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# Six new species of Afrotropical *Allodia* (Diptera: Mycetophilidae): DNA barcodes indicate recent diversification with a single origin

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## Abstract

Only one species of the genus *Allodia* has been previously recorded from the Afrotropical region, *Allodia (Brachycampta) flavorufa* Matile, 1978. Six new species are described here, all representing the nominotypical subgenus, *Allodia s.s*. The new species are described from material collected in different mountainous areas in south and east Africa; *A. jaschhofi* **sp. nov.**, *A. karkloofensis* **sp. nov.**, *A. drakensbergensis* **sp. nov.**, *A. nyeriensis* **sp. nov.**, *A. mazumbaiensis* **sp. nov.** and *A. keurbosensis* **sp. nov**. The species are morphologically very similar, and can only be separated based on minor differences in wing venation and characters of the male terminalia. The genetic differences between the species in the DNA barcode region (CO1), however, support delimitation. The origin and distribution of these Afrotropical taxa, in relation to each other and to their Holarctic relatives, is discussed.

**Key words**: Fungus gnats, Exechiini, Africa, CO1, mountains

## Introduction

Very little is known about dipteran diversity in the Afrotropical region, and estimates suggest that as much as 2/3 of the species are yet to be described (Kirk-Spriggs & Stuckenberg 2009). One family, the Mycetophilidae (true fungus gnats) is considered to be one of the most poorly known families of Diptera from the region (Kirk-Spriggs & Stuckenberg 2009). In the Afrotropical region the subfamily Mycetophilinae is considered to be relatively rare in lowland habitats, but more common in mountainous areas (Matile 1980). The fungus gnat fauna in high altitude forests of the region is more similar to that in the Holarctic regions, where the subfamily has its greatest diversity (Matile 1980). The subfamily consists of two tribes: Exechiini and Mycetophilini.

The genus *Allodia* Winnertz, 1863 belonging to the tribe Exechiini, is split into two subgenera, *Allodia s.s.* and *Brachycampta* Winnertz, 1863 (Tuomikoski 1966). The greatest species diversity is found in the Holarctic region, but the genus is considered to be sub-cosmopolitan by Bechev (1999). As for most other genera in the tribe the complete distributions of most species are uncertain. Zaitzev (1983; 1984) revised the Holarctic species of *Allodia s.s.* and *Brachycampta*, separately, and presented keys to all species. The separation of the two subgenera is based on morphological differences i.e. discal bristles, wing venation and abdominal color markings (Zaitzev 2003). The outline of the male terminalia in the two subgenera is also notably different. In addition to the species included in the revisions by Zaitzev (1983; 1984) the genus contains several names which may represent synonyms and invalid species. As such, the subgenus *Allodia* includes 41 extant species; 8 species have a Holarctic distribution, 9 are Nearctic, and 20 are Palaearctic (including 6 species described from China by Wu, Zheng & Xu (2003)). Further, two species are described from India and Ceylon (Brunetti 1912; Senior-White 1922), one from Malaysia (Edwards 1928) and one from the Palau Island, Micronesia (Colless 1966). The other subgenus *Brachycampta,* includes 41 extant species, of which 8 are considered Holarctic, 7 Nearctic, 25 Palaearctic and one Afrotropical.

As indicated above, only one species of *Allodia* has been formally described from the Afrotropical region, *A.* (*Brachycampta*) *flavorufa* Matile, 1978 from the Comoros. We have not examined specimens of this species, but the description is well illustrated and based on the morphology of the male terminalia, it obviously belongs to

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*Allodia*. It is, however, not similar to any of the species described herein. According to Søli (2017), four additional Exechiini genera are known from the Afrotropical region; *Brevicornu*, *Exechia*, *Pseudexechia* and *Rymosia*.

The mitochondrial molecular marker cytochrome c oxidase subunit I (CO1) was chosen in this study. CO1 is the standard DNA barcode region for animals (Hebert *et al*. 2003), and has previously been successful in separating closely related species in Mycetophilidae (e.g. Ševčík *et al*. 2016; Jürgenstein *et al*. 2015).

Material from the Afrotropical region is often scarce and many taxa are undescribed. In this study, the subgenus *Allodia* is recorded for the first time from the region, and six new species are described. We were able to sequence CO1 from four of the species, which, together with differences in morphology, forms the basis for separating the different species of Afrotropical *Allodia*.

## Materials and methods

The study is based on material kept in the Natural History Museum, University of Oslo, Oslo, Norway (NHMO), Tromsø University Museum, Tromsø, Norway (TMU) and in the National Museum, Bloemfontein, South Africa (BMSA). The material consists of seven males and three females, all stored in ethanol, except for one specimen preserved in glycerol and one pinned specimen. The specimens were collected in Tanzania, Kenya and South Africa between 1990 and 2009. The outgroup taxa were selected based on available CO1 sequences in the Barcode of life database (BOLD), we included 12 sequences, representing six species of *Allodia* from the Holarctic (Appendix 1).

**Slide preparation**. All specimens were dissected in ethanol and glycerol. The terminalia were heated in lactic acid to remove soft tissue, and then transferred to glycerol. Temporary slide mounts were prepared by using glycerol as a medium. The temporary slides were studied and photographed using Zeiss Axio imager M2, fitted with the camera Axiocam 506 color. The drawings were made by the use of pictures and microscope in Adobe Illustrator. After being studied, the terminalia was permanently preserved on glycerol in microvials, and stored together with the pinned specimens.

