *A. inae* **sp. nov.** and *A. valerieae* **sp. nov.**, are described.

This work forms part of a comprehensive study of *Afroleius* in South Africa. A key to all known species and additional notes on the genus will be presented in a follow-up publication.

# Material and methods

All material was collected in South Africa. Soil fauna was extracted by Berlese-Tullgren funnels. Collection data are given as follows: locality (nearest town), latitude, longitude, date collected, collector. Coordinates of localities were determined using Google Earth. Habitat is discussed collectively for all samples of each species. Collectors are indicated by their initials (LC—Louise Coetzee; DdS—Dawie de Swardt; JJBD—J.J.B. Dreyer; RE— Roy Earl; AJE—A.J. Els; CME—C.M. Engelbrecht; IE—Ina Engelbrecht; JPE—J.P. Eksteen; LG—Lorinda Grobler; DJK—D.J. Kok; BK—Binu Koshy; LNL—L.N. Lotz; SvdML—S. vdM. Louw; GN—G. Nel; CMO—C.M. Oosthuizen; BSR—B.S. Rubidge; RvP—R van Pletzen; JW—J. Watson). Specimens were temporarily mounted on cavity slides with glycerol for study purposes and thereafter stored in 70% alcohol. Measurements were taken from rostral tip to posterior margin of notogaster as seen in dorsal view (length) and across the notogaster posterior to the pteromorph (width) (temporary slides). All measurements are presented in µm, with the range (minimum and maximum) in brackets where applicable.

A Nikon Eclipse 50i light microscope equipped with a DS-Fi1 digital camera and NIS-Elements imaging software were used for light microscope images. The following procedure was followed for Scanning Electron Microscope micrographs: samples were fixed in 70% ethanol for a minimum of 24 hours, dehydrated in a graded ethanol series of 80%, 90% and 2X 100% for 30 minutes each. The samples were critical point dried in liquid carbon dioxide, mounted with double sided carbon tape on SEM-stubs and coated with 15 nm gold/palladium in a sputter coater, and viewed under a FEI Quanta 250 FEG SEM at 5 kV under high vacuum mode. (Images by Dr. Lourens Tiedt, North West University, South Africa).

All holotypes and some paratypes are deposited in the Acarology Collection of the National Museum, Bloemfontein. Some paratypes are deposited in the Invertebrate Collection, Department of Natural Sciences, KwaZulu-Natal Museum, Pietermaritzburg, South Africa.

# **Generic diagnosis**

# Afroleius Mahunka, 1984

Adults. Integument foveate or reticulate; lamella marginal; rostral seta short to medium length; lamellar seta medium to long; interlamellar seta minute; pteromorph large, fully hinged; octotaxic system consisting of four pairs of saccules or porose areas; ten pairs of minute notogastral setae present; posterior notogastral tectum present, undivided; genal notch and broad genal tooth usually present; axillary saccule of subcapitulum present; epimeral setae *3c* and *4c* absent; discidium present; custodium absent; epimeral border 4 heavily sclerotized; apodemata II, *sj* and III parallel; pedotectum I large, broad; genital plates with six pairs of setae; adanal setae ( $ad_{1-3}$ ) inserted close to each other near postero-lateral border of anal plates, posterior to *iad*; preanal organ of medium length, stem broadening towards base; postanal porose area usually present; tutorium consisting of dorsal ridge with deep incurvation in basal part; all legs heterotridactylous; tarsi I and II with dorsal dens proximal to tectal setae, varying from small point to large well-developed spur; genua I and II distally with one or two prominent cusps positioned antiaxially; porose regions on femora I–IV and trochanters III–IV present; lateral seta *l'* on femur III absent (i.e. Fe III with two setae). For a comprehensive description see Coetzee & Tiedt 2013.

# Juvenile instars (only A. floridus comb. nov. known)

Humeral organ absent, axillary saccule absent, pygidial sclerite large, unsclerotized, covering dorsal side of gastronotum; lateral sclerites absent.

# Afroleius floridus (Mahunka, 1985) comb. nov.

(Figs 1-4, 11)

Magyaria florida Mahunka, 1985 Mahunka 1985b Folia Entomologica Hungarica 46(1): 92, Figs 42–47 **Species diagnosis**. Dorsal and ventral surfaces with irregularly shaped foveae, inner surfaces of cavities with fine, irregular sculpture; surfaces of prodorsum and pteromorph reticulate, with some cerotegument; octotaxic system consisting of saccules; bothridial seta clavate, head sparsely barbed, stalk thin, directed antero-dorsally; rostral seta short, coarsely barbed; lamellar seta long, minutely barbed, thin; postanal porose area present; dorsal dens on tarsi I and II large; genu I distally with dorsal and ventral cusps antiaxially; genu II distally with lateral cusp.

**Dimensions**. Males (n = 6): length 238 (233–243), width 161 (148–175). Females (n = 14): length 256 (249–265), width 175 (166–187).

**Prodorsum** (Figs 1A–D, 3A). Rostral margin anteriorly with two small notches, transparent U-shaped area between notches (Fig. 1B); prodorsal surface reticulate with some cerotegument on reticulation; lamella wide, extending over lateral margin of prodorsum; rostral seta (ro) short (~19), coarsely barbed, inserted at anterior apex of tutorium; lamellar seta (le) long (~40), minutely barbed, thin; interlamellar seta (in) minute (~6); bothridial opening directed ventro-laterally, with overlapping slit in ventral wall of bothridium; bothridial seta (bo) clavate, head sparsely barbed, stalk long, thin, directed antero-dorsally (Fig. 3A); length of bothridial seta from point of emerging from bothridium to tip of seta ~63 (55–77).

**Notogaster** (Figs 1A, D, 3A, C). Surface of notogaster with irregularly shaped foveae, inner surface of cavities with fine, irregular sculpture (Fig. 3C); octotaxic system consisting of saccules; notogastral setae minute (~6); orifice of opisthosomal gland (*gla*) very small, situated laterally to lp (Fig. 3C); surface of pteromorph reticulate with some cerotegument.

**Podosoma and gnathosoma** (Figs 1C, 1D, 3B). Surface of mentum and epimeral region similar to that of notogastral surface; subcapitular seta a of medium length, smooth, m of medium length, finely barbed, h shorter, smooth; paraxial lateral seta on palptarsus thick (Fig. 3B, indicated by arrow), barbed; genal notch present, genal tooth short, broad (see Coetzee & Tiedt 2013, their Fig. 5); epimeral setae very short; epimeral setation (epimeres I–IV) 2-1-2-2; surface of pedotectum I reticulate with some cerotegument.

**Ventral plate** (Fig. 1C). Surface of genital plate smooth; surface of anal plate foveate; ventral plate with large foveae, structure of foveae similar to that of dorsal surface; postanal porose area present, small, round, covered by posterior notogastral tectum.

Legs (Figs 2A–D, 3D). Setal formula (trochanter to tarsus, famulus included): Leg I 1-5-3-4-20; Leg II 1-5-3-4-15; Leg III 2-2-1-3-15; Leg IV 1-2-2-3-12. Solenidial formula (genu to tarsus): Leg I 1-2-2; Leg II 1-1-2; Leg III 1-1-0; Leg IV 0-1-0.

Legs relatively short; dorsal integument of tarsi I, II, IV and tibiae I, II, IV thick; medial claw of all legs strong, lateral claws thin; femora I–IV, trochanters III–IV antiaxially with fine striae. Leg I: large, pointed, distally directed dens present on dorsal side of tarsus proximal to tectal setae; antiaxial fastigial seta (ft") short and thin; famulus ( $\varepsilon$ ) short; genu distally with large ventral and slightly smaller dorsal cusp antiaxially. Leg II: tarsus short, broad; large, pointed distally directed dens present on dorsal side of tarsus proximal to tectal setae; genu distally with large lateral cusp (Figs 2B, 3D). Leg III: relatively short, dorsal integument of all segments thin; setae finer than on other legs; l' on femur absent. Leg IV: tarsus antiaxially with weakly developed diagonal tectum running from insertion of ft towards proximo-lateral base of segment; femur ventrally with wide tectum; dorsal seta (d) on femur thick, roughened.

Juvenile instars. Measurements: Length: larva 142, protonymph 152, deutonymph 201, tritonymph 228.

**Tritonymph** (Figs 4A–E). **Prodorsum**: Aspis micropunctate; porose sclerite present between insertions of *in* and *bo* and posterior prodorsal border; *ro* of medium length (~25), barbed; *le* (~42), *in* (~45) long, robust, with midrib, regularly-spaced lateral barbs along entire length of seta; axillary saccule absent; *ex* very short (~6), fine; *bo* long (~34), club-shaped, barbed, directed antero-laterally; all setae (except *ex*) inserted on tubercles.

**Gastronotic region**: Humeral organ absent; pygidial sclerite *PY* ill-defined, very weakly sclerotized, microtuberculate, large, covering dorsal side of gastronotum, bearing setae  $c_1$ ,  $c_2$ , da, dm, dp, la, lm, lp,  $h_1$ ,  $h_2$ ,  $h_3$ ,  $p_1$ ; transverse tegumental fold present posterior to insertion of  $c_1$ ; 15 pairs of gastronotic setae present; setae similar to *le* and *in*; setal lengths:  $c_1 \sim 52$ ,  $c_2 \sim 46$ ,  $c_3 \sim 10$ ,  $da \sim 58$ ,  $dm \sim 62$ ,  $dp \sim 54$ ,  $la \sim 47$ ,  $lm \sim 54$ ,  $lp \sim 53$ ,  $h_1 \sim 59$ ,  $h_2 \sim 44$ ,  $h_3 \sim 55$ ,  $p_1 \sim 46$ ,  $p_2 \sim 17$ ,  $p_3 \sim 15$ ; all setae (except  $c_3$ ,  $p_2$ ,  $p_3$ ) inserted on robust tubercles;  $c_3 p_2$ ,  $p_3$  short, proportionally smaller than other setae, not inserted on pygidial sclerite, inserted on smaller tubercles than rest of setae; opisthonotal gland *gla* medial to *lp*.

**Ventral region**: See Table 1 for development of coxisternal, genital, anal and adanal setae; all ventral setae minute, fine, smooth.



FIGURE 1. Afroleius floridus comb. nov. A) Dorsal view. B) Rostrum, anterior view (not to scale). C) Ventral view. D) Lateral view. Scale bar 100 µm.



**FIGURE 2.** *Afroleius floridus* **comb. nov.** Legs I–IV. A) Leg I, antiaxial aspect (trochanter not illustrated). B) Leg II, antiaxial aspect (trochanter not illustrated). C) Leg III, paraxial aspect. D) Leg IV, paraxial aspect. Scale bar 50 µm.





**FIGURE 3.** *Afroleius floridus* **comb. nov.** SEM images A) Dorsal view. B) Palptarsus, antiaxial view, arrow to paraxial lateral seta. C) Postero-lateral part of notogaster. D) Leg II, pedotectum I, arrow to dorsal dens; note striae on femur.

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**FIGURE 4.** *Afroleius floridus* **comb. nov.** Tritonymph. C–E Light microscope images. A) Dorsal view. B) Ventral view. C) Seta  $h_i$  showing central core. D) Leg I, showing anterodorsal apophysis on tibia. E) Dorsal view. Scale bar 100  $\mu$ m.
	Larva	Protonymph	Deutonymph	Tritonymph	Adult
Gastronotal	12	15	15	15	10
Coxisternal	3-1-2	3-1-2-1	3-1-2-2	3-1-2-2	2-1-2-2
Genital	0	1	3	5	6
Aggenital	0	0	1	1	1
Anal	0	0	0	2	2
Adanal	0	0	3	3	3

TABLE 1. Development of gastronotal and ventral setae of Afroleius floridus comb. nov.

**Legs**: See Table 2 for development of leg setation. Tibia I of all juvenile instars with large anterodorsal apophysis, bearing solenidion  $\phi_1$  (Fig. 4D).

**TABLE 2.** Development of leg setae and solenidia of *Afroleius floridus* **comb. nov.** (setae are noted opposite the instar in which they first appear; parentheses indicate setal pairs).

Stase	Trochanter	Femur	Genu	Tibia	Tarsus
Leg I					
Larva	-	d bv	(l) σ	(l) v' $\phi_1$	(ft) (pv) (pl) s (a) (u) (p) (tc) $\varepsilon \omega_1$
Protonymph	-				$\omega_2$
Deutonymph	-	(1)		$\phi_2$	
Tritonymph	v'		v'	V	(it)
Adult		v			1 v'
Leg II					
Larva	-	d bv	(l) σ	l' ν' φ	(ft) (pv) s (a) (u) (p) (tc) $\omega_1$
Protonymph	-				
Deutonymph	-	(1)		1	ω <sub>2</sub>
Tritonymph	v'		v'	V	(it)
Adult		v			
Leg III					
Larva	-	d ev'	l' σ	$v'  \phi_{\scriptscriptstyle 1}$	$(\mathrm{ft})(\mathrm{pv}) \mathrm{s}(\mathrm{a})(\mathrm{u})(\mathrm{p})(\mathrm{tc})$
Protonymph	-				
Deutonymph	1'			1'	
Tritonymph	v'			1	(it)
Adult					
Leg IV					
Protonymph	-	-	-	-	ft (p) (u) (pv)
Deutonymph	-	d ev'	d	$v'  \phi_{\scriptscriptstyle 1}$	(tc) (a) s
Tritonymph	v'		1'	(1)	
Adult					

**Other instars**. Setae and structures of larva, protonymph and deutonymph similar to tritonymph, but proportionally smaller. See Table 1 for development of dorsal and ventral setae.

**Remarks**. The large and unusually shaped setae consist of a central core, covered by a pigmented layer ornamented with regularly spaced, almost triangular protuberances (Fig. 4C). This layer can be stripped from the core by manipulation of specimens. Slight manipulation of exuviae for mounting and studying purposes causes the pygidial sclerite to detach from the aspis.

Material examined (Fig. 11). Paratype TM 14870 Magyaria florida Mahunka, 1985, on loan from Ditsong

National Museum of Natural History (formerly Transvaal Museum), Pretoria. Collection data: Lottering forest, Tsitsikamma Mts, 33°57'S, 23°43'E; 12.xii.1977; from soil traps, leg. Dr. S. Endrődi.

