

## Pre-testicular spermathecal pores and unusual setal arrangement in the South African endemic microchaetid earthworms of presumed Gondwanan origin (Oligochaeta: Microchaetidae)

*In memoriam of BRIAN STUCKENBERG (1930–2009), whose enthusiasm and support allowed the extension of knowledge of South African earthworms.*

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### ABSTRACT

The microchaetid species with spermathecal pores anterior to the testicular segment, intrasegmental or intersegmental, in 8/9, 9, or 9/10 and 10/11, were studied. These characters were observed in species of three genera: in proandric *Proandricus* (8 spp.), and holandric *Geogenia* (3 spp.) and *Tritogenia* (1 sp.). Some irregularities in setal arrangement, seldom observed in South African microchaetids but occurring in these proandric species, are considered in the *lesothoensis* species-group. Unusual for the South African microchaetids, although known in the South American glossoscolecids, the locations of the spermathecae and their pores, a discrepancy in the arrangement of setae, and the species' geographical distribution all suggest a relationship with the Glossoscolecidae and a presumed Gondwanan origin. The distribution of the species in Gondwana ancestral to the Drakensberg is discussed. Two holandric species with spermathecae and their pores in the testicular and post-testicular segments are compared.

**KEY WORDS:** Microchaetidae, *Proandricus*, *Geogenia*, *Tritogenia*, *lesothoensis* species-group, spermathecal pores, setal arrangement, microchaetids, Gondwana, South Africa.

### INTRODUCTION

The post-testicular location of the spermathecal pores is one of the taxonomic characters distinguishing the South African Microchaetidae from the Glossoscolecidae. This status has been accepted by the majority of researchers (Michaelsen 1900, 1918, 1928; Stephenson 1930; Gates 1959; Sims 1982; Omodeo 1956, 1998, 2000; Plisko 1996, 2006a, b). However, in several species presently accredited to the Microchaetidae and characterized by a complex of the microchaetid features, the spermathecae and their pores have been observed anterior to the testicular segment in addition to the pair located in the testicular segment. In seven proandric species, viz. *Proandricus lesothoensis* (Reinecke & Ryke, 1969); *P. pajori* Plisko, 1993; *P. bourqini* Plisko, 1996; *P. sani* Plisko, 2002; *P. adami* Plisko, 2003; *P. amphius* Plisko, 2003; and *P. oresbiosus* Plisko, 2003, during dorsal longitudinal dissections the spermathecae were noted in pre-testicular segment 9 and in testicular segment 10, with their pores located intrasegmentally or intersegmentally anterior to the testicular segment. Curiously, in some of these species, at the ventral clitellar area some discrepancy in the setal arrangement of *ab* setae was observed. Considering the occurrence of these features, unusual for microchaetids, the seven species were distinguished and grouped in the *lesothoensis* species-group, designated by Plisko (1996, 2003, in press). It is known from the description of *Proandricus timmianus* (Michaelsen, 1933) that during external specimen observation the spermathecal pores were found in post-testicular intersegmental furrows 11/12, 12/13, 13/14; however, on the histological slides obtained from one individual, some rudimentary

spermathecae were present in front of the testicular segment, although no clear indication on their pores was externally established. In the other individuals dissected by Michaelsen (1933) no rudimentary spermathecae anterior to the testes were noted, although partly displaced *ab* setae in the clitellar area were mentioned. This information suggested a possible species relationship with the *lesothoensis* species-group, and *timmianus* was used for the species-group name, but later it was corrected and the name *lesothoensis* was applied (Plisko 1996, 2003).

During the present investigation, the spermathecae and their pores have been found pre-testicularly in several holandric species: *Geogenia namaensis* (Michaelsen, 1908), *G. distamosa* (Plisko, 2003), *G. quaera* (Plisko, 2003), and *Tritogenia phinda* Plisko, 2005, although the setae occur in four regular rows. *G. benhami* (Rosa, 1891) and *G. mkuzi* (Plisko, 1992) are exceptional among the microchaetids in having spermathecae in the testicular and post-testicular segments. It should be said that the occurrence of spermathecae in front of the testes or in the testis segments was described from limited material, often obtained only from the type locality. The aim of this paper is to re-