**Terminology**. The general terminology follows Søli (1997). For the terminalia, the terminology is primarily in accordance with Kjærandsen (2006; 2009); deviations are listed under the results section.

**DNA extraction, amplification and sequencing**. DNA was extracted from one leg of each of the 10 individuals using the Tissue DNA Spin Protocol of E.Z.N.A® Tissue DNA Kit. One leg from each individual was minced using micro beads in TL 200 µL TL Buffer and incubated at 55?C, the lysis proceeded overnight. We followed steps 4 - 24 of the protocol. The last elution steps (24–27) were done once with 100 µL Elution Buffer incubated at 60 ?C for 10 min to obtain higher DNA concentration. All DNA extracts were deposited and stored at -80?C in the DNA bank at the Natural History Museum, University of Oslo (see Table 1).

The DNA concentration was measured with Qubit 2.0 Fluorometer (Invitrogen). The amount of DNA extract in the PCR reaction was adjusted to the quantified DNA concentration because of low concentrations of DNA (< 0.117 ng/µL) in the samples. The final concentration was set to 0.5 ng DNA.

The Folmer primers LCO1490 (forward) and HCO2198 (reverse) were used to amplify a fragment of 658 base pairs (bp) from the CO1 at the 5' end (Folmer *et al*. 1994). The PCR setup for a total reaction volume of ~25 µL included; 12.3 µL $dH_2O$, 2.5 µL Buffer, 0.75 $MgCl_2$, 0.75 µL dNTP, 0.75 µL of each primer, 0.5 µL DMSO, 0.1 µL Platinum® Taq polymerase (Invitrogen) and 0.5 ng DNA. The PCR was set up with the following protocol: Initial denaturation at 94°C for 1 min, followed by 40 cycles of denaturation at 94°C for 30 sec, synthesis at 51°C for 30 sec and elongation at 72°C for 1 min, with a final elongation step at 72°C for 7 min. To control, check for product and length of CO1 sequences, gel electrophoresis was performed. The PCR products for two of the samples, representing two hypothesized species, did not show on the gel. Therefore, we continued with 8 samples for sequencing.

The sequencing was performed in the DNA laboratory, at the Natural History Museum, University of Oslo; the PCR products were purified with ExoSAP-IT (Stratagene), cleaned through ethanol precipitation and sequenced using BigDye 3.1 on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

**Sequence editing**. Sequences were edited and assembled using CodonCode Aligner version 4.2.7 (CodonCode Corporation, USA). The sequences were aligned using the MUSCLE algorithm (Edgar 2004) together with 12 sequences, representing six outgroup taxa, retrieved from BOLD (see Appendix 1). The Neighbor joining (NJ;

Saitou & Nei 1987) analysis was computed using the p-distance method (Nei & Kumar 2000), with bootstrap test using 1000 replicates (Felsenstein 1985). The p-distance method was selected based on the results of Sivathsan and Meier (2011), and Collins *et al*. (2012), as the Kimura-2-parameter model gave qualitatively the same result. Additionally, the interspecific p-distances (between group distances) were calculated. The alignment, calculation of p-distances and NJ analysis was done in MEGA version 7 (Kumar *et al*. 2016). The sequences have been uploaded to Genbank, with accession numbers KY370970–KY370977.

**TABLE 1.** Specimen information. The collection number, species names, locality in short, sex, accession number in the DNA bank (The Natural History Museum, University of Oslo), the measured DNA concentration (Qubit 2.0 Fluorometer (Invitrogen)) in DNA extract and Genbank accession number are given. **Abbreviations**: TMU = Tromsø University Museum; NHMO = Natural History Museum, University of Oslo; BMSA = National Museum Bloemfontein, South Africa.

| Collection number | Species | Locality | Sex | Acc. number DNA extract | DNA concentration (ng/ µL) | Acc. number Genbank |
|---|---|---|---|---|---|---|
| TSZD-JKJ-101654 (TMU) | *A. jaschhofi* | **South Africa**, KwaZulu-Natal, Central Drakensberg | M | NHMO-DAR-9365 | 0.073 | KY370977 |
| TSZD-JKJ-101655 (TMU) | *A. jaschhofi* | **South Africa**, KwaZulu-Natal, Central Drakensberg | F | NHMO-DAR-9366 | 0.072 | KY370976 |
| TSZD-JKJ-101653 (TMU) | *A. karkloofensis* | **South Africa**, KwaZulu-Natal, Howik district | M | NHMO-DAR-9367 | 0.064 | N/A |
| TSZD-JKJ-104243 (TMU) | *A. drakensbergensis* | **South Africa**, KwaZulu-Natal, Northern Drakensberg | M | NHMO-DAR-9368 | 0.083 | KY370975 |
| TSZD-JKJ-104239 (TMU) | *A. nyeriensis* | **Kenya**, Nyeri, Mount Kenya National Park | M | NHMO-DAR-9369 | 0.089 | KY370974 |
| TSZD-JKJ-104240 (TMU) | *A. nyeriensis* | **Kenya**, Nyeri, Mount Kenya National Park | M | NHMO-DAR-9370 | 0.065 | KY370973 |
| TSZD-JKJ-104241 (TMU) | *A. nyeriensis* | **Kenya**, Nyeri, Mount Kenya National Park | F | NHMO-DAR-9371 | 0.117 | KY370972 |
| TSZD-JKJ-104242 (TMU) | *A. nyeriensis* | **Kenya**, Nyeri, Mount Kenya National Park | F | NHMO-DAR-9372 | 0.104 | KY370971 |
| 251659 (NHMO) | *A. mazumbaiensis* | **Tanzania**, Tanga region, West Usambara mts. | M | NHMO-DAR-9373 | n.a. (<0.05) | N/A |
| Allodia01/loanBMSA (BMSA) | *A. keurbosenis* | **South Africa**, Western Cape, Keurbos forest | M | NHMO-DAR-9374 | 0.077 | KY370970 |