Additional material: Litter from well-vegetated habitats such as forests (also pockets of forest in the grassland biome), savanna and coastal vegetation (indigenous and exotic) from the southern and south-eastern regions of South Africa (habitat types according to Mucina & Rutherford 2006). Coastal vegetation & Fynbos: Betty's Bay 34°20'S 18°55'E 17.xi.1982 CME; Cintsa 32°50'S 28°06'E 1.xii.1989 CME; East London 33°02'S 27°52'E 19.i.1963 RvP; Hermanus (Fernkloof Nature Reserve) 34°23'S 19°15'E 29.xii.2013 LC; Kogel Bay 34°14'S 18°51'E 4.vii.1987 RE; Kogel Bay 34°14'S 18°51'E 23.ii.1983 BSR; Kogel Bay 34°14'S 18°51'E 4.v.1983 CME; Mossel Bay 34°11'S 22°08'E 4.ix.1963 RvP; Port Elizabeth 33°47'S 25°40'E 21.i.1963 RvP; Port Elizabeth (Summerstrand) 33°47'S 25°40'E 25.v.1982 CME; Thornhill 33°55'S 25°03'E 24.i.1963 RvP. Exotic (Pinus) plantations: George (Witfontein) 33°57'S 22°32'E 29.1.1963 RvP; Knysna (Kruisfontein) 34°00'S 23°11'E 27.i.1963 RvP. Forest (coastal): Garden of Eden 34°00'S 23°11'E 5.i.2013 IE; George (Glentana) 34°03'S 22°18'E 31.xii.1981 CME; George (Saasveld) 33°57'S 22°32'E 29.i.1963 RvP; Harkerville 34°05'S 23°14'E 27.xii.2012 IE; Bloukrans Pass 33°57'S 23°38'E 26.i.1963 RvP; Karatara (Koobooberry farm) 33°58'S 22°49'E 28.xii.2012 LC; Tzitzikamma 33°58'S 23°53'E 25.i.1963 RvP; Wilderness National Park 33°59'S 22°36'E 27.v.2005 LG; Woodville Reserve 33°56'S 22°38'E 20.xii.2012 LC. Forest (mistbelt): Hogsback 32°35'S 26°55'E 1.vi.1994 BK. *Fynbos*: Grabouw (Houhoek Pass) 34°12'S 19°09'E 4.ix.1963 RvP; Bredasdorp 34°31'S 19°57'E 17.xi.1982 CME; Stormsvlei 34°04'S 20°05'E 17.xi.1982 CME. Grassland (pockets of forest): Penhoek Pass 31°27'S 26°42'E 17.i.1963 RvP; Olifantskop Pass 33°19'S, 25°56'E 26.v.1982 CME. Savanna: Cookhouse 32°40'S 2°549'E 26.v.1982 CME; Dordrecht 31°22'S 27°02'E 1.iii.1964 RvP; Grahams Town 33°18'S 26°34'E 15.iv.1963 CMO; Grahams Town district 33°25'S 26°03'E 26.v.1982 CME; King Williamstown 32°53'S 27°23'E 1.vi.1963 RvP; Cookhouse 33°23'S 2°541'E 26.v.1982 CME; Whittlesea 32°29'S 26°46'E 24.v.1982 CME.

**Remarks**. On some specimens the unique structure of the notogastral foveae are replaced by small, round foveae in the mid-notogastral area only, peripheral foveae are unchanged.

Balogh (1963) instituted the genus *Magyaria* with *Scheloribates reticulatus* Balogh, 1958 as type species, collected from the Kasai District in the Democratic Republic of the Congo. Currently *Magyaria* consists of 17 species (excluding *A. floridus* **comb. nov.**) (Subías 2004, 2014). These species all have a narrow rostrum with long, marginal lamella, usually with prolamella, adanal seta *ad*<sub>3</sub> invariably inserted anteriorly to *iad*, four or five pairs of genital setae and the ambulacrum mono-, bi- or tridactyle. No mention is made of dens on tarsi I and II or cusps on genua. Species lack a notogastral tectum posteriorly. *Magyaria* is mainly distributed in the Oriental Region (12 species) and the northern part of the Ethiopian Region (five species) (Balogh 1963, Balogh 1970a, Balogh 1970b, Balogh & Mahunka 1974, Corpuz-Raros 1979, Corpuz-Raros 1991, Hammer 1979, Mahunka 1978, Mahunka 1988, Mahunka 2008, Pérez-Íñigo 1981, Pérez-Íñigo 1983).

*A. floridus* **comb. nov.** differs from *Magyaria* in having shorter, more parallel, broad lamellae, large pedotectum I, apodemes II, *sj* and III more or less parallel (contrary to those of *Magyaria* which are slanted towards the genital plates),  $ad_3$  inserted posterior to lyrifissure *iad* and six pairs of genital setae. This species also has the characteristic strongly sclerotized epimeral border IV and tutorium as in *Afroleius*, dens on tarsi I and II, cusps on genua I and II and a posterior notogastral tectum. These characters are all consistent with *Afroleius*.

Juveniles of *A. floridus* **comb. nov.** are the first to be discovered in the genus. The first association between juveniles and adults was made by the collection of an adult which was still connected to the nymphal exuvium. Further collection of juveniles followed after this recognition of juvenile morphology. Breeding experiments were also conducted. See Discussion for further remarks on juvenile morphology.

Afroleius floridus comb. nov. is a species of forests and well-vegetated areas of the southern regions of South Africa.

# *Afroleius amieae* sp. nov. (Figs 5, 6, 11)

Species diagnosis. Notogastral surface with small, round foveae; ventral surface with larger, round foveae; surfaces of prodorsum and pteromorph reticulate, with some cerotegument; octotaxic system consisting of

saccules; bothridial seta clavate, sparsely barbed, stalk thin, directed antero-dorsally; rostral seta long, barbed; lamellar seta long, minutely barbed, thin, apices touching; postanal porose area present; large dorsal dens on tarsi I and II; genu I distally with dorsal and ventral cusps antiaxially; genu II distally with lateral cusp.



FIGURE 5. Afroleius amieae sp. nov. A) Dorsal view. B) Ventral view. C) Lateral view. Scale bar 100 µm.





**FIGURE 6.** *Afroleius amieae* **sp. nov.** A–B SEM images. C Light microscope image. A) Dorsal view. B) Tarsus I, dorsal dens indicated by arrow .(C) Epimeral region, epimeral border 4 indicated by arrow.

**Dimensions**. Holotype: (male) length 282, width 177: Paratypes: males (n = 8): length 268 (262–282), width 177 (166–186); females (n = 7): length 280 (276–284), width 190 (181–196).

**Prodorsum** (Figs 5A, C, 6A, B). Rostral margin anteriorly with two small notches, transparent U-shaped area between notches (similar to *A. floridus* **comb. nov.** Fig. 1B); prodorsal surface reticulate (Fig. 6B); lamella wide, extending over lateral margin of prodorsum; rostral seta (*ro*) long, barbed (~38) (Fig. 6B), inserted at anterior apex of tutorium; lamellar seta (*le*) long (~44), minutely barbed, thin, apices medially touching; interlamellar seta (*in*) minute (~5); bothridial opening directed ventro-laterally, with overlapping slit in ventral wall of bothridium; bothridial seta clavate, sparsely barbed, stalk long, thin, directed antero-dorsally, length of bothridial seta from point of emerging from bothridium to tip of seta ~60 (56–63).

**Notogaster** (Figs 5A, C, 6A). Surface of notogaster with small, round foveae; octotaxic system consisting of saccules, notogastral setae minute ( $\sim$ 5); orifice of opisthosomal gland (*gla*) very small, situated postero-laterally to *lp*; surface of pteromorph with thick reticulation and some cerotegument, reticulation replaced by fine granules along peripheral areas.

**Podosoma and gnathosoma** (Figs 5B, C, 6C). Surface of mentum anteriorly with shallow foveae, posteriorly smooth, some cerotegument present; subcapitular seta *a* of medium length, finely barbed, *m* slightly longer than *a*, barbed, *h* short, smooth; genal notch (*gn*) present; genal tooth broad, short; epimeral setae very short; epimeral setation (epimeres I–IV) 2-1-2-2; epimeral surface laterally with large foveae, foveae medially smaller (Fig. 6C); surface of pedotectum I reticulate with some cerotegument.

**Ventral plate** (Fig. 5B). Surface of genital plate smooth; surface of anal plate foveate; ventral plate with large, round foveae; postanal porose area present, small, round, covered by posterior notogastral tectum.

**Legs** (Fig. 6B). Setation of all legs similar to *A. floridus* **comb. nov.**; tarsi I and II proximally with small transverse ridges on dorsal surface, antiaxial surface granular; dorsal integument of tarsi I, II, IV and tibiae I, II, IV thick; femora I–IV, trochanters III–IV antiaxially with fine striae; tarsi I and II with large, pointed, distally directed dens; tarsus II short, broad; genua I and II distally with ventral and dorsal cusps antiaxially; tarsus IV antiaxially with weakly developed diagonal tectum running from insertion of *ft* towards proximo-lateral base of segment; femur ventrally with wide tectum.

**Material examined** (Fig. 11). Holotype: Database no. 1768.15.1, Edenville 27°29'S, 27°42'E, litter underneath shrubs, 19.i.1982, leg. C.M. Engelbrecht. Paratypes: from the same sample; 6 males and 5 females deposited in the Acarology Collection of the National Museum (Database no 1768.15.2); 2 males and 2 females deposited in the KwaZulu-Natal Museum (Database no: NMSA-Aca 20008, Type 4030).

Additional material: Litter associated with trees and shrubs from grassland and savanna biomes in central South Africa (habitat types according to Mucina & Rutherford 2006). *Grassland*: Jim Fouche Resort (near Oranjeville) 27°00'S 28°22'E 4.vi.1964 DJK; Zastron 30°15'S 27°01'E 31.1.1977 CME; Welkom 28°04'S 26°27'E 14.ii.1977 CME; Reddersburg 29°39'S 26°11'E 21.ii.1977 CME; Heilbron 27°16'S 28°05'E 18.i.1982 CME; Edenville 27°29'S, 27°42'E 19.i.1982 CME; Kroonstad 27°36'S 27°26'E 19.i.1982 CME; Golden Gate Highlands National Park 28°31'S 28°36'E 25.i.1982 CME; Rhodes 30°45'S 28°01'E 3.xii.1993 JPE. *Savanna*: Vryburg 27°07'S 24°19'E 26.i.1983 CME.

**Remarks**. This species is very similar to *A. floridus* **comb. nov.**, but differs in the notogastral surface having small, round foveae on the entire notogaster and in the length of the lamellar and rostral setae, with the rostral seta about twice the length of *ro* in *A. floridus* **comb. nov.** and the apices of the lamellar seta in *A. amieae* **sp. nov.** touching, whereas they are well separated in *A. floridus*. It also differs in habitat preference as it occurs mainly in the grassland biome of central South Africa.

Etymology. This species is named for my mother, Amie Botha.

*Afroleius inae* sp. nov. (Figs 7, 8, 11)

**Species diagnosis.** Dorsal surface reticulate, inner surface of cavities with fine sculpture; ventral surface with round foveae; surfaces of prodorsum and pteromorph reticulate with some cerotegument; octotaxic system consisting of saccules; bothridial seta clavate, head barbed, stalk thick, directed antero-dorsally; rostral seta of medium length, barbed; lamellar seta subequal in length to *ro*, with coarse surface; postanal porose area present;

large dorsal dens on tarsi I and II; genu I distally with dorsal and ventral cusps antiaxially; genu II distally with lateral cusp.

**Dimensions**. Holotype: (female) length 251, width 170. Paratypes: males (n = 5): length 236 (232–243) width 163 (160–169); females (n = 5): length 257 (250–270), width 179 (170–185).

**Prodorsum** (Figs 7A, C, 8A–C). Rostral margin anteriorly with two small notches, small transparent U-shaped area between notches (similar to *A. floridus* **comb. nov.** Fig.1B); genal notch absent, genal tooth fused to lateral margin of rostrum, represented by small carina; prodorsal surface reticulate with some cerotegument present; lamella wide, extending over lateral margin of prodorsum, long, reaching well over <sup>3</sup>/<sub>4</sub> of prodorsal length (Figs 8A, C); rostral seta of medium length, (~20), coarsely barbed, inserted at anterior apex of tutorium; lamellar seta of medium length (~21), coarse, inserted on lamellar apex; interlamellar seta minute (~4); bothridial opening directed ventro-laterally, with overlapping slit in ventral wall of bothridium; bothridial seta clavate, head barbed, stalk of medium length, thick, directed antero-dorsally; length of bothridial seta from point of emerging from bothridium to tip of seta ~54 (51–57).

**Notogaster** (Figs 7A, C, 8A). Surface of notogaster with reticulation, inner surface of cavities with fine sculpture; octotaxic system consisting of saccules, notogastral setae minute ( $\sim$ 5); orifice of opisthosomal gland very small, situated postero-laterally to *lp*; surface of pteromorph similar to that of notogaster, peripheral area with fine granules.

**Podosoma and gnathosoma** (Figs 7B, C). Surface of mentum anteriorly with faint foveae, posteriorly with shallow, faint grooves; subcapitular seta a of medium length, smooth, m of medium length, finely barbed, h short, smooth; genal notch absent; genal tooth represented by short carina; epimeral setae very short; epimeral setation (epimeres I–IV) 2-1-2-2; epimeral surface laterally with large foveae, medially foveae becoming smaller and fainter.

**Ventral plate** (Fig. 7B). Surface of genital plate smooth; surface of anal plate foveate; ventral plate with large foveae; postanal porose area present, covered by posterior notogastral tectum.

**Legs** (Fig. 8D). Setation of all legs similar to *A. floridus* **comb. nov.**; dorsal integument of tarsi I, II, IV and tibiae I, II, IV thick; femora I–IV, trochanters III–IV antiaxially with fine striae; surfaces of tarsi and tibiae I and II finely granulate; tarsi I and II with large, pointed, distally directed dens; tarsus II short, broad; genua I and II distally with ventral and dorsal cusps antiaxially; femur IV ventrally with wide tectum.

**Material examined** (Fig. 11). Holotype: Database no. 1930.7.1, Ballito 29°31'S, 31°13'E, litter underneath dense indigenous shrubs, 2.ix.1982, leg. C.M. Engelbrecht. Paratypes: from the same sample; 4 males and 4 females deposited in the Acarology Collection of the National Museum (Database no 1930.7.2); 1 male and 1 female deposited in the KwaZulu-Natal Museum (Database no: NMSA-Aca 20009, Type 4031).