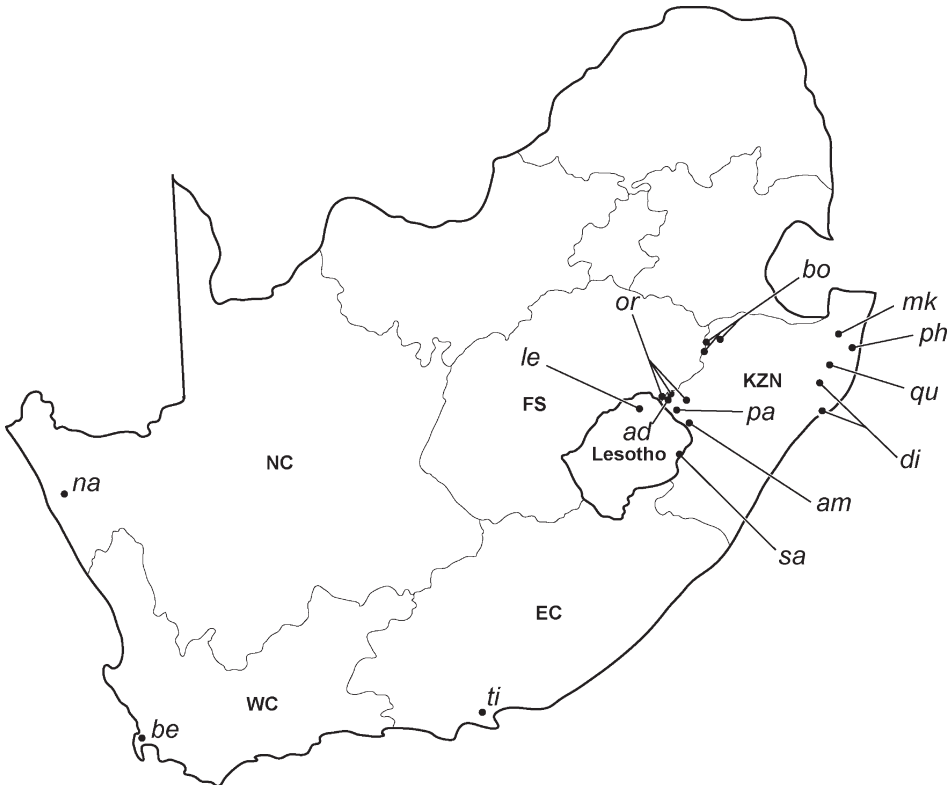


Fig. 1. Geographical distribution of endemic earthworm species with irregular locations of the spermathecal pores. Abbreviations: *ad* – *Proandricus adami*, *am* – *P. amphius*, *be* – *Geogenia benhami*, *bo* – *Proandricus bourquini*, *di* – *Geogenia distamosa*, *le* – *Proandricus lesothoensis*, *mk* – *Geogenia mkuzi*, *na* – *G. namaensis*, *or* – *Proandricus oresbiousus*, *pa* – *P. pajori*, *ph* – *Tritogenia phinda*, *qu* – *Geogenia quaera*, *sa* – *Proandricus sani*, *ti* – *P. timmianus*. South African provinces: NC – Northern Cape, FS – Free State, KZN – KwaZulu-Natal, WC – Western Cape, EC – Eastern Cape.

examine these species, including new material, and to evaluate this interesting phenomenon observed in the endemic South African microchaetids.

#### MATERIAL AND METHODS

A study was undertaken on the group of microchaetid species in the Natal Museum Oligochaeta collection, Pietermaritzburg, South Africa, in which, during longitudinal dissection, the spermathecae and their pores were found to occur in the testicular segment or anterior to this segment. The type material, and new material of the proandric species: *lesothoensis*, *pajori*, *bourqini*, *sani*, *adami*, *amphius*, *oresbiosus*, the holandric *Geogenia*: *benhami*, *namaensis*, *mkuzi*, *distasmosa*, *quaera*, and *Tritogenia phinda* were compared. For *benhami* and *namaensis* the original description and redescription (Plisko 1995) were used, and type material compared. Type material of *Proandricus timmianus* (Michaelsen, 1933) was not accessible because the species was declared 'typus amissus' by Reynolds and Cook (1976) and no new material is obtainable.

The external and internal characters of studied species are adequately given in the original descriptions in most instances, so they are not repeated. Instead, the principal features characterizing them, namely the location of spermathecae, their pores, and an arrangement of setae, were scrutinized. The setup of revised species is given in separated genera, chronologically. The arrangement of the spermathecal pores and setae for proandric species and holandric *Geogenia* species are presented in Table 1 and Table 2 respectively. Geographical species distribution is shown on the map (Fig. 1). Coordinates not taken from the original label are given in brackets.

Abbreviations and codens used in the text:

- cl – clitellate;
- HNHM – Natural History Museum, Budapest, Hungary;
- juv – juvenile;
- KZN – KwaZulu-Natal;
- MDTP – Maloti-Drakensberg Transfrontier Project;
- NHWM – Natural History Museum, Vienna, Austria;
- NMSA – Natal Museum, Pietermaritzburg, South Africa;
- USNM – National Museum of Natural History, Washington, DC, USA;
- ZMH – Zoological Museum and Institute, University of Hamburg, Germany.

#### TAXONOMY

*Proandric species:*

*Proandricus timmianus* (Michaelsen, 1933)

*Microchaetus timmianus*: Michaelsen 1933: 429; Reynolds & Cook 1976: 181.

*Proandricus timmianus*: Plisko 1992: 355; 1993: 202; 1994: 231; 2003: 293.

Type locality: South Africa: Eastern Cape, Uitenhage (33°45'S:25°25'E). The site shown on the map (Fig. 1) is only approximate.

No type material was available for examination, as its whereabouts are not known (Reynolds & Cook 1976). Michaelsen (1933) during external examination observed spermathecal pores in intersegmental furrows 11/12, 12/13, 13/14, and in 8/9, 9/10, 10/11 on microscope slides. A declaration on the species' male funnels located in 10, which is a proandric condition, allowed Plisko (1992) to transfer this species to *Proandricus* and later to couple it with species with characters found in the *lesothoensis* species-

group. In the absence of the type material, and of any other identified material, it is difficult to consider its inclusion in the *lesothoensis* species-group. Furthermore, little is known on the species' environmental status except information that it was found together with *A. rosea* (Savigny, 1826) and *Microscolex dubius* (Fletcher, 1888), both alien species introduced to South Africa. It may be supposed that specimens studied by Michaelsen might be not endemic to the type locality. Little can be said on the taxonomic position of this species until new material becomes available. At present, it may not be included in the *lesothoensis* species-group.