## Results

### The terminalia of Afrotropical *Allodia*

The male terminalia consists of tergite 9, cerci, proctiger, gonocoxites and gonostyli. Tergite 9 is divided; each part with two strong apical bristles of different length. The epiproct is usually strongly or entirely reduced, or the two cerci, shaped as two narrow lobes, represent a composite structure, including the epiproct. Two lobes below the cerci are here termed the pseudocerci, in accordance with the interpretation in *Tarnania* (Kjærandsen 2006) and *Pseudexechia* (Kjærandsen 2009). The hypoproct is present as a weakly sclerotized, bare, triangular plate, ventral of the pseudocerci. The aedeagal complex is indistinct, connected to a pair of sclerotized, slender apodemes, extending laterally (Fig. 1F). The two gonocoxites are weakly connected ventrally. The hypandrial lobe (following Kjærandsen (2006; 2007; 2009)), is present ventrally between the gonocoxites, forming an elongate structure,

whose shape varies between the species (Fig. 1F). The gonostylus consists of three lobes; here termed dorsal, median and ventral lobe respectively (Fig. 1G), all seemingly articulating basally or sub-basally. The dorsal lobe is most prominent and well sclerotized, and is laterally flattened with a membranous area at the apex, and varies in size and outline between the species. The median lobe is ~rectangular, with posterodorsal corner distinctly tapered. The ventral lobe is long and narrow and slightly club-shaped with apical setae, and is only slightly shorter than the median lobe. In addition to these three lobes, the gonostylus contains a compound basal portion, representing the posterior part of the membranous internal branch. This portion is partly divided in most species, with several short setae (Fig. 1E). A small rounded sclerite is present just anterior to the gonostylus, fused or articulating with the dorsal edge of the gonocoxite. This sclerite is illustrated in Sasakawa & Ishizaki (2003) for *A. laccariae*, but is not discussed.

The female terminalia consist of hypogynal valves with labia, cerci and gonapophyses (Fig. 2). The hypogynal valves are located apically of sternite 8 and seemingly form the bilobed end of sternite 8, each valve with several long setae and one especially long one at the apex. Between the hypogynal valves are the labia, forming a membranous, pointed lobe. The cerci are two-segmented; first segment dorsally fused, with several strong setae; second segment ovoid to round. The gonapophyses are fused into a lobe-like structure with several setae at the apex.

**Important characters.** The male genitalia hold most of the characters that can be used to separate the species. Of most importance are the membranous area of the dorsal lobe, the shape and chaetotaxy of the median and ventral lobe, and the chaetotaxy and outline of the basal part of the gonostylus.

## The species

### *Allodia nyeriensis* Magnussensp. nov.

(Figs 1, 2)

**Diagnostic characters.** *A. nyeriensis* can best be separated from the other species described here based on the following combination of characters: Rm is almost twice as long as the stem of posterior fork; the dorsal lobe of the gonostylus have a small inconspicuous area at apex, as in *A. mazumbaiensis*, *A. drakensbergensis* and *A. keurbosensis*; the median lobe is similar to *A. keurbosensis*, but with more setae on the ventral margin and a well-defined heel-shaped posteroventral corner of the lobe (Fig. 10); the basal part of the gonostylus is different from all the other species (Fig. 1G). The shape of the female hypogynal valves (Fig. 2) differs from *A. jaschhofi* (Fig. 8).

**Type material.** Holotype ♂. Kenya, Nyeri, Mount Kenya National Park, Base camp at Naro Moru River Lodge (0.17027°S, 37.21500°E, 3050 masl), sweep net, bamboo forest. 18–19 Aug. 2008. TSZD-JKJ-104239. Leg. J. Kjærandsen. (TMU). Paratypes 1 ♂, 2 ♀, data as for holotype. (TMU).

**Measurements.** Male (n = 2): Body length 3.04–3.91 mm; wing length 2.91–3.26 mm. Female (n = 2): Body length 4.00–4.35 mm; wing length 3.33 mm

**Coloration.** Head and clypeus brown. Mouthparts and palpomeres whitish yellow. Antennae brown, with scape, pedicel and basal half of first flagellomere whitish yellow. Scutum brown, with yellow lateral margin. Lateral sclerites brown. Wings clear without markings. Halteres whitishyellow. Legs whitish yellow. Abdomen brown, tergites 2–4, 2–5 in females, with lateral area yellow, broadening towards posterior margin. Terminalia yellow.