Additional material: Litter from forests or wooded patches from various biomes of the eastern and north eastern parts of South Africa (habitat types according to Mucina & Rutherford 2006). *Indian Ocean Coastal Belt*: Ballito 29°31'S 31°13'E 9.ii.1982 CME; Kwambonambi 28°41'S 32°12'E 11.xi.1993 JPE; Leisure Bay 31°00'S 31°14'E 10.ii.1982 CME; Stanger 29°20'S 31°17'E 8.ii.1982 CME. *Coastal forest*: Cape Vidal 28°07'S 32°33'E 9.xi.1993 JPE; Richard's Bay 28°51'S 32°02'E 11.xi.1993 JPE; St Lucia 28°16'S 32°29'E 8.xi.1993 JPE. *Grassland*: Frankfort 27°17'S 28°22'E 18.i.1982 CME; Golden Gate Highlands National Park 28°31'S, 28°36'E 16.x.1985 SvdML; Heilbron 27°19'S 27°58'E 18.i.1982 CME; Lydenburg 25°08'S 30°32'E 5.ii.2013 DdS; Pomeroy 28°47'S 30°24'E 27.i.1982 CME; *Savanna*: Greytown 28°47'S 30°31'E 27.i.1982 CME; Inchanga 29°42'S 30°40'E 30.iii.1982 JP; Makhado 23°02'S 30°03'E 3.viii.1982 CME; Mtubatuba 28°24'S 32°12'E 11.xi.1993 JPE; Thohoyando 23°00'S 31°38'E 3.viii.1982 CME; Vernon Crookes Nature Reserve 30°16'S 3°035'E 15.i.1992 LNL.

**Remarks**. *Afroleius inae* **sp. nov.** is recognized by the long lamellae, rostral and lamellar setae of similar length and unique dorsal sculpturing, being reticulate with fine sculpture within the cavities. The stalk of the bothridial seta is slightly thicker than in the species described above. The absence of the genal incision and the genal tooth being fused to the rostral margin is rare in Ceratozetoidea. This character state is expressed in *Nuhivabates* Niemi & Behan-Pelletier, 2003 (Mycobatidae) as well as in some *Melanozetes* species (Ceratozetidae) (Behan-Pelletier 1986; Niemi & Behan-Pelletier 2003). *Afroleius inae* **sp. nov.** is distributed widely in South Africa, occurring along the east coast region, grasslands of the interior and savanna of the northern regions.

**Etymology**. This species is named for my sister, Ina Engelbrecht, in recognition of her encouragement and provision of material.



FIGURE 7. Afroleius inae sp. nov. A) Dorsal view. B) Ventral view. C) Lateral view. Scale bar 100 µm.





**FIGURE 8.** *Afroleius inae* **sp. nov.** A–B SEM images. C–D Light microscope images. A) Dorsal view. B) Bothridial seta. C) Prodorsum, showing lamellar apices and lamellar setae. D) Leg II, showing thick integument on tarsus and tibia and dorsal dens on tarsus.

#### Afroleius valerieae sp. nov.

(Figs 9, 10, 11)

**Species diagnosis**. Notogastral and epimeral surfaces with small round foveae; surfaces of prodorsum and pteromorph reticulate; octotaxic system consisting of porose areas; opening of bothridium directed ventrally; bothridial seta clavate, head smooth, stalk short, directed dorsally; rostral seta long, thickly barbed; lamellar seta long, minutely barbed, robust, directed medially; notogaster with large depressions antero-medially to *im*; postanal porose area present; dorsal dens on tarsus I conical, dens on tarsus II spur-like; genu I distally with dorsal and ventral cusps antiaxially; genu II distally with lateral cusp.

**Dimensions**. Holotype: (female) length 333, width 218. Paratypes: males (n=18): length 304 (283–321), width 186 (158–203); females (n=16): length 326 (314–337); width 208 (192–216).

**Prodorsum** (Figs 9A, C, 10A, B, D–E). Rostral margin rounded, transparent anterior tegument forming two to three ridges; prodorsal surface reticulate; lamella wide, extending over lateral margin of prodorsum; steep decline of prodorsum anterior to lamellar apices with band of small granules; thick layer of cerotegument sometimes present on prodorsum, forming elongate hexagonal cells, easily detachable (Fig. 10B); rostral seta long (~35), thickly barbed, inserted at anterior apex of tutorium; lamellar seta long (~36), minutely barbed, robust, curved medially, apices touching; interlamellar seta minute (~6); bothridial opening directed ventrally; bothridial seta clavate, head mostly smooth, minutely barbed in some specimens, directed dorsally; length of bothridial seta from point of emerging from bothridium to tip of seta ~53 (51–56).

**Notogaster** (Figs 9A, C, 10A, C). Surface of notogaster with small, round foveae, inner surface of foveae minutely granulate; octotaxic system consisting of small, round porose areas (Fig. 10C); notogastral setae minute ( $\sim$ 6); orifice of opisthosomal gland very small, situated medially to *A1*; large depressions antero-medially to *im* present; surface of pteromorph reticulate.

**Podosoma and gnathosoma** (Figs 9B, C, 10D). Surface of mentum and epimeral region with small round foveae; subcapitular seta *a* short (~7), smooth, *m* slightly longer (~12), finely barbed, *h* short (~5), smooth; genal notch present, genal tooth short, broad; epimeral setae very short; epimeral setation (epimeres I–IV) 2-1-2-2.

**Ventral plate** (Fig. 9B). Surface of genital plate slightly uneven; surface of anal plate foveate; ventral plate with large foveae; postanal porose area small, round, situated more or less at level of border of posterior notogastral tectum.

**Legs** (Fig. 10F). Setation similar to *A. floridus* **comb. nov**.; dorsal integument of tarsi I, II and IV and tibiae I, II and IV thick; large, conical dorsal dens on tarsus I; pointed dens on tarsus II; genu I distally with large ventral and slightly smaller dorsal cusp antiaxially; genu II with large lateral cusp antiaxially; tarsus IV antiaxially with well developed diagonal tectum running from insertion of *ft* towards proximo-lateral base of segment; femur IV ventrally with wide tectum; femora I–IV, trochanters III–IV antiaxially with fine striae.

**Material examined**. Holotype: Database no. 2127.8.1, Makhado 23°02'S, 30°06'E, litter underneath dense shrubs, 3.viii.1982 leg. C.M. Engelbrecht. Paratypes: from the same sample; 16 males and 14 females deposited in the Acarology Collection of the National Museum (Database no 2127.8.2); 2 males and 2 females deposited in the KwaZulu-Natal Museum (Database no: NMSA-Aca 20010, Type 4032).

Additional material: Litter from various biomes of the eastern, northern and central parts of South Africa (habitat types according to Mucina & Rutherford 2006). *Grassland*: Bergville 28°47'S, 29°29'E 26.i.1982 CME; Frankfort 27°17'S, 28°21'E 18.i.1982 CME; Heilbron 27°15'S, 27°56'E 19.i.1982 CME; Lindley 27°52'S, 27°55'E 6.iv.1964 DJK; Middelburg 25°45'S, 29°28'E 30.viii.1982 CME; Parys 26°53'S, 27°26'E 20.x.1981 JJBD; Reitz 27°43'S, 28°13'E 18.i.1982 CME; Sasolburg 26°53'S, 27°46'E 19.i.1982 CME; Settlers 24°51'S, 28°32'E 30.viii.1982 CME; Vrede 27°55'S, 29°09'E 6.iv.1964 DJK; Winterton 28°50'S, 29°35'E 26.i.1982 CME. *Indian Ocean Coastal Belt*: Kingsburgh 30°03'S, 30°52'E 1963 AJE; Mtunzini 28°57'S, 31°45'E 19.iv.1965 GN; Scottburgh 30°16'S, 30°30'E 9.ii.1982 CME; Stanger 29°21'S, 31°16'E 8.ii.1982 CME; *Greytown 28°51'S*, 30°28'E 27.i.1982 CME; Izingolweni 30°46'S, 30°10'E 10.ii.1982 CME; Komatiepoort 25°26'S, 31°56'E 2.ix.1982 CME; Mabula Game Lodge 24°44'S, 27°55'E 9.iii.1983 CME; Mahado 23°02'S, 30°06'E 3.viii.1982 CME; Makhado District 23°02'S, 29°54'E 5.viii.1982 CME; Mbombela 25°28'S, 30°58'E 28.ii.1987 RE; Mokopane 24°10'S, 29°00'E 8.iii.1983 CME; Pietermaritzburg 29°55'S, 30°38'E 9.ii.1982 CME; Punda Maria 22°47'S, 30°54'E 3.viii.1982 CME; Rustenburg 25°42'S, 27°15'E 21.iv.1962 AJE;

Soutpansberg area 23°03'S, 30°03'E 4.viii.1982 CME; Thabazimbi 24°37'S, 27°23'E 9.iii.1983 CME; Thohoyandou 22°58'S, 30°29'E 3.viii.1982 CME; Vaalwater 24°17'S, 28°06'E 8.iii.1983 CME.



FIGURE 9. Afroleius valerieae sp. nov. A) Dorsal view. B) Ventral view. C) Lateral view. Scale bar 100 µm.



**FIGURE 10.** *Afroleius valerieae* **sp. nov.** A SEM image. B–F Light microscope images. A) Dorsal view. B) Prodorsum with cerotegument. C) Notogaster, lateral view, arrow to porose area *Aa*. D) Bothridium and bothridial seta. E) Prodorsum, lateral view. F) Leg II, tarsus and tibia, showing thick integument on tarsus and tibia and conical dens on tarsus (arrow).



FIGURE 11. Distribution of *Afroleius floridus* comb. nov. (circles), *A. amieae* sp. nov. (triangles) and *A. inae* sp. nov. (squares) and *A. valerieae* sp. nov. (crosses) in South Africa.

**Remarks**. The octotaxic system consisting of porose areas and the ventrally directed bothridium of *A*. *valerieae* **sp. nov.** are the most easily recognised characters to distinguish it from other *Afroleius* species. Some specimens display heavily sclerotized borders to all epimeres. Thick cerotegument is also present on the prodorsum and notogaster of some specimens of *Afroleius crassus* Coetzee, 2013. This species is widely distributed in grassland and savanna regions of South Africa, and is mostly absent from forests.

**Etymology**. This species is named for Dr. Valerie Behan-Pelletier in recognition of her outstanding contribution to Oribatology, particularly the Ceratozetoidea.

#### Discussion

Coetzee & Tiedt (2013) presented an analysis of adult characters of *Afroleius* and argued for placement of this genus in Mycobatidae (Ceratozetoidea), based on certain key characters summarized by Norton & Behan-Pelletier (2009), such as the presence of the axillary saccule of the subcapitulum, genal tooth, posterior notogastral tectum, and the large pedotectum I.

The discovery of juveniles of at least one species (*A. floridus* **comb. nov.**) provides further clues to the relationship of the genus. Juveniles are known for certain species of the mycobatid genera *Ceresella* Pavlitshenko, 1993, *Minunthozetes* Hull, 1916, *Mycobates* Hull, 1916, *Punctoribates* Berlese, 1908, and *Zachvatkinibates* Shaldybina, 1973 (Norton & Ermilov 2014). The humeral organ, usually present in immature Ceratozetoidea (Norton *et al.* 1997, Norton & Behan-Pelletier 2009), is absent in all immature instars of *Afroleius*. This structure is also absent in *Punctoribates sellnicki* Willmann, 1928 (Seniczak & Seniczak 2008), and absent in the larva of *Mycobates acuspidatus* Behan-Pelletier *et al.*, 2001, but developed in the nymphal stages. The axillary saccule is absent in juvenile instars of *Afroleius floridus* **comb. nov.** and develops in the adult stase. The presence or absence of the axillary saccule in juveniles had not been recorded for the known juveniles of other mycobatid genera. The pygidial sclerite *PY* is ill-defined, very weakly sclerotized and microtuberculate. This condition is present in *Zachvatkinibates epiphytos* Behan-Pelletier *et al.*, 2001. A transverse fold on the gastronotum (posterior to seta  $c_1$  in *A. floridus* **comb. nov.**) is present, but posterior to seta *da*, in *Z. epiphytos* and species of *Mycobates (M. acuspidatus* Behan-Pelletier *et al.*, 2001, *M. corticeus* Behan-Pelletier *et al.*, 2001 and *M. parmeliae* (Michael, 1884) (Behan-Pelletier *et al.*, 2001).

Subías, in the 2014 electronic update of his oribatid mite catalog (Subías 2004, 2014), includes *Afroleius* in the family Humerobatidae, but without any explanation. This placement is rejected because of the absence of microsclerites on the gastronotum of juveniles of *Afroleius*. The presence of microsclerites as well as macrosclerites on the gastronotum of immatures of *Humerobates rostrolamellatus* Grandjean, 1936 prompted Grandjean (1970) to institute the family Humerobatidae to accommodate this genus. Species in Humerobatidae are associated with living plants and are rarely found in litter (Norton & Behan-Pelletier 2009).

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## Abstract

A revised diagnosis and key to the species of *Afroleius* Mahunka, 1984 is presented. Adults of *Kilimabates polygonatus* Mahunka, 1985 are redescribed, juvenile instars are described and the species is recombined as *Afroleius polygonatus* (Mahunka). The morphology of *Afroleius undulatus* (Balogh, 1959) is discussed. A new species *Afroleius lucidus* **sp.nov.** is described and a key is given for all species of *Afroleius*.

Key to the species of *Afroleius* Mahunka, 1984 (Acari, Oribatida, Punctoribatidae), recombination of *A. polygonatus* (Mahunka, 1985), description of *A. lucidus* sp. nov. and discussion of *A. undulatus* (Balogh, 1959)

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#### Abstract

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Key words: Taxonomy, new species, new combinations, juvenile instars, key, South Africa.

#### Introduction

The genus *Afroleius* Mahunka, 1984a was described from South Africa and included three species (*A. deformis* Mahunka, 1984 (type), *A. minor* Mahunka, 1984 and *A. simplex* Mahunka, 1984). Subsequently eight new species have been added to this genus, all from South Africa (Coetzee 2013, 2014a, 2014b). Subías (2014), in his on-line catalogue, recombined *Africoribates undulatus* Balogh, 1959 in *Afroleius*, a recombination with which I

concur, and Coetzee (2014b) recombined *Magyaria florida* Mahunka, 1985 in this genus. Herein another species, *Kilimabates polygonatus* Mahunka, 1985 is recombined in *Afroleius*. It is redescribed based on type material and additional material from the collection of the National Museum, Bloemfontein. Juvenile instars are also described. A new species, *A. lucidus* **sp. nov.** is described. Coetzee and Tiedt (2013) presented an overview and diagnosis of the genus. They also discussed its systematic relationship and presented arguments for its placement in the family Punctoribatidae. Grandjean (1954) instituted the family Mycobatidae in his important paper on natural classification of Oribatida. He included four genera in this family namely *Mycobates* Hull, *Calyptozetes* Thor (now a subgenus of *Mycobates*), *Minunthozetes* Hull and *Punctoribates* Berlese. However, the name Punctoribatidae has priority as Thor (1937) had already proposed this family with *Punctoribates* as type genus. A number of character states (presence of epimeral seta *1c*, exobothridial seta and proximoventral porose areas on tarsi I–IV) are discussed further and a key to the species is presented.