*Proandricus lesothoensis* (Reinecke & Ryke, 1969)

Fig. 2

*Geogenia lesothoensis*: Reinecke & Ryke 1969: 516; Reynolds & Cook 1976: 127.

*Microchaetus lesothoensis*: Zicsi & Pajor 1992: 129 (for *Proandricus pajori*).

*Proandricus lesothoensis*: Plisko 1992: 355; 1993: 202; 1994: 229; 2003: 295.

Type locality: Lesotho: Butha-Buthe District (approx. 28°50'S:28°30'E), at ca 3300 m. This species is known from the central Drakensberg mountain range, in Lesotho.

Material examined: Lectotype NMSA/Olig.01402 and 3 paralectotypes NMSA/Olig.01403.

This species' external and internal characters are in accord with their description (Reinecke & Ryke 1969) and redescription (Plisko 1993). Re-examination of setae confirms their closely paired arrangement in four pairs, although on several pre-clitellar segments they are difficult to trace and some of *ab* are irregularly misplaced. In clitellar area *ab* are sporadically uplifted dorsally to *cd* pairs. No constant pattern was observed in four examined individuals. Usually, the discrepancies occur on four to six segments, where *ab* setae are shifted dorsally to leave the other *ab* pairs on neighbouring segments in even, regular rows. Setae *cd* always in regular rows, not misplaced. Spermathecae in 9 and 10, variable in shape and size in the same individual; usually ampulla spherical with elongated duct. Number of spermathecae one or two, in one segment. Spermathecal pores in 9/10 and 10/11. Anterior spermathecae usually empty. Presence of sperm in posterior thecae confirms activity of spermathecal pores 10/11; lack of sperm in the anterior spermathecae in the same specimen suggests that pores 9/10 might be not active during mating.

*P. lesothoensis*, identified more than three decades after Michaelsen (1933) described *timmiianus*, exhibits the possible occurrence of the spermathecae in pre-testicular segments observed on histological slides, and some irregularities in the arrangement of setae in clitellar segments. However, no comparative study between these two species was undertaken at the time of the species' description, or during the revision of the *lesothoensis* type material by Plisko (1994), because of the lack of *timmiianus* type material, and this has obscured the taxonomic position of both species. Although *lesothoensis* was first accredited to the *timmiianus* species-group, later a lack of *timmiianus* type material made this invalid. Later, when pre-testicular spermathecal location was observed in other proandric species, with some irregularities in the arrangement of setae, the species-group was proposed, and the name given after *lesothoensis*.

Present examination verifies the *lesothoensis* relationship with the small group of species having spermathecae anterior to the testicular segment, supposedly in the process of variation or evolutionary change.



Figs 2–5. Habitus and enlarged clitellar area of *Proandricus* species: (2) *P. lesothoensis*, (3) *P. pajori*, (4) *P. bourquini*, (5) *P. sani*. Scale bars = 1 cm.

*Proandricus pajori* Plisko, 1993

## Fig. 3

*Proandricus pajori*: Plisko 1993: 203; 1994: 230; 2000: 259; 2003: 295.

*Microchaetus lesothoensis*: Zicsi & Pajor 1992: 129.

Type locality: South Africa: KZN, Cathedral Peak area (approx. 28°55'S:29°09'E) at ca 1800 m. Known from the central Drakensberg mountain range, from the type locality and its close neighbourhood.

Material examined: Holotype NMSA/Olig.01202/1; 2 clitellate paratypes NMSA/Olig.01202/2.

In examined specimens internal and external characters are in agreement with their original descriptions. Two specimens deposited in the HNHM, identified by Zicsi and Pajor (1992) as *lesothoensis*, were not examined by me. However, I am of the opinion that they belong to *pajori*. These specimens, collected at the same time, from the same locality as *pajori*, had been identified before *pajori* was described. All descriptions of the characters, including the number and position of spermathecae, presented by Zicsi and Pajor (1992), justify their identity as *pajori*.

The only one pair of spermathecae in 10, with its pores immediately posterior to the testicular segment in furrow 10/11, at first suggested a relationship with the *warreni* species-group (Plisko 1993), which is characterized by one pair of spermathecae. Considering the discrepancy in the arrangement of setae and a reduction of the spermathecae to one pair located in the testicular segment, *pajori* is assigned to the *lesothoensis* species-group. These two specific characters may confirm a relationship with the other congeners accredited to this group. It is possible that *pajori* undergoes a reduction of the anterior spermathecae.

*Proandricus bourquini* Plisko, 1996

## Fig. 4

*Proandricus bourquini*: Plisko 1996: 298; 2003: 301.

Type locality: South Africa: KZN, Ncandu Forest Reserve (27°42'S:29°59'E), at ca 1830 m. Known from a broad area in the northern part of the central Drakensberg mountain range, along the banks of Ulumbi [iNcandu] R., indigenous forest on the left side of the river, and numerous sites on the grassland plateau and its vicinity (27°42'S:29°59'E; 27°45'30"S:29°42'30"E; 27°54'03"S:29°41'36"E).