**Head.** Two ocelli, near eye margin. Head covered with fine trichia, except for row of 5 short bristles near eye margin, above ocellus. Antennae in males about twice as long as thorax, in females slightly longer than thorax (1.13 mm/1.09 mm, respectively). Scape and pedicel with several setae on distal third. Flagellomeres cylindrical, densely covered with fine trichia. First flagellomere twice as long as scape.

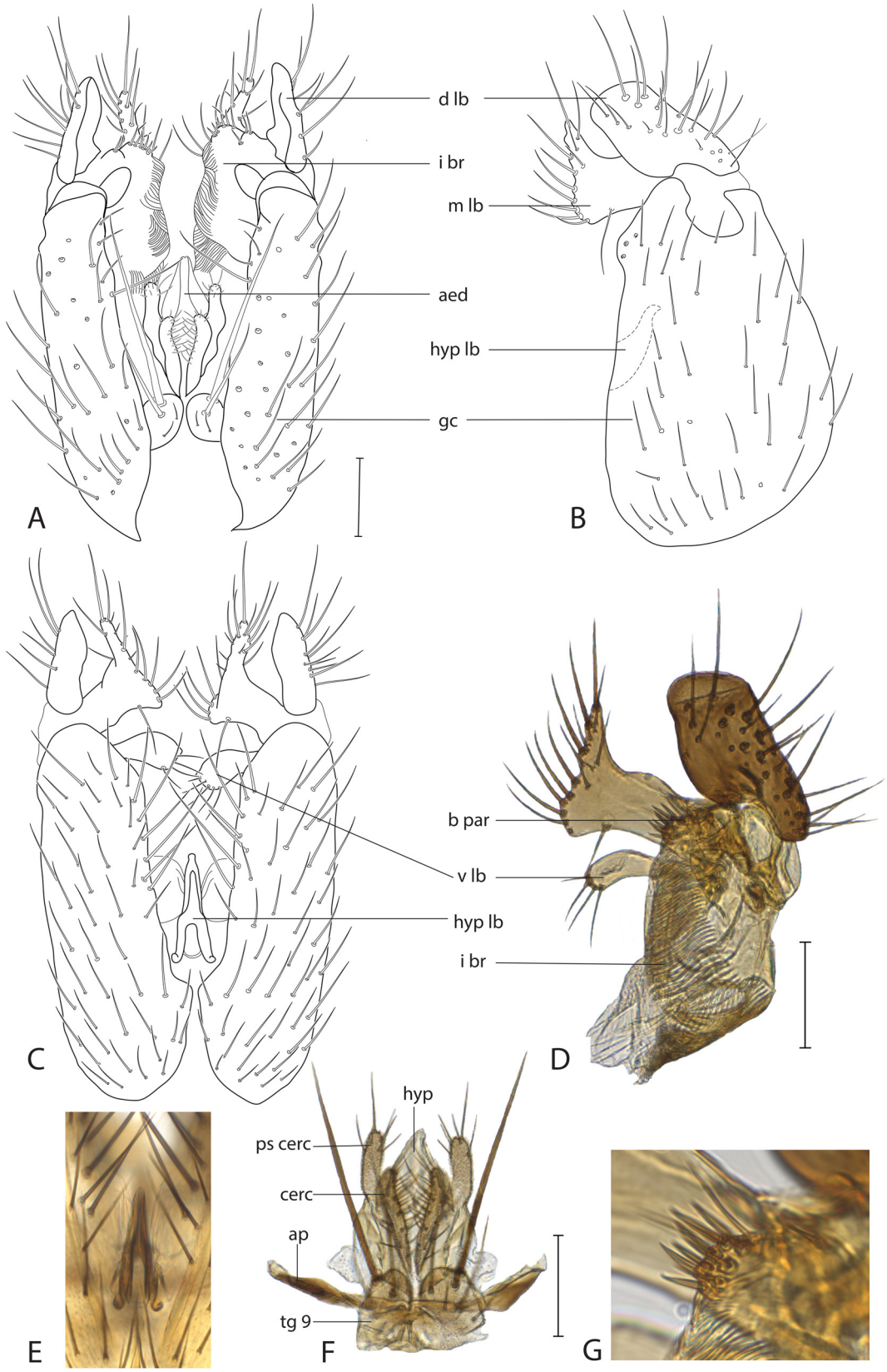


**FIGURE 1.** The male terminalia of *A. nyeriensis*. **A**, Dorsal view; **B**, Lateral view; **C**, Ventral view; **D**, Gonostylus from inner side; **E**, Hypandrial lobe; **F**, Dorsal structures; **G**, Basal part of gonostylus. **Abbreviations**: ap = apodeme, b par = basal part, cerc = cerci, d lb = dorsal lobe, gc = gonocoxite, hyp = hypoproct, hyp lb = hypandrial lobe, i br = internal branch, m lb = median lobe, ps cerc = pseudocerci, tg 9 = tergite 9. Scale bar = 1µm.