All species of *Afroleius*, with the exception of *A. undulatus* (Balogh, 1959) from Tanzania, are so far known only from South Africa. Because of the absence of data on the oribatid fauna in neighbouring countries (Mozambique, Zimbabwe, Botswana and Namibia), little can be said about the distribution of *Afroleius* in the rest of southern Africa.

## Material and methods

All material was collected in South Africa. Fauna was extracted with Berlese-Tullgren funnels from soil and litter samples. Collection data are given as follows: Locality (nearest town), latitude, longitude, date collected, collector. Habitat is grouped into vegetation biomes of South Africa according to Mucina and Rutherford (2006). Collectors are indicated by their initials (LC—Louise Coetzee; EdP—E. du Pisani; RE— R. Earlé; JPE—J.P. Eksteen; AJE— A.J. Els; CME—C.M. Engelbrecht; DJK—D.J. Kok; LNL—L.N. Lotz; SvdML—S. vdM. Louw; CMO—C.M. Oosthuizen; JOP—J.O. Pretorius; BSR—B.S. Rubidge; RvP—R. van Pletzen; AvR—Annemie van Rensburg; JW—J. Watson.

Specimens were temporarily mounted on cavity slides with glycerol or lactic acid for study purposes and thereafter stored in 70% alcohol. Measurements were taken from rostral tip to posterior margin of notogaster as seen in dorsal view (length) and across the notogaster posterior to the pteromorph (width). All measurements are presented in µm, with the range (minimum and maximum) in brackets where applicable.

A Nikon Eclipse 50i light microscope equipped with a DS-Fi1 digital camera and NIS-Elements imaging software were used for light microscope images. The following procedure was followed for Scanning Electron Microscope micrographs: Samples were fixed in 70% ethanol for a minimum of 24 hours, dehydrated in a graded ethanol series of 80%, 90% and 2X 100% for 30 minutes each. The samples were critical point dried in liquid carbon dioxide, mounted with double sided carbon tape on SEM-stubs and coated with 15 nm gold/palladium in a sputter coater, and viewed under a FEI Quanta 250 FEG SEM at 5 kV under high vacuum mode. (Images by Dr. Louwrens Tiedt, North West University, South Africa).

Revised genus diagnosis

Afroleius Mahunka, 1984

## Integument

Darkly sclerotized, dorsally and ventrally foveate, polygonate or reticulate with the exception of the lateral propodosoma and genital plates (with some exceptions); epimeral region microtuberculate, with or without foveae.

#### Size

Medium-sized, ranging between 250-420  $\mu m$ .

#### Dorsal side

Lamella (*L*) marginal; very short lamellar cusp sometimes present; rostral seta (*ro*) short or of medium length; lamellar seta (*le*) of medium length to long; interlamellar seta (*in*) minute; bothridium cup-shaped with lateral / ventral slit of which the lobes may or may not overlap; bothridial seta (*bo*) clavate; exobothridial seta (*ex*) mostly very short, sometimes long, curved; very narrow anterior notogastral tectum present, no medial process; pteromorph fully hinged; octotaxic system consisting of four pairs of saccules or porose areas; ten pairs of notogastral setae present, all setae minute; lyrifissures of medium length, lyrifissure *ia* situated on ventral surface of pteromorph, lyrifissures *im*, *ih*, *ips* and *ip* in usual positions; undivided posterior notogastral tectum present.

#### Ventral side

Genal notch and broad genal tooth (*gn*) present, genal tooth sometimes secondarily fused to genal border; axillary saccule of subcapitulum (*ax*) present; subcapitulum without mental tectum; epimeral surface microtuberculate, sometimes with foveae; apodemes II, *sj* and III short, parallel; number of setae on epimeres from anterior to posterior: 3-1-2-2; custodium absent; discidium large, triangular; epimeral border 4 darkly sclerotized; ventral plate foveate; genital plates with six pairs of setae, integument microtuberculate, sometimes with faint striae or foveae; one pair of aggenital setae present (except *A. deformis* Mahunka, 1984, aggenital seta absent); anal plates foveate with two pairs of setae; lyrifissure *iad* anterolaterally of anal plates; three pairs of adanal setae present, inserted close to each other near posterolateral border of anal plates (except *A. deformis* and *A. deformatoides* Coetzee, 2013); pre-anal organ with narrow stem, broadening slightly towards base; post anal porose area present or absent, when present small, round.

#### Lateral view

Tutorium (*tu*) consisting of ridge, anteroventrally with granular area; pedotectum I broad, covering acetabulum I, dorsally reaching exobothridial seta; pteromorph large, distal edge rounded, with complete hinge.

## Legs

All legs heterotridactylous; dorsal integument of tibiae and tarsi of legs I, II and IV thickened; tarsi I and II usually with dorsal dens (varying from small point to large well-developed spur, occasionally absent) proximal to tectal setae; genu I with dorsal and ventral cusps antiaxially; genu II distally with lateral cusp; tarsus IV proximally with tectum running dorsally from seta *ft*" to proximoventral end of tarsus (Fig. 2C); femur IV with ventral projection curving towards antiaxial side; femora I-IV and trochanters III-IV with porose areas; tarsi I–IV proximoventrally with porose areas; femora I–IV and trochanters III–IV antiaxially with fine striae.

#### Juvenile instars

Apheredermous; aspis posteriorly with area of porosity; humeral organ absent; axillary saccule absent; *ro* of medium length; *le* and *in* long, robust, inserted on tubercles; hysterosomal macrosclerite very weakly sclerotized, ill-defined, covering dorsal side of hysterosoma; lateral sclerites absent; gastronotal setation unideficient (larva with 12 pairs of setae; nymphs with 15 pairs of setae); in larva setae  $c_1$ ,  $c_2$ , da, dm, dp, la, Im, Ip and  $h_1$  long, robust, inserted on tubercles, setae  $c_3$ ,  $h_2$ ,  $h_3$  of different shape, much shorter, finer, not inserted on tubercles; in nymphs setae  $c_1$ ,  $c_2$ , da, dm, dp, la, Im, lp,  $h_3$ ,  $p_1$  long, robust, inserted on tubercles; setae  $c_3$ ,  $p_2$ ,  $p_3$  of different shape, much shorter, finer, not inserted on tubercles, setae  $c_3$ ,  $p_2$ ,  $p_3$  of different shape, much shorter, finer, not inserted on tubercles; tibia I of all instars with large anterodorsal apophysis, bearing solenidion  $\varphi_1$ .

## Afroleius polygonatus (Mahunka, 1985) com. nov.

Kilimabates polygonatus Mahunka, 1985

Mahunka, S. 1985. Oribatids from Africa (Acari: Oribatida) II. *Folia Entomologica Hungarica* 46(1): 73–113, page 102, figures 72–77.

Type locality: Western Cape, Hawequas, 33°34'S, 19°08'E.

#### Description based on type material and additional South African specimens

(Figures 1–2, 5)

## **Species diagnosis**

Integument reticulate/polygonate, darkly sclerotized; octotaxic system consisting of porose areas; *ro* of medium length, thickly barbed; *le* seta long; roughened; *bo* clavate, head short, barbed, stalk long, thin; genal tooth wide; notogastral setae minute; notogaster without depressions; post anal porose area absent; epimeral surface centrally with fine striae; genital plates smooth; anal plates reticulate; dorsal dens on tarsi and tibiae I–II absent.

## Dimensions

Holotype (TM 14877) (female) length 329, width 255; paratypes (TM 14897) males (n=8): length 303 (range 297–314), width 229 (range 220–238); females (n=8) length 324 (range 313–336), width 243 (range 228–255).

Additional material: Males (n=6): length 309 (range 301–319), width 233 (range 218–241); females (n=6) 323 (range 310–329), width 253 (range 243–266).

## Prodorsum (Figures 1A–C, 2A)

Rostral border anteriorly with shallow indentation (visible in ventral view); prodorsal surface reticulate, darkly sclerotized; lamella wide extending over lateral prodorsal margin; prodorsal profile with steep slope at level of apex of lamella (see Remarks of *A. undulatus*); *ro* of

medium length, thickly barbed (~33), *le* long, roughened (~49), *in* minute (~9); bothridium directed laterally, ventral slit with overlapping lobes; *bo* clavate, head short, barbed, stalk long, thin (~45 from where *bo* emerges from bothridium to tip of seta), directed dorsolaterally (Fig. 2A).

## Notogaster (Figures 1A, C, 2A–B)

Surface of notogaster polygonate, darkly sclerotized; octotaxic system consisting of porose areas; orifice of opisthosomal gland (*gla*) small, gland large (Fig. 2B); notogastral setae very short (~9); notogaster without depressions; surface of pteromorph reticulate.

## Podosoma and gnathosoma (Figures 1B–C, 2E)

Surface of mentum finely foveate; subcapitular setae *a*, *h* short, smooth, *m* longer, finely barbed; *gn* wide (Fig. 2E); *ex* long, curved (~35); epimeral setae short; epimeral surfaces centrally with fine striae.

## Ventral plate (Figure 1B)

Surface of genital plate smooth; anal plate reticulate; ventral plate polygonate; post anal porose area absent.

## Legs

Setal formula (trochanter to tarsus, famulus included): Leg I 1–5–3–4–20; Leg II 1–5–3–4– 15; Leg III 2–2–1–3–15; Leg IV 1–2–2–3–12. **Solenidial formula** (genu to tarsus): Leg I 1– 2–2; Leg II 1–1–2; Leg III 1–1–0; Leg IV 0–1–0.

Tarsi I and II without dorsal dens; tarsi and tibiae I, II and IV with thick integument, proximoventral porose areas on tarsi I to IV not clearly defined.

#### Juvenile instars (Figures 2D)

Measurements (length): Larva (n=1)158, protonymph (n=2) 197, deutonymph (n=1) 238, tritonymph (n=1) 343.

**Larva**: Humeral organ absent; axillary saccule absent; undefined area of porosity between insertion of *in*, *bo* and posterior prodorsal border; *ro* of medium length, barbed; *le*, *in* long, robust, with long, widely spaced branches; setae inserted on robust tubercles (Fig. 2D); *bo* long, narrowly clavate, sparsely barbed; hysterosomal macrosclerite punctate, very weakly sclerotized, ill-defined, covering dorsal side of hysterosoma; lateral sclerites absent; 12 pairs of gastronotic setae present; setae  $c_1$ ,  $c_2$ , da, dm, dp, la, lm, lp,  $h_1$ , similar to *le* and *in*, setae  $c_3$ ,  $h_2$ ,  $h_3$ , very short, straight, smooth, not inserted on macrosclerite, not inserted on tubercles; Claparède's organ large, round; all ventral setae very short, straight, smooth. Tibia I with large anterodorsal apophysis, bearing solenidion  $\varphi_1$ .

**Nymphal instars**: Setae and appearance of all nymphal instars similar, differing only proportionally in size; humeral organ absent; axillary saccule absent; undefined area of porosity between insertion of *in*, *bo* and posterior prodorsal border; *ex* very short (~6); *ro* of medium length, barbed; *le*, *in* long, robust, with long, widely spaced branches (similar to those of larva), setae inserted on robust tubercles; *bo* long, narrowly clavate, sparsely barbed; hysterosomal macrosclerite very weakly sclerotized, ill-defined, covering dorsal side of hysterosoma, integument punctate; lateral sclerites absent; 15 pairs of gastronotic setae present; setae  $c_1$ ,  $c_2$ , da, dm, dp, la, lm, lp,  $h_1$ ,  $h_2$ ,  $h_3$ ,  $p_1$  similar to *le* and *in*, setae  $c_3$ ,  $p_2$ ,  $p_3$ very short (~6), straight, smooth, not inserted on macrosclerite, not inserted on tubercles. All ventral setae very short, straight, smooth. See Table 1 for development of gastronotal, coxisternal, genital, anal and adanal setae. Tibia I of all instars with large anterodorsal apophysis, bearing solenidion  $\varphi_1$ . See Table 2 for development of leg setation.

#### Material examined (Figure 5)

Holotype (TM 14877) and paratypes (TM 14897) on loan from Ditsong National Museum of Natural History (formerly Transvaal Museum). Collection data: Rep. of South Africa, Western Cape, Hawequas, 33°34'S, 19°08'E, 5.xi.1973, sifted humus, leg. Dr. S. Endrődy-Younga.

Additional material: *Fynbos*: Van Stadens Pass (near Port Elizabeth) 33°55'S, 25°11'E 24.i.1963 RvP; Cango Caves (near Oudtshoorn) 33°23'S, 22°12'E 30.i.1963 RvP. *Coastal vegetation and Fynbos*: Steenbras River Mouth 34°11'S, 18°49'E 3.iv.1963 RvP; Kogel Bay 34°14'S, 18°51'E 3.iv.1963 RvP; Mossel Bay 34°11'S, 22°08'E 9.iv.1963 RvP; Gansbaai 34°34'S, 19°21'E 17.xi.1982 CME; Kogel Bay, WC 34°14'S, 18°51'E 4.v.1983 CME; Gordon's Bay 34°09'S, 18°52'E 4.v.1983 CME; Kogel Bay 34°14'S, 18°51'E 4.v.1983 BSR; Kogel Bay 34°14'S, 18°51'E 4.v.1983 RE; Schoenmakerskop (near Port Elizabeth) 34°02'S, 25°35'E 14.iii.2014 LC. *Savanna*: Grahamstown, Seven Fountains 33°18'S, 26°34'E 15.iv.1963 CMO.

#### Remarks

Mahunka (1985) placed this species in *Kilimabates* Mahunka, 1984b (with a question mark) and remarked that the placement of the species is problematic. He concluded that the "reduced custodium, the wide genal teeth and shape of the tectum" indicate a relationship with *Kilimabates. Kilimabates* was proposed by Mahunka (1984b) for the species *K. processus* Mahunka, 1984 collected at Mweka, a base camp in the montane forest belt on Mt. Kilimanjaro, Tanzania. Mahunka (1984b) regarded *Kilimabates* as a member of the family Ceratozetidae but that the relationship to other genera is uncertain. Balogh and Balogh (1992) synonymized *Kilimabates* with *Africoribates* Evans (together with *Anellozetes* Hammer and *Baloghobates* Hammer) in the family Ceratozetidae. Subías (2004) synonymized *Kilimabates* with *Anellozetes* in the family Humerobatidae, while he recognized *Africoribates* Sellnick. *Afroleius polygonatus* **com. nov.** has a posterior notogastral tectum and thus cannot be included in either Ceratozetidae or Humerobatidae. Also, the nature of the macrosclerite in juveniles (lacking porose regions) precludes placement in Humerobatidae.

The absence of the custodium and wide genal tooth are both character states found in *Afroleius* (Coetzee and Tiedt 2013). The third character listed by Mahunka (1985) that prompted him to place the species in *Kilimabates*, is the shape of the "tectum". The tectum (i.e. tutorium) differs significantly between *Africoribates* (lamelliform) and *Afroleius* (a ridge). A reexamination of the type material of *A. polygonatus* shows the shape of the tutorium to be similar to that of *Afroleius*.