Material examined: Holotype NMSA/Olig.02313, 1 cl paratype NMSA/Olig.02312; 4 cl NMSA/Olig.03489.

External and internal characters are in accord with the species description. Setae moderate in size, conspicuous, paired in four regular rows on the whole body length. In the clitellar area a distance between *ab* pairs is slightly smaller than preclitellarly, although no irregular uplifting of setae was noted. In all mature individuals the spermathecae are similar in appearance, different in size, one to three pairs per segment, in pre-testicular segment 9, and in testicular segment 10. Ampullae globular with elongated ducts. Anterior spermathecal pores in intersegmental furrow 9/10, posterior in 10/11. In the majority of specimens collected in January (local summer), sperm is present in all ampullae; in material collected in October (local early spring) the ampullae are usually empty.

This species, although most similar to *lesothoensis* and *adami* in having both spermathecal pores immediately next to testicular segment, in 9/10 and 10/11; differs in the arrangement of setae, not manifesting any discrepancy in the clitellar area. From *oresbiosus*

it differs in the lack of additional spermathecae in segment 9 with its intrasegmental pores.

This species is distantly isolated from the other species of the group. It is found at lower altitudes than other species of the group in the central Drakensberg, occurring on plateau covered by grasses, bushes and small patches of indigenous forest. Being abundant, *P. bourquini* shows some gradual changes in reduction of the anterior spermathecae, and discrepancy of the setae.

*Proandricus sani* Plisko, 2002

Fig. 5

*Proandricus sani*: Plisko 2002: 185; 2003: 295.

Type locality: Lesotho: Sani Plateau at 2906 m (29°35'S:29°16'E). Known only from the type locality in the southern part of the central Drakensberg mountain range, located in Lesotho.

Material examined: Holotype NMSA/Olig.02715.

External and internal characters are in accord with the species description. Spermathecae spherical, with long ducts, in 9 and 10. Spermathecal pores intrasegmentally in 9 and intersegmentally in 10/11. Anterior spermathecae of segment 9 empty, with duct indicating intrasegmental opening in 9, not detected externally. Posterior spermathecae much larger than anterior, with slight iridescence signifying pore activity during mating. Similar intrasegmental pores in 9 are known in *oresbiosus* and *amphius*, although some relationship might be expected with *amphius*, in which also the spermatheca of 9 is not active. Differences between these species are in the shape and commencement of the intestine, which for *sani* is segment 12, with no loops, but in *amphius* it is 13 with developed loops in 21–27. Setae are irregularly shifted in the clitellar area, confirming the species' accreditation to the *lesothoensis* species-group.

*Proandricus adami* Plisko, 2003

Fig. 6

*Proandricus adami*: Plisko 2003: 295.

Type locality: South Africa: KZN, Royal Natal National Park, Bank of Devil's Hoek River, approx. 28°42'S:28°54'E. Known from numerous sites located in the central Drakensberg mountain range.

Material examined: Holotype NMSA/Olig.02530; 3 cl paratypes NMSA/Olig.00918 and NMSA/Olig.00919. New material: South Africa, KZN, Royal Natal National Park, forest, 13 cl and 5 juv collected during March–April 2006 from 6 slightly distanced sites by MDTP KwaZulu-Natal University students group: NMSA/Olig.04203, NMSA/Olig.04204, NMSA/Olig.04215, NMSA/Olig.04246, NMSA/Olig.04249, NMSA/Olig.04252a, NMSA/Olig.04254, NMSA/Olig.04304, NMSA/Olig.4307, NMSA/Olig.04308.

The external morphological characters in all examined material agree with the original description. Although, preclitellarly and postclitellarly the setae are closely paired in regular rows, and in the clitellar area some of the pairs of *ab* are shifted up between *bc*, forming an irregular arrangement, some additional abnormality in setal arrangement was noted. On segment 16 and 18 in inter-setal space between *ab* and *cd*, a pair of additional setae occurred, making five pairs of setae per segment; in the other two specimens, two additional pairs of setae were placed above *ab*, giving six pair per segment

on three clitellar segments. This abnormal phenomenon has been noted in three clitellate specimens and on two juvenile, selected from the population of 18 individuals collected in the Royal Natal National Park forested area. Spermathecae variable in shape and size. Anterior pairs elongated, always evidently smaller than posterior, often containing sperm. Posterior oval, large, with thin, elongated duct. In the majority of specimens examined, the posterior spermathecae are iridescent, filled with sperm and transparent through the body wall. Spermathecal pores in intersegmental furrows 9/10 and 10/11, although the pores of 9/10 are externally difficult to trace. Clitellum slightly resembling this of *amphius*, although on various individuals it was variably developed, unequally encircling segments ventrally.

*P. adami*, although noticeably similar to the species of the *lesothoensis* species-group, manifests irregularity in the setal arrangement extending to abnormality. Similar to *bourquini*, and *lesothoensis*, but differs by peculiarly arranged setae.

*Proandricus amphius* Plisko, 2003

Fig. 7

*Proandricus amphius*: Plisko 2003: 297.

Type locality: South Africa: KZN, Injasuthi (29°06'51"S:29°26'34"E), wooded grassland.

Material examined: Holotype NMSA/Olig.03620; 9 paratypes NMSA/Olig.03621. Known only from the type locality in the central Drakensberg mountain range.