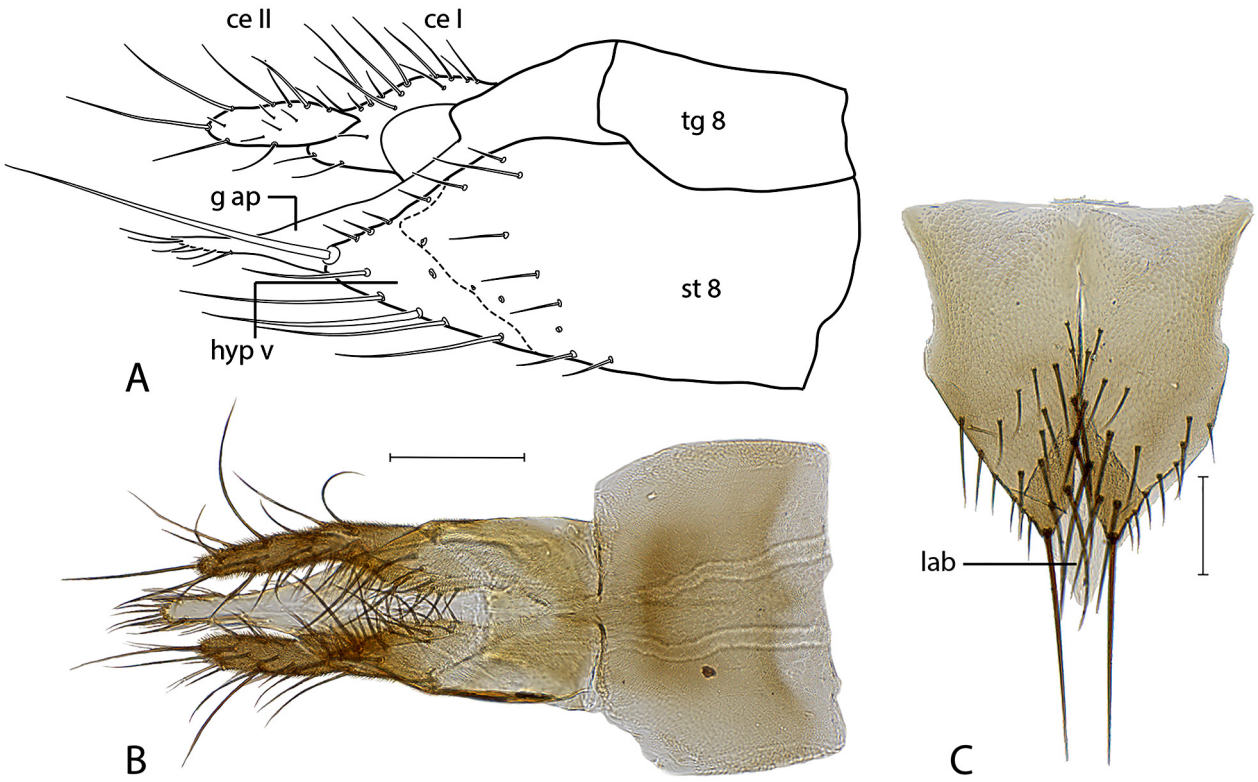


**FIGURE 2.** The female terminalia of *A. nyeriensis*. **A**, Lateral view; **B**, Dorsal view; **C**, Hypogynium, ventral view. **Abbreviations**: ce = cerci, g ap = gonapophysis, hyp v = hypogynal valves, lab = labia, st = sternite, tg = tergite. Scale bar = 1µm.

**Thorax.** Antepronotum with 5–6 strong setae. Scutum covered with uniform small, pale setae, with strong lateral prealar and postalar setae, more numerous close to wing base. Discal bristles absent. Scutellum with 2 strong bristles. Laterotergite with 2 long and 3 shorter setae. Other lateral sclerites bare.

**Legs.** All tibiae with short setae arranged in rows. Mid tibia with 5–7 anterodorsal and 25–28 posterodorsal bristles, on distal 3/4 of segment. Hind tibia with 5–7 anterodorsal and 8 posterodorsal bristles.

**Wings.** Sc short, ending in R, rm almost twice as long as stem of posterior fork, base of anterior fork slightly before base of posterior fork. $R_1$ with setae on distal 2/3 and $R_5$ with setae on distal 3/4.

**Male terminalia** (Fig. 1). Tergite 9 medially divided, each part rounded, covered with minute trichia, with one strong apical bristle, two short and three longer setae. Cerci covered with fine trichia, with longer setae apically; pseudocerci with several long setae medially and apically (Fig. 1F). Dorsal lobe of gonostylus oblong, apex rounded with small, roundish, pouch-like membranous structure internally; outer surface with numerous strong setae. Median lobe with heel-shaped posteroventral corner; 9 strong and 4 shorter setae on posterior margin, plus 4 minute setae on interior surface; one long seta on ventral edge, with short distance from ventral margin (about 1/6 of total distance). Ventral lobe elongated, slightly arched, with 5–6 setae apically. Basal part of gonostylus with ventral semi-circular structure, bearing about 8 short setae, and second small pointed dorsal structure with one short seta (Fig. 1G). Hypandrial lobe heavily sclerotized, slender, protruding inwards at apex (Fig. 1E).

**Female terminalia** (Fig. 2). Tergite 8 elongated. Cerci two-segmented; first segment oblong, arcuate, fused at basis; second segment oblong, narrower than first segment, with several long setae. Gonapophyses fused, membranous, tongue-shaped with small setae around edge. Hypogynal valves elongated, triangular shaped, pointed towards apex; one long seta on tip; labia membranous.

**Etymology.** Named after the region of the type locality.

**Remarks.** The specimens have become pale after years of storage in ethanol. The measurements were made on ethanol preserved specimens. Wing length for females was measured on one specimen only.