The type locality, Hawequas, is a mountain near Paarl in the Western Cape, part of which falls in the Hawequas (also known as Limietberg) Nature Reserve, a protected area in the Cape Floral Region. Vegetation is dominated by scrub, asteraceous and proteoid fynbos (SANBI 2011). The additional material had been collected from the Fynbos Region, mainly from coastal vegetation, and also from a small patch of savanna in the Eastern Cape.

#### Afroleius lucidus sp .nov.

(Figures 3–5)

#### Species diagnosis

Integument of prodorsum and pteromorph reticulate, notogaster and ventral plate foveate, notogastral foveae shallow with deep central pit; fine granular cerotegument sometimes present; octotaxic system consisting of porose areas; *bo* clavate, finely barbed, stalk short, directed laterally; *ro* robust, densely barbed, *le* slightly thinner, longer, roughened; genal tooth fused to genal border; post anal porose area present; dorsal dens on tarsi and tibiae I–II absent; proximoventral porose areas on tarsi I–IV clearly evident.

#### Dimensions

Holotype: (female) length 377, width 275. Paratypes: Males (n=10): length 358 (range 338– 377), width 247 (range 236–265); females (n=10) length 377 (range 362–401), width 261 (range 244–284).

## **Prodorsum** (Figures 3A–C, 4A, C, E)

Rostral border anteriorly with shallow indentation (visible in ventral view); prodorsal surface reticulate; lamella wide, extending over lateral prodorsal margin, laterally microtuberculate; steep slope in profile at level of apex of lamella; *ro* robust, densely barbed, of medium length (~35), inserted at apex of tutorium; small dens ventral to *ro* insertion; *le* slightly thinner and longer than rostral seta, roughened (~44); *in* very short (~15); bothridium directed laterally, ventrally with overlapping slit (Fig. 4C); *bo* clavate, finely barbed, stalk short (~39 from where *bo* emerges from bothridium to tip of seta), directed laterally.

## Notogaster (Figures 3A, C, 4A–C, F)

Surface of notogaster foveate, sometimes with granular cerotegument; foveae shallow with deep central pit (Fig. 4B without cerotegument, Fig. 4C with cerotegument); thin integument below central pits causes foveae to appear bright, shiny, in transmitted light; notogaster laterally depressed along margin, rendering central elevation (Fig. 4A); octotaxic system consisting of porose areas (Fig. 4F); notogastral setae very short (~14); orifice of opisthosomal gland (*gla*) small.

#### Podosoma and gnathosoma (Figures 3B–C, 4C)

Surface of mentum faintly foveate; epimeral region microtuberculate with small, faint foveae; subcapitular setae short; genal tooth fused to genal border, represented by carina; *tu* proximodorsally with short grooves (Fig. 4C, arrow); epimeral setae short; surface of pedotectum I reticulate.

## Ventral plate (Figures 3B, 4D)

Surface of genital plate foveate (Fig. 4D); anal plate foveate; ventral plate with large foveae; post anal porose area (*ap*) present.

#### Legs

**Setal formula** (trochanter to tarsus, famulus included): Leg I 1–5–3–3–20; Leg II 1–5–3–4– 15; Leg III 2–3–1–3–15; Leg IV 1–2–2–3–12. **Solenidial formula** (genu to tarsus): Leg I 1– 2–2; Leg II 1–1–2; Leg III 1–1–0; Leg IV 0–1–0.

Genu I distally with small dorsal and ventral cusps antiaxially; genu II distally with large lateral cusp; tarsi I and II without dorsal dens; proximoventral porose areas on tarsi I to IV large, clearly evident; antiaxial lateral seta *I*' present on femur III; antiaxial lateral seta *I*'' absent from tibia I.

## Material examined (Figure 5)

Holotype: Database no. 2088.6.1, Cradock, Eastern Cape Province, 32°11'S, 25°37'E, litter underneath tree in grassland biome; 26.v.1982, C.M. Engelbrecht. Paratypes from the same sample; 8 males, 8 females deposited in the Acarology Collection of the National Museum (Database no. 2088.6.2); 2 males, 2 females deposited in the KwaZulu-Natal Museum, Pietermaritzburg, South Africa.

Additional material: *Fynbos*: Albertinia 34°12'S, 21°34'E 9.iv.1963 RvP; Barrydale 33°54'S, 20°42'E 17.ii.1982 CME; Calitzdorp 33°32'S, 21°41'E 18.xi.1982 CME; Cango Caves (near Oudtshoorn) 33°23'S, 22°12'E 30.1.1963 RvP; Ceres 33°22'S, 19°18'E 16.xi.1982 CME; Darling 33°23'S, 18°22'E 15.x.1987 LNL; De Rust 33°29'S, 22°31'E 18.xi.1982 CME; George (Outeniqua Pass) 33°53'S, 22°24'E 4.ix.1963 RvP; Ladismith 33°29'S, 21°16'E 18.xi.1982 CME; Montagu 33°46'S, 20°06'E 17.xi.1982 CME; Napier 34°28'S, 19°53'E 17.xi.1982 CME; Oudtshoorn 33°36'S, 22°12'E 18.xi.1982 CME; Swellendam 34°02'S, 20°25'E 9.iv.1963 RvP. *Fynbos (Coastal vegetation)*: Agulhas 34°49'S, 20°00'E 17.xi.1982 CME; Betty's Bay, 34°20'S, 18°55'E 17.xi.1982 CME; Gansbaai 34°35'S, 19°21'E 17.xi.1982 CME; Gordon's Bay 34°10'S, 18°52'E 7.iv.1987 RE; Kleinmond 34°19'S, 19°00'E 17.xi.1982 CME; Kogel Bay 34°14'S, 18°51'E 3.iv.1963 RvP; Mossel Bay 34°11'S, 22°08'E 3.iv.1963 RvP; Port Elizabeth 33°47'S, 25°40' 23.i.1963 RvP; Simon's Town 34°11'S, 18°26'E 31.x.1986 RE;

Stilbaai 34°22'S, 21°24'E 18.xi.1982 CME; Stormsvlei 34°04'S, 20°05'E 17.xi.1982 CME; Thornhill 33°55'S, 25°03'E 24.i.1963 RvP. Grassland: Jim Fouche Resort (near Oranjeville) 27°00'S, 28°22'E 6.iv.1964 DJK; Arlington 28°01S, 27°51'E 6.iv.1964 DJK; Bethlehem 28°14'S, 28°17'E 2.ii.1963 AJE; Bloemfontein 29°06'S, 26°12'E 11.xi.1962 AJE; Brandfort 28°42'S, 26°27'E 5.iv.1963 AJE; Bultfontein 28°17'S, 26°08'E 10.iii.1983 CME; Cradock 32°11'S, 25°37'E 5.ii.1986 AvR; Cradock 32°11'S, 25°37'E 26.v.1982 CME; Excelsior 28°55'S, 27°03'E 18.i.1982 CME; Ficksburg 28°50'S, 27°52'E 24.i.1977 CME; Florisbad 28°46'S, 26°05'E 18.ix.1981 JOP; Fouriesburg 28°37'S, 28°12'E 17.xii.1963 RvP; Glenco 28°11'S, 30°08'E 27.i.1982 CME; Golden Gate Highlands National Park 28°31'S, 28°36'E 12.xii.1963 RvP; Graaf-Reinet 32°14'S, 24°31'E 19.xi.1982 CME; Jamestown 31°00'S, 26°49'E 24.v.1982 CME; Kestell 28°19'S, 28°42'E 2.ii.1963 AJE; Lady Grey 30°42'S, 27°14'E 8.xi.1984 JPE; Lindley 27°19'S, 27°58'E 6.iv.1964 DJK; Lindley 27°19'S, 27°58'E 18.i.1982 CME; Marquard 28°40'S, 27°25'E 24.i.1977 CME; Middelburg 31°31'S, 25°00'E 19.xi.1982 CME; Olifantskop Pass (near Paterson) 33°19'S, 25°56' 26.v.1982 RvP; Petrus Steyn 27°39'S, 28°08'E 18.i.1982 CME; Queenstown 31°54'S, 26°52'E 24.v.1982 CME; Reitz 27°43'S, 28°13'E 6.iv.1964 DJK; Rosendal 28°30'S, 27°55'E 24.i.1977 CME; Rouxville 30°25'S, 26°50'E 8.iv.1964 RvP; Sasolburg 26°52'S, 27°53'E 19.i.1982 CME; Smithfield 30°13'S, 26°33'E 21.ii.1977 CME; Soutpan 28°45'S, 26°04'E 19.i.1977 CME; Sterkspruit 30°31'S, 27°22'E 9.ix.1977 CME; Virginia 28°06'S, 26°51'E 14.ii.1977 CME; Wepener 29°44'S, 27°02'E 31.i.1977 CME; Willem Pretorius Nature Reserve (near Winburg) 28°18'S, 27°13'E 18.i.1982 CME; Winburg 28°32'S, 27°00'E 30.xi.1984 CME; Zastron 30°17'S, 27°03'E 31.i.1977 CME. Nama-Karoo Beaufort West 32°24'S, 22°28'E 16.xi.1982 CME; Colesberg 30°44'S, 25°06'E 15.xi.1982 CME; Noupoort 31°11'S, 24°57'E 19.xi.1982 CME; Oppermansgronde 29°25'S, 24°44'E 29.x.1982 EdP; Petrusburg 29°06'S, 25°24'E 29.i.1985 CME; Richmond 31°25'S, 23°56'E 15.xi.1982 CME; Van der Kloof Dam (near Petrusville) 29°59'S, 24°43'E 7.iv.1982 DJK. Savanna: Cookhouse 33°23'S, 25°41'E 26.v.1982 CME; Griekwastad 28°51'S, 23°15'E 25.x.1982 CME; Inchanga 29°42'S, 30°40'E 30.iii.1982 JW; King Williamstown 32°53'S, 27°23'E 31.v.1962 AJE; Koopmansfontein 28°16'S, 24°01'E 24.1.1983 CME; Kuruman 27°29'S, 23°25'E 26.i.1983 CME; Mafikeng 25°52'S, 25°38'E 27.1.1983 CME; Reivilo 27°32'S, 24°10'E 28.i.1983 CME; Rustenburg 25°42'S, 27°15'E 6.i.1963 AJE; Whittlesea 32°29S, 26°46'E 24.v.1982 CME. Succulent Karoo Garies 30°33'S, 17°59'E 18.ix.1982 SvdML; Graskom 30°18'S, 17°22'E 18.ix.1982 SvdML; Port Nolloth 29°14'S, 16°54'E 13.ix.1982 SvdML; Vanrhynsdorp 31°37'S, 18°44'E 20.ix.1982 SvdML.

#### Remarks

The pitted notogastral foveae separate this species from all other *Afroleius* species. The presence of granular cerotegument on some specimens may obscure the appearance of the unique, shiny foveae of the notogaster. It also differs on the setal counts for Fe III and Ti I (see above). In *A. lucidus* **sp. nov.** the antiaxial lateral seta *I*' on Fe III is present (absent from other South African species of *Afroleius*) and the antiaxial lateral seta *I*" on Ti I is absent

(present in other South African species of *Afroleius*). Formulae of leg setae for *A. undulatus* are unknown.

*Afroleius lucidus* **sp. nov.** is widely distributed in South Africa, mainly in the grassland and savanna biomes. It is an ecologically tolerant species as it occurs in fynbos, coastal vegetation, grassland, savanna and also in the much drier Nama-Karoo (western and central South Africa) and Succulent Karoo (in the far west) biomes where it is the only representative of *Afroleius* recorded so far. It is absent from the far eastern regions and forests.

## Etymology

The species name *lucidus* refers to the bright appearance of the central pits of the notogastral foveae in transmitted light, caused by the thin integument below the pits.

## Afroleius undulatus (Balogh, 1959)

Africoribates undulatus Balogh, 1959

Balogh, J. 1959. Some oribatid mites from Eastern Africa (Acari: Oribatidae). *Acta Zoologica Hungaricae* 5(1-2): 13–32, page 16, figures 22–26. *Afroleius undulatus* (Balogh, 1959) Subías 2014 p. 368.

Type locality: Kibosha [sic] /Kibosho, Tanzania

## Remarks

This species was described by Balogh (1959) from material collected early in the 20th century in Tanzania, eastern Africa, by K. Kittenberger. The material was derived from Kibosha [sic], but no indication of habitat nor number of specimens examined was given. Kibosho is a settlement located on the southern slopes of Mt. Kilimanjaro, just south of the

equator (3°15'S, 37°19'E, 1430 m a.s.l.). It lies in the bushland/savanna zone, bordering the montane forest belt that encircles the mountain (Dalao 2011).

Balogh's description (1959), in Latin, includes five figures (dorsal, ventral and lateral views, sensillus (i.e. bothridium and bothridial seta) and genu, tibia and tarsus of leg I. The type material is housed in the Hungarian Natural History Museum, Budapest. Current local logistical problems prevented a paratype being sent to South Africa for investigation. However, information obtained from the Hungarian Natural History Museum (Dr. Jan Mourek pers. comm.) indicates that key features such as the shape of the tutorium, absence of the custodium and absence of the translamella are in accordance with *Afroleius*.

Balogh (1959) described the translamella as "optime expressa" but a steep slope of the prodorsum at the level of the lamellar apices in several *Afroleius* species gives the impression of a translamella when viewed dorsally in transmitted light. Behan-Pelletier (1984) described a similar "appearance of a translamella" due to the abrupt change in slope of the prodorsum in *Ceratozetes pacificus* Behan-Pelletier and also in *C. cuspidatus* Jacot (Behan-Pelletier 1984; Behan-Pelletier & Eamer 2009). The paratype of *A. undulatus* also displays such a steep slope.

## Discussion

In previous work on *Afroleius* epimeral seta *1c* had been reported as absent (Coetzee & Tiedt 2013, Coetzee 2013, Coetzee 2014a, Coetzee 2014b). However, a reexamination of all the species described so far shows that *1c* is present. The position of the seta on pedotectum I, together with the mostly rough, foveate surface and very small size of seta *1c* rendered it easily overlooked.

The exobothridial seta (*ex*) is obscured by the pteromorph, pedotectum I and femur I. In five species (*A. polygonatus* (Mahunka, 1985) **com. nov.,** *A. floridus* (Mahunka, 1985), *A. decurvatus* Coetzee, 2013, *A. amieae* Coetzee, 2014b and *A. valerieae* Coetzee, 2014b) *ex* is long and curved, while in the rest of the species it is minute. *Ex* is situated near the dorsal end of the base of pedotectum I. It is visible in dissected specimens.