External and internal characters are in accord with the species description. Present examination confirms the presence of spermathecae in 9 and 10, the anterior pair with not fully developed ducts located intrasegmentally in segment 9, empty. The posterior pair with ducts deeply embedded in body tissues, entering intersegmentally furrow 10/11, always iridescent, containing sperm. The lack of sperm in the anterior spermathecae suggests that the ducts are not open, or are not used during mating. Spermathecal pores in 10/11, although externally not noted, being deeply embedded in body tissues, active. Similar to *sani*, although some morphological differences were observed (compare notes under *P. sani*).

*Proandricus oresbious* Plisko, 2003

Fig. 8

*Proandricus oresbious*: Plisko 2003: 299.

Type locality: South Africa: KZN, Royal Natal National Park, north side of Devil's Hoek River (28°41'S:28°53'E). Known from a few sites in the central Drakensberg mountain range.

Material examined: Holotype NMSA/Olig.02440; 3 cl paratypes NMSA/Olig.02434 from Bergville area (28°42'S:29°22'E). New material: NMSA/Olig.03878, 2 cl *Free State*: ca 70 km north of Mont-aux-Sources, on extended grassland, slightly below 2500 m (28°38'10.62"S:28°57'39.84"E).

External and internal characters are in accord with the species description. However, it should be added that the intestine in segments 21–27, 28 is covered by twisted, paired folds, similar to those observed in *adami*. Setae, although minute on type material, are slightly larger in the clitellar area on the newly examined specimens, but their irregular shifting on the few segments is difficult to locate. Spermathecae in all dissected specimens are minute, ampullae oval, ducts elongated on bulbous basis. Two pairs of spermathecae are located in the anterior and posterior parts of segment 9; always smaller than the pair

in 10. Ectal parts of anterior pair of 9 enter the body wall intrasegmentally between segmental ringlets, or in intersegmental furrow 8/9, with pores not noted externally. Spermathecal pores of the posterior pair of 9 open intersegmentally in 9/10. Pores of spermathecae located in 10 are in 10/11. Although examined individuals reveal external and internal maturity, having well developed clitella, tubercula pubertatis and spermathecae, iridescence was observed in tiny parts of the ampullae in only two individuals. This species, having three pairs of spermathecae, of which two pairs are in one segment opening intrasegmentally and intersegmentally, differs markedly from others.

The Mont-aux-Sources area, located in the northern part of the central Drakensberg, is adjacent to the Royal Natal National Park, and the new collecting site lies in the



Figs 6–8. Habitus and enlarged clitellar area of *Proandricus* species: (6) *P. adami*, (7) *P. amphius*, (8) *P. oresbiosus*. Scale bars = 1 cm.

neighbourhood of the species' type locality. Both sites are located in the area of the water source for the Vaal, Orange and Thukela rivers, which descend west to the Atlantic Ocean, and east to the Indian Ocean respectively. It is possible, that, in addition to other factors, the distribution of this species is correlated with rainfall and plunged rivers.

*Holandric species:*

*Geogenia benhami* (Rosa, 1891)

Fig. 9

*Microchaeta benhami*: Rosa 1891: 382; Beddard 1895: 673.

*Microchaetus benhami*: Michaelsen 1891: 382; 1900: 451; 1913: 538; 1918: 316; Pickford 1975: 2; Reynolds & Cook 1976: 77; Plisko 1992: 342; 1993: 235; 1995: 46 [designation of lectotype, deposited in ZMH]; 2003: 288.

*Geogenia benhami*: Plisko 2006: 43.

Type locality unknown. Type material declared as "*typus amissus*" by Reynolds and Cook (1976). Lectotype designated by Plisko (1995) on the material deposited in ZMH, collected in: South Africa, Western Cape, Bergvleit near Constantia (34°01'S:18°27'E).

Material examined: 7 mature and 5 semimature specimens deposited in USNM, with no. 52.831, lectotype with no V 183, deposited in ZMH, 1 deposited in NMHW with no. 4811, and 2 mature specimens NMSA/Olig.03925 collected recently in Western Cape, Stellenbosch area.

Although the locality was not indicated at the time of the species description, Michaelsen (1891) and Pickford (1975) reported its occurrence in the Western Cape: the Constantia area, Cape Town, and Stellenbosch, and some of the collected material is deposited in ZMH and USNM. In examined specimens, the anterior spermathecae and their pores occur in intersegmental furrow 10/11, with the following in 11/12, 12/13, 13/14, 14/15, 15/16. Spermathecae in this species are multiple in one segment, usually being more than three, sometimes five or six. Setae are arranged in four even pairs, showing no discrepancy.

Although the anterior spermathecal pores in this species are not located anterior to the testicular segment, their unusual position is of great interest. Only *mkuzi* is similar to this species, though it differs anatomically and in distribution.

Considering this species' holandric condition, the presence of spermathecae in both testicular segments (10 and 11), and also the numerous spermathecae posterior to testes in segments 12, 13, 14, 15, and the regular arrangement of setae, no similarity to the species accredited to *lesothoensis* species-group has been found. Its distant occurrence from the sites of species distributed in the Drakensberg mountain range confirms its exclusion.

From verbal information provided by Frana Fourie who deposited new material in the NMSA, the species occurs in the Stellenbosch area in large populations. Recorded from natural, not disturbed sites, and also from an arable cultivated field.