***Allodia mazumbaiensis* Magnussen sp. nov.**
(Fig. 3)

**Diagnostic characters.** *A. mazumbaiensis* can best be separated from the other species described here, based on the following combination of characters: Rm as long as stem of the posterior fork; the dorsal lobe of the gonostylus have a small inconspicuous area at apex, as in *A. nyeriensis*, *A. drakensbergensis* and *A. keurbosensis*; the median lobe is narrower than in the other species, with fewer setae on the ventral margin and one especially long and pronounced on the dorsal tip (Fig. 10); basal part of gonostylus differs from all the other species (Fig. 3C).

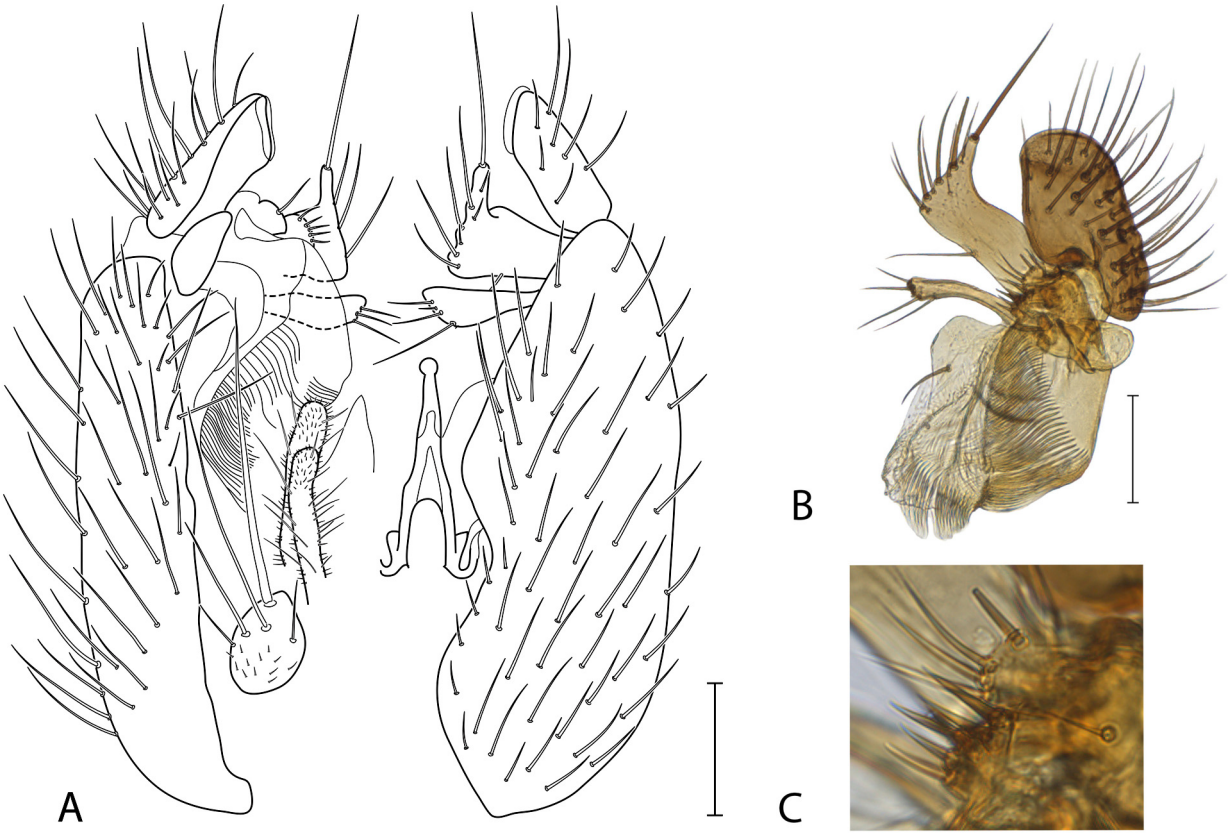


**FIGURE 3.** The male terminalia of *A. mazumbaiensis*. **A**, left: dorsal view, right: ventral view; **B**, Gonostylus from inner side; **C**, Basal part of gonostylus. Scale bar = 1µm.

**Type material.** Holotype ♂. Tanzania: Tanga region, West Usambara mountains, Mazumbai, 1500 masl. Malaise trap, loc. O., ZBM's Tanzania expedition. 29 Sep. –3 Des. 1990. Leg. G. Søli. (NHMO).

**Measurements.** Male: Body length 4.44 mm; wing length 3.33 mm.

**Coloration.** Head and clypeus brown. Mouthparts and palpomeres whitish yellow. Antennae brown, with scape, pedicel and first half of first flagellomere whitish yellow. Scutum brown, with yellow lateral margin. Antepronotum whitish, lateral sclerites otherwise brown. Wings clear without markings. Halteres whitish yellow. Legs whitish, with brown basal area ventral on femur. Abdomen brown, tergites 2–4 with lateral area whitish yellow. Terminalia yellow.

**Head.** Two ocelli, located close to eye margin. Head covered with fine trichia, except for row of 5 short bristles near eye margin, above ocellus. Antennae about twice as long as thorax. Scape and pedicel with several setae on distal third. Flagellomeres cylindrical, densely clothed with fine trichia. First flagellomere twice as long as scape.