Porose areas on the proximoventral surfaces of tarsi and tibiae I–IV are usually present in adults of Oripodoidea but are less common in Ceratozetoidea (Behan-Pelletier 1986; Norton *et al.* 1997). These porose areas are referred to by Norton *et al.* (1997) as the tibio-tarsal system. In *Afroleius* the tibio-tarsal system is expressed partially, as the porose areas are present only on the tarsi. Its presence on tarsi and tibiae I–IV in Ceratozetidae species had been recorded in *Diapterobates humeralis* Hermann, *Melanozetes meridianus* Sellnick and *Neogymnobates marilynae* Behan-Pelletier, on tarsi I–IV in *Trichoribates striatus* Hammer and on tarsus and tibia I in *lugoribates gracilis* Sellnick (Behan-Pelletier 1986; Behan-Pelletier 2000). In Humerobatidae they are present on tarsi I–IV of adults and tritonymphs of *Humerobates setosus* Behan-Pelletier and Mahunka (Behan-Pelletier & Mahunka 1993). Behan-Pelletier (1986) hypothesized that their widespread occurence in Oripodoidea suggest a plesiomorphic character state in Ceratozetoidea with subsequent loss in most species. In a reexamination of *Afroleius* species these porose areas were found to be present on tarsi I–IV of all species and absent on tibiae.

Fine striae on femora I–IV and trochanters III–IV (present on antiaxial surfaces of femora I– IV and trochanters III–IV of all species of *Afroleius*) is widely found in most families of Ceratozetoidea, particularly in Punctoribatidae. This feature had been reported by Behan-Pelletier (1984, 1985, 1986, 1988, 1994, 1996, 1998, 2000), Behan-Pelletier and Eamer (2003, 2005, 2008, 2009), Behan-Pelletier, Eamer and Clayton (2001) and Niemi and Behan-Pelletier (2004) for almost all Ceratozetoidea of North America.

## Key to the species of *Afroleius* Mahunka, 1984

1.	Ventral profile convex (see Mahunka 1984a, Fig. 132; Coetzee 2013, Fig. 16)2
-	Ventral profile straight3
2.	Medial part of notogaster foveate, octotaxic system consisting of porose areas, apices
	of lamellar setae reaching rostral apex, aggenital seta present
	A. deformatoides Coetzee, 2013
-	Medial part of notogaster smooth, octotaxic system consisting of saccules, apices of
	lamellar setae not reaching rostral apex, aggenital seta absent
	A. deformis Mahunka, 1984
3.	Octotaxic system consisting of porose areas4
-	Octotaxic system consisting of saccules8
4.	Posterior margin of notogaster with caudal protuberance (males and females), lamellar
	seta very long, ribbon-shaped
-	Posterior margin of notogaster slightly undulate or smooth, lamellar seta setiform 5
5.	Dorsal dens on tarsus I and tibia I conical, rostral and lamellar setae almost equal in
	length, bothridium directed ventrally A. valerieae Coetzee, 2014
-	Dorsal dens on tarsus I and tibia I absent, rostral seta slightly shorter than lamellar
	seta, bothridium directed laterally6
6.	Notogaster without lateral depressions, genal tooth wide, post anal porose area absent
	<i>A. polygonatus</i> (Mahunka, 1985)
-	Notogaster with lateral depressions, genal tooth fused, post anal porose area present
	7
7.	Notogastral foveae with deep central pit, genital plates foveate, bothridial seta directed
	laterally
-	Notogastral foveae without central pit, genital plates smooth, bothridial seta directed
	anteromedially
8.	Saccules tubular, bothridial seta long, thin, decurvedA. decurvatus Coetzee, 2013
-	Saccules round, bothridial seta not decurved9

9.	Post anal porose area absent, rostral seta short, thick, anterior notogastral border
	straight A. natalensis Coetzee, 2014
-	Post anal porose area present, rostral seta short to long, slender, anterior notogastral
	border arched 10
10.	Lamellae long, reaching ¾ of prodorsal length11
-	Lamellae of medium length, not reaching <sup>3</sup> / <sub>4</sub> of prodorsal length
11.	Notogaster without depressions or ridges, bothridial stalk robust, genital plates smooth
-	Notogaster with depressions or ridges, bothridial stalk slender, genital plates striate or
	punctate
12.	Notogaster with depressions, genital plates striate, bothridial stalk long, rostral and
	lamellar setae equal in length A. minor Mahunka, 1984
-	Notogaster with marginal ridges, genital plates punctate, bothridial stalk short, rostral
	seta much shorter than lamellar seta A. crassus Coetzee, 2013
13.	Bothridial seta directed posterolaterally, genal tooth fused, genital plates faintly striate
-	Bothridial seta directed anterodorsally, genal tooth wide, genital plates smooth 14
14.	Notogastral surface irregularly foveate, rostral seta much shorter than lamellar seta
	(less than half the length of <i>le</i> )
-	Notogastral surface with small round foveae, rostral seta long (about <sup>3</sup> / <sub>4</sub> length of <i>le</i> )

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	Larva	Protonymph	Deutonymph	Tritonymph	Adult
Gastronotal	12	15	15	15	10
Coxisternal	3-1-2	3-1-2-1	3-1-2-2	3-1-2-2	3-1-2-2
Genital	0	1	3	5	6
Aggenital	0	0	1	1	1
Anal	0	0	0	2	2
Adanal	0	0	3	3	3

Table 1: Development of gastronotal and ventral setae of Afroleius polygonatus com. nov.Lid of Claparède's organ included in setal count for epimere I of larva.

Table 2: Development of leg setae and solenidia of *Afroleius polygonatus* **com. nov.** (setae are noted opposite the instar in which they first appear; parentheses indicate setal pairs). (La-Larva; PN-Protonymph; DN-Deutonymph; TN-Tritonymph; Ad-Adult)

Stase	Trochanter	Femur	Genu	Tibia	Tarsus
Leg I					
La	-	d, bv"	(I), σ	(I), ν', φ1	(ft), (pv), (pl), s, (a), (u), (p), (tc), ε, ω <sub>1</sub>
PN	-				ω <sub>2</sub>
DN	-	l"		φ2	
TN	V'	ľ	V'	v"	(it)
Ad		V"			l", v'
Leg II					
La	-	d, bv"	(I), σ	l', ν' φ	(ft), (pv), s, (a), (u), (p), (tc), ω <sub>1</sub>
PN	-				
DN	-	(I)		l"	ω <sub>2</sub>
TN	V'		v'	V"	(it)
Ad		٧"			
Leg III					
La	-	d, ev'	l', σ	ν', φ <sub>1</sub>	(ft), (pv), s, (a), (u), (p), (tc)
PN	-				
DN	l'			ľ	
TN	V'			"	(it)
Ad					
Leg IV					
PN	-	-	-	-	ft", (p), (u), (pv)
DN	-	d, ev'	d	ν', φ <sub>1</sub>	(tc), (a), s
TN	v'		l'	(I)	
Ad					

#### Figures

- Figure 1: *Afroleius polygonatus* (Mahunka, 1985) **com. nov.** A) Dorsal view. B) Ventral view.C) Lateral view. Scale bar 100 μm
- Figure 2: Afroleius polygonatus (Mahunka, 1985) com. nov. A SEM image. B–E Light microscope images. A) Anterolateral view. B) Notogaster, showing opisthosomal gland. C) Leg IV, antiaxial view, arrow to proximolateral tectum on tarsus. D) Larva, dorsal view. E) Gnathosoma, arrow to genal tooth.
- Figure 3: *Afroleius lucidus* **sp. nov.** A) Dorsal view. B) Ventral view. C) Lateral view. Scale bar 100 μm
- Figure 4: Afroleius lucidus sp. nov. A–C SEM images. D–F Light microscope images. A)
  Dorsal view. B) Notogastral integument, without cerotegument. C) Bothridium and
  bothridial seta, notogaster and pteromorph with cerotegument, arrow to proximodorsal
  grooves on tutorium. D) Genital plates. E) Lamellar setae in dorsal view. F) Porose
  area Aa and seta la.
- Figure 5: Distribution of *A. lucidus* **sp. nov.** (triangles) and *A. polygonatus* (Mahunka,1985) **com. nov.** (circles) in South Africa.



Figure 1: *Afroleius polygonatus* (Mahunka, 1985) **comb. nov.** A) Dorsal view. B) Ventral view. C) Lateral view. Scale bar 100 µm



Figure 2: *Afroleius polygonatus* (Mahunkla, 1985) **comb. nov.** A SEM image. B-E Light microscope images. A) Anterolateral view. B) Notogaster, showing opisthosomal gland. C) Leg IV, antiaxial view, arrow to proximolateral tectum on tarsus. D) Larva, dorsal view. E) Gnathosoma, arrow to genal tooth.



Figure 3: *Afroleius lucidus* **sp. nov.** A) Dorsal view. B) Ventral view. C) Lateral view. Scale bar 100 µm



Figure 4: Afroleius lucidus sp. nov. A-C SEM images. D-F Light microscope images.
A) Dorsal view. B) Notogastral integument, without cerotegument. C) Bothridium and bothridial seta, notogaster and pteromorph with cerotegument, arrow to proximodorsal grooves on tutorium. D) Genital plates. E) Lamellar setae, dorsal view. F) Porose area Aa and seta la.



Figure 5. Distribution of *Afroleius lucidus* **sp. nov.** (triangles) and *A. polygonatus* (Mahunka, 1985) **comb. nov.** (circles) in South Africa.

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# **Chapter 6**

### Molecular Phylogeny of Afroleius

#### Abstract

In this section the results of a molecular phylogenetic analysis, based on segments of the ribosomal gene 28S rDNA, are presented. Sequence processing of *Afroleius* samples from the National Museum's mite collection was performed by a commercial laboratory. Additional sequences of representative genera of Ceratozetoidea and Oribatellidae outgroups were retrieved from GenBank. Sequences were aligned in MUSCLE and analysed in MEGA6. The tree topology was partly incongruent with morphological evidence for the species involved. However, representative species of *Afroleius* (*A. deformis, A. floridus, A. polygonatus* and *A. minor*), formed a deeply divergent clade with *Mycobates tridactylus* Willmann, 1929 and *Trichoribates trimaculatus* (Koch, 1836), and *Afroleius* was shown to be a valid genus.

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### Introduction

Phylogenetic systematics involves methods used to obtain the best estimates of evolutionary history. It is practiced on two levels, i.e. using genetic information (molecular phylogenetics) and morphological characters, and grouping organisms by the distribution of shared genetic or morphological character states. Different methods such as Bayesian Logic, Maximum Likelihood or Parsimony are applied in a variety of computer programmes to estimate the relationships among members of a group of organisms.

The science of phylogenetic systematics became popular (albeit controversial) during the 1950s and 1960s through the work of Willi Hennig (Schmidt 2003). Initially these methods were applied only to datasets of morphological characters. The development of methods to extract DNA from biological material, and especially the method developed by Mullis *et al.* (1986) to replicate small amounts of DNA into workable amounts by the polymerase chain reaction (PCR) (Bartlett & Stirling 2003), made it practical to use genetic information for

phylogenetic analyses. Molecular phylogenetics, providing much larger datasets than those used in morphological analyses, gradually became the dominant technique in phylogenetic systematics (Cruickshank 2002; Prendini 2005; San Mauro & Agorreta 2010; Wägele *et al.* 2009). However, the broad-scale application of only molecular methods has its opponents (see Discussion below).

The rapid increase in the application of molecular phylogenetics in Acari is demonstrated by Dabert (2006) who showed that in 1993 only 49 sequences of Acari were available in GenBank, whereas in 2006 there were almost 93 000 sequences, and this number has now risen to over 1 million (NCBIa).

Molecular techniques have been applied in several oribatid studies, at species and family levels (Heethoff *et al.* 2011; Lienhard *et al.* 2013; Pfingstl *et al.* 2010; Salomone *et al.* 1996; Schäffer *et al.* 2009), in population and ecological studies (Heethoff *et al.* 2006; Maraun *et al.* 2009), as well as for deep phylogenies at higher taxonomic levels (Dabert *et al.* 2010; Domes *et al.* 2007; Maraun *et al.* 2004; Salomone *et al.* 2002; Schäffer *et al.* 2010). None of these studies addresses the phylogeny of Ceratozetoidea, or that of any other poronotic Brachypylina.

*Afroleius* specimens in the collection of the National Museum were processed (DNA extraction, PCR and sequencing) at a commercial laboratory (Inqaba Biotec, Pretoria). The results were not optimal as few sequences were successfully retrieved. A comprehensive analysis including all *Afroleius* species is therefore not possible, but the available DNA information was used, together with sequences from GenBank, to determine the relationships of *Afroleius*.

### Materials and methods

#### Materials

*Afroleius* specimens from the collection of the National Museum were sequenced at Inqaba Biotec, Pretoria. Representatives of all *Afroleius* species (except *A. undulatus*) were selected for sequencing, but only four were successful (Table 1). Sequences (28S rDNA) of representatives of Ceratozetoidea and Oribatellidae were retrieved from GenBank (Table 2). Oribatellidae was selected as outgroup as they are poronotic Brachypylina but there is no evidence that these taxa are referable to Ceratozetoidea.

Database no.	Species	Locality & Habitat	Date collected	Number of base pairs
4580	<i>A. floridus</i> (Mahunka,1985)	Koobooberry Farm, Sedgefield, Western Cape Province; litter from	28.xii.2012	246
4603	<i>A. defomis</i> Mahunka, 1984	Schoenmakerskop, Port Elizabeth, Eastern Cape Province: litter from coastal fynbos	14.iii.2014	245
4603	<i>A. polygonatus</i> (Mahunka, 1985)	Schoenmakerskop, Port Elizabeth, Eastern Cape Province, litter from coastal fynbos.	14.iii.2014	246
4590	<i>A. minor</i> Mahunka, 1984	Garden of Eden, Plettenberg Bay, Western Cape Province, litter from forest.	5.xii.2013	245

Table 2: 28S rDNA sequences retrieved from GenBank.

GenBank accession number	Family	Species	Number of base pairs
EF989719.1	Ceratozetidae	Ceratozetes pacificus Behan-Pelletier, 1984	245
AY273522.1	Ceratozetidae	Fuscozetes bidentatus (Banks, 1895)	246
HM582384.1	Ceratozetidae	Trichoribates trimaculatus (Koch, 1836)	245
AF515426.1	Chamobatidae	Chamobates borealis Trägårdh, 1902	242
AY273520.1	Chamobatidae	Chamobates cuspidatus (Michael, 1884)	242
AF515427.1	Chamobatidae	Chamobates voigtsi (Oudemans, 1902)	243
DQ090821.1	Euzetidae	Euzetes globulus (Nicolet, 1855)	244
HM582374.1 isolate	Euzetidae	Euzetes globulus (Nicolet, 1855)	244
AY273524.1	Punctoribatidae	Mycobates tridactylus Willmann, 1929	245

#### Table 2 (continued)

### Outgroup

GenBank accession number	Family	Species	Number of base pairs
AF515428.1	Oribatellidae	<i>Oribatella calcarata</i> (Koch, 1835)	247
AF515429.1	Oribatellidae	Oribatella quadricornuta (Michael, 1880)	247

### **Gene selection**

Cruickshank (2002) advised that widely used molecular markers were preferable because sequences from other studies could be used for broad-scale phylogenies and for comparative studies. GenBank (NCBIb) was consulted for Ceratozetoidea sequences and suitable outgroups.