*Geogenia namaensis* (Michaelsen, 1908)

*Microchaetus namaensis*: Michaelsen 1908: 40; 1918: 319; Reynolds & Cook 1976: 143; Plisko 1992: 339; 1995: 48 [redescription].

*Geogenia namaensis*: Plisko 2006: 44.

Type locality: South Africa: Northern Cape, Komaggas, Namaqualand (29°48'S:17°30'E). Species type locality given by Michaelsen is 'Klein-Namaland, Kamaggas'.

The absence of spermathecae, reported in the species description by Michaelsen (1908), obscured its relationship with other microchaetids known at that time. Plisko

(1995), re-examining type material, found spermathecae, although small, in segments 10, 11, 12, with their ducts close to intersegmental furrows 9/10, 10/11, 11/12. The location of spermathecae in the testicular segments, with one pair of pores anterior to the testes, is unique in holandric species, and to date it has been observed only in this species, and in two species of *Geogenia* and one of *Tritogenia*, which are also discussed in this paper. The arrangement of setae described by Michaelsen and confirmed by myself does not reveal any irregularities in the clitellar area.

This species obviously differs from the proandric species accredited to the *lesothoensis* species-group in being holandric, with setae arranged in four regular rows and no discrepancy in the arrangement. The location of spermathecae and their pores is unique, with the anterior pair in furrow 9/10.

Not much is known about the species' type locality, other than that it was found in the northern part of Namaqualand. From the data given for the other species mentioned in the species description, it might be concluded that *namaensis* was found together with the introduced *Microscolex phosphoreus* (Dugès, 1837) and *Aporrectodea caliginosa* (Savigny, 1826), possibly in a newly created garden, or introduced from some other site.



Figs 9, 10. Habitus of *Geogenia benhami* (9) and *G. mkuzi* (10). Scale bars = 1 cm.

As only the type material is known, and no new specimens have been found in the type locality area or other parts of South Africa, it is difficult to speculate on its relationship with its congeners.

*Geogenia mkuzi* (Plisko, 1992)

Fig. 10

*Microchaetus mkuzi*: Plisko 1992: 341; 1998: 273; 2003: 281.

*Geogenia mkuzi*: Plisko 2006: 44.

Type locality: South Africa: KZN, Mkuzi Game Reserve (27°36'S:32°15'E).

Material examined: Holotype NMSA/Olig.00630, 8 paratypes NMSA/Olig.00632; 5 cl NMSA/Olig.01759 from KZN Itala Game Reserve.

The species is known from two sites, both in the northern part of KZN, much distant from the similar, possibly related, *benhami*. In *mkuzi*, the spermathecae and their pores occur in a similar position to those in *benhami*, e.g. in 10/11, 11/12, 12/13, 13/14, 14/15, 15/16. However, in one specimen, the first spermathecal pores on the right side are found anterior to first pair of testes in 9/10, having the following five in 10/11, 11/12, 12/13, 13/14, 14/15. On the left side they are in 10/11 and the following furrows. Setae are arranged in regular rows.

The presence of spermathecal pores in both testicular segments in holandric species is unusual for microchaetids, and was noted only in *benhami* and *mkuzi*. Both species, however, show no similarity to the *lesothoensis* species-group.

*Geogenia distasmosa* (Plisko, 2003)

Fig. 11

*Microchaetus distasmosus*: Plisko 2003: 282.

*Geogenia distasmosa*: Plisko 2006: 44.

Type locality: South Africa: KZN, Mapelane Nature Reserve (28°54'S:31°55'E). Known from the type locality and a slightly distanced site in the Umfolozi Game Reserve (28°22'S:31°52'E).

Material examined: 8 paratypes, mature specimens NMSA/Olig.00626a, collected on the bank of the White Mfolozi River.

As the holotype was slightly injured after extended dissection during its description, eight paratypes have been examined. External and internal characters are in accord with the species description. Spermathecae small, one to two per segment, in 8 and 9. Ampullae small, variable in size; elongated, ducts short, embedded in body wall, close to septa 8/9 and 9/10. Spermathecal pores in 8/9 and 9/10, externally difficult to locate. No iridescent was observed in the spermathecae, although all examined individuals have clitella. Setae in four regular rows.

The location of the anterior spermathecae and their pores in pre-testicular segments is unique among holandric species, and in *Geogenia* is known only in *namaensis*, *distasmosa* and *quaera*. Possibly these two last species are related, differing only in the shape and location of the clitellum and tubercula pubertatis. The distribution of this species might be controlled by the water of the White Mfolozi River.

*G. distasmosa* differs from species accredited to the *lesothoensis* species-group in being holandric, having the setae arranged in four regular rows and not demonstrating

discrepancy in their arrangement. Also its geographical occurrence is distant. The location of spermathecae and their pores in pre-testicular segments possibly may be accounted for by variation or divergence occurring between holandric species.

*Geogenia quaera* (Plisko, 2003)

Fig. 12

*Microchaetus quaerus*: Plisko 2003: 286.

*Microchaetus querus* [printing error]: Plisko 2006: 45.

*Geogenia quera* [printing error]: Plisko 2006: 45.

Type locality: South Africa: KZN, Hluhluwe Game Reserve Mbombe Forest (28°03'15"S: 32°03'12"E), at 484 m, forest valley. Known only from the type locality.