**Thorax.** Antepronotum with 5 strong setae. Scutum covered with uniform small, pale setae, with strong lateral prealar and postalar setae. Discal bristles absent. Scutellum with 2 strong bristles. Laterotergite with 2 long and 4 minute setae. Other lateral sclerites bare.

**Legs.** Fore-, mid and hind tibia with short setae arranged in rows. Mid tibia with 14 anterodorsal and 26 posterodorsal bristles, on distal 3/4 of segment. Hind tibia with 8 anterodorsal and 9 posterodorsal bristles.

**Wings.** Sc short, ending in R, length of rm equal to stem of posterior fork, base of anterior fork slightly before base of posterior fork. $R_1$ and $R_5$ with setae.

**Male terminalia** (Fig. 3). Tergite 9 medially divided, each part rounded, covered with minute trichia, with two strong apical bristles and three long setae. Cerci clothed with fine trichia, with longer setae apically; pseudocerci with several long setae (Fig. 3A). Dorsal lobe of gonostylus oblong, apex rounded with small, inconspicuous, membranous area internally; outer surface with numerous strong setae. Median lobe slender, ventral side straight; with 4 strong and 4 thin setae on posterior margin, apical seta especially prominent; 2-3 small setae on lobe surface; one long seta on ventral side, located with approximately equal distance from tip and basis of lobe. Ventral lobe slender, slightly arched, with 5-6 setae apically. Basal part of gonostylus with two structures of equal size, both bulbous with 5-6 setae (Fig. 3C). Hypandrial lobe complex, elongated, curved inwards (Fig. 3A)

**Etymology.** Named after the type locality.

**Remarks.** The specimen has become pale after years of storage in glycerol. Measurements were made on specimen in ethanol.

### *Allodia drakensbergensis* Magnussen sp. nov.

(Fig. 4)

**Diagnostic characters.** *A. drakensbergensis* can best be separated from the other species described here, based on the following combination of characters: Rm about 1.5 times as long as stem of the posterior fork; the dorsal lobe of gonostylus is similar to *A. nyeriensis*, *A. mazumbaiensis* and *A. keurbosensis*; the median lobe most similar to *A. keurbosensis*, but with fewer setae on the ventral margin and the shape of ventral lobe differs from *A. keurbosensis* (Fig. 10); the basal part of the gonostylus is different from all the other species (Fig. 4C).

**Type material.** Holotype ♂. South Africa: Northern Drakensberg, KwaZulu-Natal, Royal Natal National Park, Gudu forest (28.6691°S, 28.9188°E, 1630–1730 masl), sweep net, old-growth indigenous forest. 29 Nov. 2005. TSZD-JKJ-104243. Leg. M. Jaschhof. (TMU).

**Measurements.** Male: body length 4.44 mm; wing length 3.33 mm.

**Coloration.** Head and clypeus brown. Mouthparts and palpomeres whitish yellow. Antennae brown, with scape, pedicel and first half of first flagellomere whitish yellow. Scutum brown. Lateral sclerites brown. Wings clear without markings. Halteres whitish at basis, brown on club. Legs whitish. Abdomen brown. Terminalia yellowish brown.

**Head.** Two ocelli, located close to eye margin. Head covered with fine trichia, except for row of about 7 short bristles near eye margin, above ocellus. Antennae just over twice as long as thorax. Scape and pedicel with several setae on distal third. Flagellomeres cylindrical, densely clothed with fine trichia. First flagellomere twice as long as scape.

**Thorax.** Antepronotum with 5 strong setae. Scutum covered with uniform small, pale setae, with strong lateral prealar and postalar setae. Discal bristles absent. Scutellum without strong bristles. Laterotergite with 3 long and 3 minute setae. Other lateral sclerites bare.

**Legs.** Fore-, mid and hind tibia with short setae arranged in rows. Mid tibia with 14 anterodorsal and 26 posterodorsal bristles on distal 3/4 of segment. Hind tibia with 8 anterodorsal and 9 posterodorsal bristles.

**Wings.** Sc short, ending in R, rm 1.5 times as long as stem of posterior fork, base of anterior fork slightly before base of posterior fork. $R_1$ and $R_5$ with setae, absent at basis of veins.

**Male terminalia** (Fig. 4). Tergite 9 medially divided, each part rounded, covered by minute trichia, with one strong bristle, two shorter bristles and three setae. Cerci clothed with fine trichia, with long setae apically and some medially, pseudocerci with several long setae medially and apically. Dorsal lobe of gonostylus oblong, apex rounded with small, round membranous area internally; outer surface with several strong setae. Median lobe with slightly curved posteroventral corner; 7 strong and 2 shorter setae on posterior margin, one seta on interior surface; one long seta on ventral side, about one third of total distance from posterior side. Ventral lobe distinctly club-shaped, with 8 setae apically. Basal part of gonostylus with two bulbous structures of equal size, dorsal structure with 5 long setae, ventral structure with about 8 stout setae (Fig. 4C). Hypandrial lobe elongated, with strong sclerotized lateral apodemes (Fig. 4A).

**Etymology.** Named after the type locality.

**Remarks.** The specimen has become pale after years of storage in ethanol. Measurements were made on specimen in ethanol.