### Nuclear ribosomal gene 28S rDNA

The structure of the 28S rDNA gene consists of conserved core segments and hypervariable expansion segments (Dabert 2006). The conservative region can be used for deep branch investigations (higher taxonomic levels) and the hyper-variable region for investigations of closely related taxa (Cruickshank 2002; Dabert 2006; Maraun *et al.* 2004).

## Polymerase Chain Reaction (PCR) and sequencing

Genomic DNA from individual specimens was extracted and amplified using the Phire Thermo Direct Kit (http:// www.thermoscientificbio.com). PCR conditions were as follows (according to Inqaba Biotec, Pretoria): an initial denaturation step at 98°C for 5 min, followed by 40 amplification cycles (98°C for 5 s, 50°C for 5 s, 72°C for 15 s) and a final elongation step at 72°C for 1 min; primers according to Maraun *et al.* 2004. PCR products were sequenced using ABI BigDye Terminator v3.1 Cycle Sequencing Kit (https://www.lifetechnologies.com) and analysed on ABI 3500XL.

#### BLAST

Basic Local Alignment Search Tool (BLAST) is a method for performing sequence comparisons (Altschul *et al.* 1990). The program searches sequence databases to find regions of local similarity between sequences. Various algorithms have been developed for different research questions. For example, BLASTn compares a given nucleotide sequence to other nucleotide sequences in the DNA database specified by the user. The most similar sequences are returned together with the statistical significance of matches. In this way undetermined sequences can be identified or failed DNA sequences can be detected (e.g. where the DNA of contaminants was amplified instead of the organism under consideration). All *Afroleius* sequences were subjected to a BLASTn search to verify their origin.

#### Data analysis

Sequences were aligned using MUSCLE (Edgar 2004) and the analysis was performed in MEGA6 (Tamura *et al.* 2013). Evolutionary history was inferred by Maximum Likelihood (ML) based on the Kimura 2-parameter model (Kimura 1980). To assess the robustness of relationships, 1000 bootstrap replicates were performed. Bootstrap is a statistical method to evaluate the confidence or accuracy of phylogenetic trees (Efron *et al.* 1996; Felsenstein 1985). The number indicated above the branches is the percentage of trees in which the associated taxa clustered together. A value of 70% or higher is often accepted as an indication of a reliable grouping (Baldauf 2003). Although there are concerns and controversy over interpretation of bootstrap values, the usefulness and wide application of this method of estimation of accuracy ensures its continued use (Soltis & Soltis 2003).

#### Results

Figure 1 is a cladogram produced by the Maximum Likelihood method for 15 sequences of 28S rDNA based on the Kimura 2-parameter model.



0.005

Figure 1: Estimated phylogeny of selected Ceratozetoidea species inferred by Maximum Likelihood. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values are shown above the branches. Bootstrap values are all above 50%, but the majority are below 70%.

The separation between Ceratozetoidea (in this analysis represented by members of the families Ceratozetidae, Chamobatidae, Euzetidae and Punctoribatidae) and the outgroup (Oribatellidae) is strongly supported (bootstrap value 99%). *Ceratozetes pacificus* (Ceratozetidae) is separated from the rest of the Ceratozetoidea by a strongly supported branch (99%). *Afroleius (A. deformis, A. floridus, A. polygonatus* and *A. minor*) and the sister grouping of *Mycobates tridactylus* + *Trichoribates trimaculatus* form a strongly supported clade. This clade is deeply divergent as indicated by the long branch in Figure 1. The inclusion of *T. trimaculatus* in this clade is unexpected. In terms of the relationship between *Afroleius* and *Mycobates tridactylus* + *Trichoribates trimaculatus*, the 90% bootstrap value indicates that they are clearly separate groups. Little can be said about the relationships of

the rest of the taxa (*Fuscozetes bidentatus*, *Chamobates borealis*, *C. cuspidatus*, *C. voigtsi* and *Euzetes globulus*) because of low bootstrap support values. However, the clade formed by *Euzetes globulus* (Euzetidae) and the species of *Chamobates* (Chamobatidae) forms a polytomy (i.e. unresolved) and *Fuscozetes bidentatus* (Ceratozetidae) is the sister taxon to the monophyletic group formed by *Afroleius* + *Mycobates tridactylus* + *Trichoribates trimaculatus*. A larger dataset may improve the statistical support at the nodes (Kimball & Braun 2014; Soltis & Soltis 2003).

#### Discussion

Molecular data indicates that the four species of *Afroleius* included in this analysis are closely related, and the status of *Afroleius* as a distinct genus is therefore supported. The close relationship of *Afroleius* and *Mycobates* is supported by their morphology. The deeply divergent clade recovered (Fig. 1) supports the separation of this clade from the rest of the taxa. However, the unexpected inclusion of the ceratozetid *Trichoribates trimaculatus* as sister species to *Mycobates tridactylus* suggests that *T. trimaculatus* is in fact a member of Punctoribatidae or, alternatively, that the identity of this sequence should be confirmed. The key morphological difference between Punctoribatidae and Ceratozetidae is the presence or absence of a posterior notogastral tectum which is present (divided or complete) in Punctoribatidae and absent in Ceratozetidae. *Mycobates tridactylus* and *T. trimaculatus* are both widely distributed in the Holarctic Region (Bayartogtokh & Schatz 2008; Bayartogtokh *et al.* 2011; Behan-Pelletier 1993; Marshall *et al.* 1987), while *Afroleius* is restricted to Africa.

The other two Ceratozetidae species included in this analysis (*Fuscozetes bidentatus* and *Ceratozetes pacificus*) also branched into different clades, indicating that Ceratozetidae is paraphyletic.

*Euzetes globulus*, in the monogeneric family Euzetidae, and species of *Chamobates* (*C. borealis, C. cuspidatus* and *C. voigtsi*) in the family Chamobatidae, form a monophyletic group, contradictory to their morphology. In Euzetidae the posterior notogastral tectum is absent, the subcapitular mental tectum is present, and epimere IV is neotrichous (i.e. four or more pairs of setae). This family has a further autapomorphy in the presence of two hollows laterally on the propodosoma of immatures into which legs I and II can be retracted (Grandjean 1954, Norton & Behan-Pelletier 2009). In Chamobatidae the posterior notogastral tectum is present, the subcapitular mental tectum is absent, and epimere IV has the plesiomorphic three pairs of setae. In this family the lamellar seta is inserted on the prodorsal surface (lamellar cusp absent) (Grandjean 1954, Norton & Behan-Pelletier 2009).

Buhay (2009) outlined some problems that may occur during molecular analyses, such as the presence of *numts* (nonfunctional copies of mitochondrial DNA in the nuclear genome "behaving as junk DNA"), pseudogenes (dysfunctional genes that have lost their protein-coding ability), contaminated DNA, and poor sequence quality or editing. Another problem that arises frequently is long-branch attraction artifacts, resulting in inaccurate trees (Baldauf 2003; Bergsten 2005; Dabert *et al.* 2010). These are issues that can be recognised and dealt with by trained molecular biologists. The reliability of GenBank sequences must also be questioned, as many erroneous identifications have been pointed out, and sequences may be of low quality (Buhay 2009; Harris 2003; Wägele *et al.* 2009).

Some questionable phylogenies in various animal groups have recently been discussed (e.g. Buhay 2009; Mooi & Gill 2010; Wägele *et al.* 2009) and criticism boils down to questionable molecular data, different methods that are being applied, and the lack of taking morphological evidence into account. Mooi & Gill (2010), with examples from fish taxonomy, emphasised the danger of ignoring or misunderstanding the importance of character distribution, homology and the meaning of evidence, and the treatment of characters and organisms as "statistical values and models".

Molecular and morphological data should be used together for phylogenetic and taxonomic studies, as has been applied successfully by some authors. Examples in oribatid studies where other data sources were used together with molecular data (but analysed separately) to delineate species are those of Heethoff *et al.* (2011) (morphological, chemical and molecular data) and Pfingstl *et al.* 2010 (morphological, morphometrical and molecular data). Wahlberg *et al.* (2005) combined sequence data for three genes and a data matrix of morphological characters to resolve the phylogeny of butterflies and skippers (Lepidoptera) and showed that a combined analysis of morphological and molecular data enhances the phylogenetic signal. However, Wortley & Scotland (2006) analysed published phylogenetic analyses where molecular and morphological data sets were combined and concluded that addition of morphological data improves resolution (number of clades) but does not necessarily improve support of the clades.

The current analysis is limited in scope, as it is based only on 28S rDNA sequences of a few taxa. It results in more questions than answers as far as the phylogeny of Ceratozetoidea is concerned, but the monophyly of the genus *Afroleius* is confirmed by a strongly supported clade. The conflict between the present DNA-based inference and the morphology of Ceratozetoidea emphasises the need for a large-scale, taxon-inclusive phylogenetic analysis based on morphological and molecular data, as suggested by Behan-Pelletier & Eamer (2009). The lack of detailed descriptions (especially in the older literature) hampers morphological studies of this nature and points to the need for co-ordination of effort to resolve the phylogeny of Ceratozetoidea.

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# Discussion

The genus *Afroleius* Mahunka, 1984 is essentially South African, with all but one species occurring south of the Limpopo River. Mahunka (1984) described three species in the genus (*A. deformis* [type], *A. minor*, and *A. simplex*) from Nature's Valley, a forested area in the southern part of the Western Cape Province.

The family relationship of the genus was uncertain from the start. Mahunka (1984) placed it in the family Haplozetidae (Oripodoidea), but with a question mark. Subsequently it was treated as a member of Haplozetidae as well as a "ceratozetoid" genus by Balogh & Balogh (1992), and moved to Humerobatidae (Ceratozetoidea) by Subías (2014). In the first part of this study the genus was re-described and its family relationship investigated with additional characters. Coetzee & Tiedt (2013) concluded that *Afroleius* is a member of Ceratozetoidea, in the family Punctoribatidae (= Mycobatidae).

*Afroleius* specimens in the Acarology Collection of the National Museum are mostly referable to new species described during this study (*A. crassus* Coetzee, 2013; *A. decurvatus* Coetzee, 2013; *A. deformatoides* Coetzee, 2013; *A. natalensis* Coetzee, 2014; *A. caudatus* Coetzee, 2014; *A. amieae* Coetzee, 2014; *A. inae* Coetzee, 2014; *A. valerieae* Coetzee 2014; *A. lucidus* Coetzee, In Press). Two species were recombined from other genera, namely *A. polygonatus* (Mahunka, 1985) from the genus *Kilimabates* Mahunka, 1984 and *A. floridus* (Mahunka, 1985) from the genus *Magyaria* Balogh, 1963. Subías (2014) recombined *Africoribates undulatus* Balogh, 1959 from Tanzania as *Afroleius undulatus*. The genus now comprises 15 species. A valuable addition to our knowledge of Afroleius is the first discovery and description of juvenile instars of this genus. Juveniles of two species (A. floridus and A. polygonatus) have been retrieved from litter samples and positively associated with adults. A newly-hatched adult of A. floridus with nymphal skin still attached was found, and for A. polygonatus an adult female was found with a fully developed larva in the abdominal cavity. The importance of knowledge of juveniles in studies of phylogenetic relationships has been emphasised by Grandjean (1954) and Norton & Ermilov (2014). The absence of microsclerites (small porose sclerites) on the gastronotic sclerite supports the rejection of the affiliation of Afroleius with Humerobatidae (Ceratozetoidea) as well as Haplozetidae (Oripodoidea). Grandjean (1970) instituted the family Humerobatidae on the basis of the presence of microsclerites as well as macrosclerites on the gastronotum of juveniles. At the time of description of the genus, Mahunka (1984) proposed membership of Afroleius in Haplozetidae. Juveniles of Haplozetidae, as in almost all Oripodoidea, have microsclerites (also called excentrosclerites) at the base of at least some of the gastronotic setae and the absence of macrosclerites. Furthermore, the first Scanning Electon Micrographs of species of Afroleius have contributed to interpretation of adult morphology.

Species of *Afroleius* are mostly concentrated in the humid eastern and southern parts of South Africa. All are litter-dwelling, with the possible exception of *A. caudatus* which may be arboreal. *Afroleius floridus* is very abundant, especially in forest litter of the southern regions of the Eastern- and Western Cape Provinces, and is also present in litter of coastal vegetation and a few patches of savanna in the southern regions. *Afroleius polygonatus* shares the geographical distribution of *A. floridus*, but is absent from forests. Of the species of Mahunka (1984) the type species (*A. deformis*) is distributed along the southern and eastern regions, mainly in forests and coastal vegetation, but is scarce; the distribution of *A. minor* is restricted to the forests and coastal regions between Cape Town and Port Elizabeth where it is relatively abundant; *A. simplex* has a much wider distribution, from Cape Town along the southern coastal regions towards KwaZulu-Natal and in the eastern and northern Grassland and Savanna Biomes (as defined by Mucina & Rutherford 2006). Afroleius crassus is a species of the coastal forests of the Indian Ocean Coastal Belt (as defined by Mucina & Rutherford 2006). This species is widely distributed along the east coast but occurs in low numbers. Three species with very limited distributions are A. deformatoides (high montane grassland), A. natalensis (savanna) and A. caudatus. The latter is suspected to be arboreal. In one sample it was recorded feeding on the underside of leaves, and its morphology supports an arboreal lifestyle (globular shape of the bothridial seta). Two specialists of the Grassland Biome are A. decurvatus and A. amieae. These two species, which are not abundant, are found in the northern and eastern grasslands of the country. Afroleius valerieae is widely distributed in the northern and eastern Savanna Biome and is also present in the eastern highlying Grassland Biome and Indian Ocean Coastal Belt. Afroleius inae occurs mainly in the coastal regions of KwaZulu-Natal (Indian Ocean Coastal Belt) with a few records from the eastern highlying grasslands and northern lowlying savanna. The most widely distributed species, although not the most abundant, is A. lucidus. It is widely distributed in the grasslands of the Free State, Eastern- Northern- and Western Cape Provinces, with a few records from savanna of the North West Province and Limpopo Province. It is also found in the Fynbos Biome and in coastal vegetation in the southern regions of the Western Cape Province. This is the only Afroleius species to be found in the arid western regions-the Nama-Karoo and Succulent Karoo Biomes (as defined by Mucina & Rutherford 2006). The only Afroleius species currently known from outside South Africa is A. undulatus which occurs in Tanzania (Balogh 1959). The oribatid faunas of neighbouring countries (Mozambique, Zimbabwe, Botswana and Namibia) are unknown and little can be said about the distribution of *Afroleius* in the rest of southern Africa.