Material studied: Holotype NMSA/Olig.03659; 1 cl paratype NMSA/Olig.03664.

External and internal characters are in accord with species description. Location of spermathecal pores, although being difficult to trace, in 8/9 and 9/10, is known also in holandric *distasmosa*. Both species might be related, although differ in the shape and location of clitellum and tubercula pubertatis. (Compare notes under *G. distasmosa*.)

*Tritogenia phinda* Plisko, 2005

*Tritogenia phinda*: Plisko 2005: 112.

Type locality: South Africa: KZN, Phinda (27.87726°S:32.33983°E). Known only from the type locality.

Unfortunately, the type specimen NMSA/Olig.03907 is in unsatisfactory condition for examination. From the original species description it is known that one pair of small spermathecae occurs in segment 9, and a single spermatheca at the right side in segment 10. Their spermathecal pores have been observed during species dissection in



Figs 11, 12. Habitus and enlarged clitellar area of *Geogenia distasmosa* (11) and *G. quaera* (12). Scale bars = 1 cm.

intersegmental furrow 9/10 and 10/11, which is unique in the holandric genus *Tritogenia*. Considering the abnormal condition with the only one spermathecae at one side in segment 10, it might be supposed that this individual may have been undergoing some abnormal development. Until more specimens confirming described characters are available for further study, no decision on the taxonomical position of this species can be taken.

#### DISCUSSION

The occurrence of spermathecal pores in pre-testicular intersegmental furrows or intrasegmentally is rare in the South African indigenous Microchaetidae. This character, known in the South American sister family Glossoscolecidae, has been accepted as one of the distinctive characters separating these two families. This unique occurrence, although it is seldom found in microchaetids, has been recognized up to now in seven proandric taxa and in four species in two holandric genera. In *Proandricus* this includes *lesothoensis*, *pajori*, *bourquini*, *sani*, *adami*, *amphius*, and *oresbiosus*; and among holandric species, three of *Geogenia*: *namaensis*, *distasmosa*, *quaera*, plus *Tritogenia phinda*. In the proandric species, additional to the locations in pre-testicular spermathecal pores, some irregularity in the arrangement of the *ab* setae, also a unique character, has been observed. These two characters (Table 1) and the geographical distribution of the species (Fig. 1) confirm the grouping of these seven taxa in the *lesothoensis* species-group, as documented by Plisko (in press). This species-group occurs in the central Drakensberg mountain region, extending through the northern part of Lesotho, its surrounding area stretching to KwaZulu-Natal and neighbouring parts of the Free State, within Gondwanaland. The formation of the Drakensberg mountains started with the volcanic activity at the beginning of the breakup of Gondwana some 180–190 million years ago (Duncan & Marsh 2006; Watkeys 2006). Uplift, periodic earth movements, water erosion and many other factors later shaped the steep ridges and other mountain formations, setting up the whole Drakensberg mountain range from the southern area of the Eastern Cape to Zimbabwe. Most of the central Drakensberg mountain range lies on the plateau at *ca* 3000 m, with its highest peaks in Lesotho (Thabana Ntlenyana, 3482 m) and South Africa (Mont-aux-Sources, 3290 m). Both peaks are the water source for the Vaal and Orange rivers, which descend in the west to the Atlantic Ocean, and the Thukela River, which runs east to the Indian Ocean. The whole area is well known for its endemic flora and fauna.

The species ascribed to the *lesothoensis* species-group occur on moist slopes at altitudes over 1500 m, often reaching more than 3000 m. They tolerate a wide range of temperature, as the climatic changes from very hot in the summer months to very cold in winter, with snowfalls in July–August. Little is known about the biology of these species, except that some of them occur in shallow, rocky, leached soil and basaltic sand, under rocks, or in the litter from afro-montane grasses, various other plants, and the fallen leaves of indigenous and alien trees and bushes. They are also found on the grasslands plateau, in valleys and at riverbanks at high-altitude sites in the central Drakensberg, while they also extend to the Drakensberg foothills. Most of the species, as currently known, seem to have a somewhat restricted distribution, but it must be said that the whole area where they have been found has been relatively poorly sampled. For a more accurate assessment of their range, further sampling should be done.

TABLE 1

Location of spermathecal pores and arrangement of setae in the *lesothoensis* species-group, and first anterior pair in other proandric species.

Species name	Spermathecal pores in	Arrangement of setae	Distribution
<i>oresbiosus</i>	8/9 9 9/10 10/11	slightly irregular	28°41'S:28°53'E, 28°42'S:29°22'E
<i>amphius</i>	9 10/11	regular	29°06'51"S:29°26'34"E, at ca 1540 m
<i>sani</i>	9 10/11	irregular	29°35'S:29°16'E, at ca 2906 m
<i>lesothoensis</i>	9/10 10/11	irregular	approx. 28°50'S:28°30'E, at ca 3300 m
<i>adami</i>	9/10 10/11	irregular + anomalia	28°42'S:28°54'E, and in close vicinity
<i>bourquini</i>	9/10 10/11	regular	27°42'S:29°59'E; 27°45'30"S:29°42'30"E; 27°54'03"S:29°41'36"E.
<i>pajori</i>	10/11	irregular	approx. 28°55'S:29°09'E, at ca 1800 m
Not <i>lesothoensis</i> group			
<i>notabilis</i>	10/11 11/12 12/13	regular	27.77751°S:32.3107°E at sea level
<i>timmianus</i>	? 8/9 9/10 10/11 11/12 12/13 13/14	irregular	[33°45'S:25°25'E]
Other proandric species	First pair in: 11/12 + or 12/13 +	regular regular	