This study also includes a molecular phylogenetic analysis from sequences prepared by a commercial laboratory (Inqaba Biotec, Pretoria). Challenges in this regard are that technicians may not be familiar with special techniques required for certain organisms and financial constraints do not allow for trial and error. The limited number of *Afroleius* 

sequences obtained (28S rDNA of *A. floridus*, *A. deformis*, *A. polygonatus* and *A. minor*) were applied in a phylogenetic analysis, together with sequences of other taxa retrieved from GenBank, to shed light on the phylogeny of *Afroleius* and its relationships in Ceratozetoidea. The strongly supported monophyletic group formed by species of *Afroleius*, *Mycobates* and *Trichoribates* indicates that these genera are sister taxa (although *Trichoribates* is currently regarded as a member of the family Ceratozetidae). This very limited phylogenetic analysis also indicates that Ceratozetidae is paraphyletic.

The microscope opened up the micro-world, allowing us to see small organisms. Molecular biology took science a step further—allowing us to "see" inside organisms. Molecular techniques are now within reach of more and more taxonomists (Dabert 2006) and analyses of DNA sequences are being applied to resolve evolutionary phylogenies, in population studies, for discovering cryptic species, and also to answer ecological questions (Klompen 2009; Maraun *et al.* 2009).

The "DNA revolution" also has its challenges. It seems that it is often forgotten that for every sequence there must be a properly identified organism. One only needs to look at GenBank (NCBI) to see how many sequences are databased only to the genus level. Hebert *et al.* (2003) outlined the advantages of establishing a database of barcodes for species, using the cytochrome oxydase I (COI) mitochondrial gene as basis. They argued that for many groups taxonomic expertise is collapsing and that a high level of expertise is needed to correctly identify species with the use of keys. Furthermore, barcodes can uncover cryptic species and also identify immature specimens. An example of this approach in the Acari is provided by Young *et al.* (2012).

Much has been written for (Blaxter 2004; Tautz *et al.* 2003) and against (Ebach & de Carvalho 2010; Ebach *et al.* 2011a; Prendini 2005; Wheeler 2005) the concept of DNA barcoding, and a more balanced view is provided for example by Schander & Willassen

(2005) and Padial *et al.* (2010). The Barcode of Life Consortium encourages DNA barcoding as "a new tool in the taxonomists' toolbox supplementing their knowledge as well as being an innovative device for non-experts who need to make a quick identification" (Barcode of Life). The link between taxonomic data and DNA sequences, and the importance of voucher specimens is crucial (Pleijel *et al.* 2008; Puillandre *et al.* 2012). Small organisms such as mites are often destroyed in the process of DNA extraction. It is advisable that non-destructive methods such as those proposed by Ota *et al.* (2011) be explored.

Natural history collections are an important source of information not only for taxonomists, but for all branches of biological sciences (Drew 2011; Suarez & Tsutsui 2004). Some, or probably most, of these collections date from long before the advent of high-tech methods in taxonomy. Many specimens are therefore not stored in conditions suitable for molecular studies (Puillandre *et al.* 2012). Although new techniques are continually being developed to extract and sequence degraded DNA from unsuitably stored material (Alda *et al.* 2007; Campos & Gilbert 2012), curators should be encouraged to adjust storage methods to facilitate extraction of sufficient, good quality DNA. Best results for Acari are obtained from specimens in 95% ETOH and stored at -20°C (Corriveau *et al.* 2009). Fast-changing techniques may make it impossible to envisage exactly what will be needed in the long term, but at least current requirements should be employed in order to retain the relevance and usefulness of natural history collections.

Species are the basic unit that underpins taxonomy, but to make sense of the multitude of described species, their relationships and evolutionary history need to be understood. There has been a tug-of-war among phylogeneticists about the suitability of morphological data versus molecular data to reconstruct phylogenies (Jenner 2004; Scotland *et al.* 2003; Wiens 2004). In a review of the use of both data types, Wortley & Scotland (2006) concluded that the adaptation of techniques to enable the use of morphological data and combined data implies the demand and need for such research. The software *Mesquite* (Maddison &

Maddison 2014) is designed to analyse comparative data with the emphasis on phylogenetic analysis and includes a model for analysing morphological data. The era of web-based communication and the "cloud" also facilitate data sharing (Cranston *et al.* 2014) so that datasets are available to other researchers who wish to make use of the same data.

Is molecular phylogeny the panacea to resolve relationships? There are many pitfalls that may lead to conflicting results for example, studies on the phylogenetic relationship between Astigmata and Oribatida (Dabert *et al.* 2010; Domes *et al.* 2007). Norton (1994, 1998, 2007) provided strong morphological evidence for the origin of Astigmata within Oribatida. The molecular analysis by Domes et al. (2007) based on ribosomal 18S DNA and the nuclear elongation factor 1 alpha (ef1a) with 28 species of Oribatida, eight of Astigmata, two of Prostigmata and two of Endeostigmata, and outgroups from members of Opilioacariformes, Parasitiformes and Ricinulei, does not support the hypothesis of Norton (1994, 1998, 2007). On the other hand, the analysis by Dabert et al. (2010) based on ribosomal 18S DNA and the mitochondrial cytochrome oxidase I (COI) with a much larger taxon set (56 species of Astigmata, 51 of Oribatida, 29 of Trombidiformes and six of Endeostigmata) and outgroups from members of Parasitiformes, Amblypygi, Araneae, Opiliones, Palpigradi, Pseudoscorpionida, Ricinulei, Schizomida, Scorpiones, Solifugae and Limulidae, corroborates Norton's hypothesis. Unbalanced taxon sampling, differences in alignment strategies, and the effect of long branch attraction (LBA) may greatly influence the outcome (Dabert et al. 2010). The reliability of GenBank sequences has also been questioned, and users are cautioned when using publicly available sequences (Buhay 2009; Harris 2003; Wägele et al. 2009).

Strong criticism of molecular phylogeny came from Mooi & Gill (2010), illustrating (with examples in fish taxonomy) that the move away from an understanding of morphological evidence, character distribution and homology lay at the root of conflicting phylogenies estimated only on the basis of molecular data. These points are further elaborated by Ebach

*et al.* (2011b), drawing attention to "classification based on homology" and "grouping based on similarity". In their view, practitioners of the latter (molecular systematists) have adopted the technology and ignored the theory. However, they recognise that molecular phylogeny has great potential once molecular homology is brought into the methods. A holistic approach, taking morphology, molecular data and ecology into account, should be a goal.

Certainly, the last word on phylogenetic relationships in Ceratozetoidea has not been written yet. A large-scale phylogenetic study is needed to resolve familial, or even superfamilial relationships, for example of the family Zetomimidae, currently regarded as a family of Ceratozetoidea (Behan-Pelletier & Eamer 2003). Behan-Pelletier & Eamer (2003, 2009) encourage a large-scale phylogenetic study based on morphological as well as molecular data to resolve the phylogeny of Ceratozetoidea. A challenge in using morphological data may be to obtain sufficient unambiguous characters and character coding.

Taxonomists are mostly employed at government institutions such as museums or universities worldwide and therefore are at the mercy of policy makers and funding bodies. Recent decades saw a decline in the number of acarologists (Walter & Proctor 1999), especially taxonomists, and this trend is continuing. However, it is not only acarology that suffers from this "extinction". As long as 20 years ago Daly (1995) indicated a decline in doctoral students in entomology systematics in the United States of America and Hopkins & Freckleton (2002) demonstrated the decline in number of amateur and professional taxonomists in entomology in the United Kingdom. Others suggest that numbers of taxonomists have increased, but that they produce fewer species descriptions (Joppa *et al.* 2011). Problems relating to the future of taxonomy and the failure to attract newcomers to the field, including funding, decreasing job opportunities, low impact factors of taxonomic journals, lack of interest by the younger generation, and fewer institutions teaching classical taxonomy, have been outlined (Guerra-García *et al.* 2008; Wheeler 2004). Proposed remedies include approaches such as "turbo-taxonomy" where greater use is made of molecular methods, shorter concise descriptions, digital imaging and web-publication (Riedel *et al.* 2013), and the incorporation of citizen scientists and parataxonomists (Pearson *et al.* 2011). Citizen scientists may play an important role in recording the distribution of, for instance, butterflies, birds and reptiles, but "invisible" species such as mites or fish parasites may not attract the same—if any—level of interest. Capacity building should be the main focus to ensure that there are trained and competent taxonomists to put a name and a face to the species that are being barcoded (Smith & Figueiredo 2009).

Oribatid mite taxonomists are particularly vulnerable because our subjects are not the culprits causing large-scale agricultural damage or diseases of man and beast, apart from a small group involved in transmitting anoplocephalid tapeworms (Schuster & Coetzee 2012; Sengbusch 1977; Stunkard 1937). The "invisibility" of mites, especially oribatid mites mostly hidden in litter and soil, makes it difficult to convince decision makers of the need for their study, and their ecological importance as decomposers is not readily understood by non-scientists.

As for acarology, Klompen (2009) expresses his optimism in the future of this field "because there are so many truly remarkable things left to be discovered, whether it is in systematics, physiology, ecology or genetics."

Traditional taxonomy is a science that has stood the test of time since the days of Linnaeus. Let us hope that our efforts will ensure that Robert Hooke's "little pretty creatures" will retain their rightful place in the scientific literature of the future.

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## Abstract

The genus *Afroleius* Mahunka, 1984 originally comprised three species (*A. deformis* Mahunka, 1984 (type), *A. minor* Mahunka, 1984 and *A. simplex* Mahunka, 1984), but with an uncertain relationship, at first placed in the family Haplozetidae (Oripodoidea). In this study the genus is redescribed and its relationship investigated. Specimens from the acarology collection of the National Museum referable to new species are described, two species originally described in other genera are recombined, a key to the species of the genus is proposed and its phylogeny investigated on the basis of molecular data.

It is concluded that *Afroleius* is a member of the superfamily Ceratozetoidea, based on the presence of the axillary saccule, genal tooth, and the shape of pedotectum I. Its relationship in the family Punctoribatidae is based on the presence of the posterior notogastral tectum and desclerotized hinge of the pteromorph.

Nine new species are described (*A. crassus* Coetzee, 2013; *A. decurvatus* Coetzee, 2013; *A. deformatoides* Coetzee, 2013; *A. natalensis* Coetzee, 2014; *A. caudatus* Coetzee, 2014; *A. amieae* Coetzee, 2014; *A. inae* Coetzee, 2014; *A. valerieae* Coetzee 2014; *A. lucidus* Coetzee, In Press). Two species are recombined namely *A. polygonatus* (Mahunka, 1985) from the genus *Kilimabates* Mahunka, 1984 and *A. floridus* (Mahunka, 1985) from the genus *Magyaria* Balogh, 1963. *Afroleius undulatus* (Balogh, 1959) (previously recombined from *Africoribates*) from Tanzania is discussed. Juvenile instars of two species (*A. floridus* and *A. polygonatus*) are described. The genus now comprises 15 species. The phylogenetic analysis based on molecular data of 28S rDNA of *A. floridus*, *A. deformis*, *A. minor* en *A. polygonatus*, additional Ceratozetoidea sequences and outgroup sequences retrieved from GenBank, indicates *Mycobates* and *Trichoribates* as the sister genera of *Afroleius*. Ceratozetidae is shown to be paraphyletic. This analysis emphasises the need for a large-scale phylogenetic analysis of Ceratozetoidea including morphological and molecular data.

## Opsomming

Die genus *Afroleius* Mahunka, 1984 het uit drie spesies bestaan (*A. deformis* Mahunka, 1984 (tipe), *A. minor* Mahunka, 1984 en *A. simplex* Mahunka, 1984), en die verwantskap was onseker. Dit is oorspronklik in die familie Haplozetidae (Oripodoidea) geplaas. In hierdie studie is die genus herbeskryf en die verwantskap bestudeer. Eksemplare van hierdie genus in die akarologie-versameling van die Nasionale Museum wat nuwe spesies uitmaak, is beskryf, twee spesies wat oorspronklik in ander genera beskryf is, is herkombineer, 'n sleutel tot die spesies in die genus is opgestel en 'n molekulêre filogenetiese studie is gedoen.

Dit is bevind dat *Afroleius* aan die superfamilie Ceratozetoidea behoort, gegrond op die teenwoordigheid van die oksellêre sakkulus, laterale insnyding aan die gnathosoma, en die vorm van die eerste pedotektum. Die verwantskap aan die familie Punctoribatidae is gegrond op die teenwoordigheid van 'n posterior notogastrale tektum en die beweegbaarheid van die pteromorf.

Nege nuwe spesies is beskryf (*A. crassus* Coetzee, 2013; *A. decurvatus* Coetzee, 2013; *A. deformatoides* Coetzee, 2013; *A. natalensis* Coetzee, 2014; *A. caudatus* Coetzee, 2014; *A. amieae* Coetzee, 2014; *A. inae* Coetzee, 2014; *A. valerieae* Coetzee 2014; *A. lucidus* Coetzee, In Druk). Twee spesies is na *Afroleius* oorgeplaas naamlik *A. polygonatus* (Mahunka, 1985) vanaf die genus *Kilimabates* Mahunka, 1984 en *A. floridus* (Mahunka, 1985) vanaf die genus *Kilimabates* Mahunka, 1984 en *A. floridus* (Mahunka, 1985) vanaf die genus *Kilimabates* Mahunka, 1984 en *A. floridus* (Mahunka, 1985) vanaf die genus *Kilimabates* Mahunka, 1984 en *A. floridus* (Mahunka, 1985) vanaf die genus *Magyaria* Balogh, 1963. *Afroleius undulatus* (Balogh, 1959) (voorheen oorgeskuif vanaf *Africoribates*) van Tanzanië is bespreek. Onvolwasse stadia van twee spesies (*A. floridus* en *A. polygonatus*) is beskryf. Die genus bestaan nou uit 15 spesies.

Die filogenetiese ondersoek gegrond op molekulêre data van 28S rDNA van *A. floridus*, *A. deformis*, *A. minor* en *A. polygonatus*, bykomende Ceratozetoidea data en buitegroep data wat van GenBank afgelaai is, dui daarop dat *Mycobates* en *Trichoribates* die sustergenera van *Afroleius* is. Die analise dui ook aan dat Ceratozetidae parafileties is. Hierdie analise benadruk die behoefte aan 'n grootskaalse filogenetiese ondersoek van die Ceratozetoidea wat morfologiese en molekulêre data bevat.