The type locality for *lesothoensis* in the Butha-Buthe District at ca 3300 m (approx. 28°50'S:28°30'E) lies in the neighbourhood of the Royal Natal National Park, where *adami* (28°42'S:28°54'E) and *oresbiosus* (28°41'S:28°53'E) were found. On the Sani plateau, in the southern part of the central Drakensberg, at ca 2900 m, south of both previously mentioned sites, *sani* was collected. On the grassland plateau in the Ncandu Forest Reserve (27°42'S:29°59'E) outlying to the north at ca 1830 m, *bourquini* occurs at numerous sites on both sides of the Ulumbi River. In the Drakensberg foothills, *pajori* occurs at ca 1800 m (28°55'S:29°09'E), and *amphius* at 1540 m (29°06'51"S:29°26'34"E). The species are usually found near water and, in addition to other factors, their distribution from higher altitudes maybe determined by plunged rivers and rain-water. Multiple samples of *P. adami*, *oresbiosus* and *bourquini* confirm their occurrence in the eastern, western and northern central Drakensberg areas, extending to the foothills. The distribution of those species known only from the type locality, or described from only one specimen, cannot be established. To assess the distribution of *lesothoensis*, *pajori*, *amphius*, and *sani*, more material is needed.

The members of the *lesothoensis* species-group differ noticeably from the other known South African microchaetids. Differences are in the location of spermathecae and their pores, and in the arrangement of *ab* setae, and these characters are sometimes coupled. In examined individuals, the spermathecae are sometimes found fully developed, with openings able to accept partner's sperm during mating, or are not fully

formed, with intrasegmentally marked, non-functional pores. The shape and size of the spermathecae in specimens undergoing maturation, or before mating, or after discharging sperm, differ from those filled with sperm. These states may mislead correct estimation of their development. In each case when only one examined individual showed these two specific dispositions, the other characters currently accepted for species taxonomical valuation have been inspected. However, for more precise species taxonomical recognition, more up-to-date methods are required.

The phylogenetic significance of the spermathecae in the Oligochaeta has been disputed for decades, and the problem is probably insoluble (Brinkhurst & Jamieson 1971). However, the quality of the spermathecae and their locations are commonly used for taxonomic purposes at the species, generic, or family levels (Michaelsen 1928; Omodeo 1998, 2000). Michaelsen (1918) regarded the pre-testicular condition as ancestral to all glossoscolecids (*sensu lato*) and applied them in the microchaetid separation from glossoscolecids (*sensu lato*). Though Brinkhurst and Jamieson (1971) opposed this, the character is still in use as one, together with others, to distinguish Microchaetidae from Glossoscolecidae. The discovery of the pre-testicular and testicular locations of spermathecae in a group of the microchaetid species occurring in the part of Gondwana, which is now the central Drakensberg mountain range, has a significant value. The character, known in South American glossoscolecids and now found in South African microchaetids, suggests a closer relationship than is commonly accepted between the two families. The discrepancies in setal arrangement observed in some species of the *lesothoensis* species-group might be comparable with the setal irregularities noted by Moreno (2004) in seven genera of Glossoscolecidae, and in ten of 19 species of *Pontoscolex* (Schmarda, 1861).

Whether the species of the *lesothoensis* species-group originated from one ancestral species that later undergone diversification, or different species developed unique features described herein independently, they demonstrate a unique combination of characters. This syndrome could have been inherited from a common ancestor of Microchaetidae+Glossoscolecidae or developed after the separation of the continent from the rest of Gondwana.

The holandric species *Geogenia distasmosa* (28°54'S:31°55'E) and *quaera* (28°03'15"S:32°03'12"E) with testes in segments 10 and 11, and their spermathecal pores in intersegmental furrows 8/9 and 9/10, although having pores in the pre-testicular segments, differ from species accredited to the *lesothoensis* species-group in being holandric and having a regular arrangement of the setae. The two species are similar to each other and may be related, although they differ in other features. They also differ from their other congeners, in which anterior spermathecal pores usually occur in 11/12, or 12/13, or 13/14. In two other, exceptionally interesting, species of this genus, *benhami* (34°01'S:18°27'E) and *mkuzi* (27°36'S:32°15'E), anterior pores occur in 10/11, e.g. between the segments containing testes (Table 2), and five other pores posterior to the testes. Both species, similar to each other in external and internal morphology and exceptionally differing from other congeners, occur in different biotopes, separated by over 2000 km, and live under different climatic and ecological conditions. The intended or coincidental introduction of these species is not expected, leaving the distribution of the species under puzzled question.

TABLE 2

Location of anterior spermathecal pores in holandric *Geogenia* species.

Species name	Anterior spermathecal pores in
<i>distasmosa</i>	8/9 and 9/10
<i>quaera</i>	8/9 and 9/10
<i>namaensis</i>	9/10 10/11 11/12
<i>benhami</i>	10/11 +
<i>mkuzi</i>	10/11 +
other species of this genus	11/12 + 12/13 + 13/14 +

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