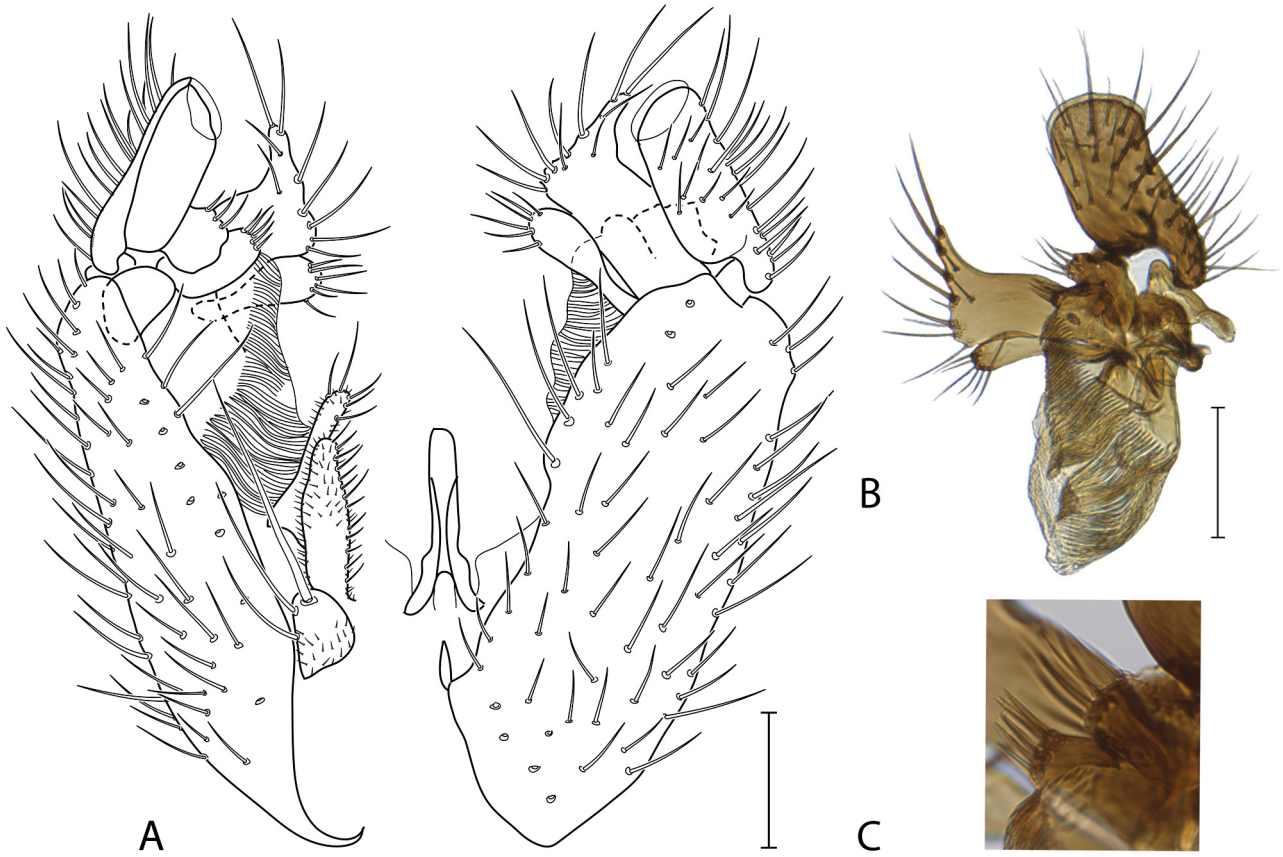


**FIGURE 4.** The male terminalia of *A. drakensbergensis*. **A**, left: dorsal view, right: ventral view; **B**, Gonostylus from inner side; **C**, Basal part of gonostylus. Scale bar = 1µm.

***Allodia keurbosensis* Magnussen sp. nov.**
(Figs 5, 6)

**Diagnostic characters.** *A. keurbosensis* can best be separated from the other species described here, based on the following combination of characters: Rm almost twice as long as stem of the posterior fork; the dorsal lobe of gonostylus is similar to *A. nyeriensis*, *A. mazumbaiensis* and *A. drakensbergensis*; the median lobe is most similar to *A. drakensbergensis*, but with more setae on the ventral margin (Fig. 10); the shape of the ventral lobe is different to that of *A. drakensbergensis* (Fig. 4B); the basal part of gonostylus different from all the other species (Fig. 6C).

**Type material.** Holotype ♂. South Africa: Eastern Cape, Keurbos forest (33.9072°S, 23.7285°E, 500 masl), malaise trap, indigenous montane forest. 28–30 Mar. 2009. Leg. A.H. Kirk-Spriggs; S. Otto. (BMSA).

**Measurements.** Male: Body length 3.91 mm; wing length 3.23 mm.

**Coloration.** Head and clypeus brown. Mouthparts and palpomeres yellow. Antennae brown, with scape, pedicel and first half of first flagellomere yellow. Scutum brown. Antepronotum whitish anteriorly, lateral sclerites otherwise brown. Halteres whitish. Legs whitish. Wings clear without markings. Abdomen brown, with small yellow triangular area on tergites 3 and 4. Terminalia yellow.

**Head.** Two ocelli, located close to eye margin. Head covered with fine trichia, except for row of about 8 short bristles near eye margin, above ocellus. Antennae about twice as long as thorax. Scape and pedicel with several setae on distal third. Flagellomeres cylindrical, densely clothed with fine trichia. First flagellomere twice as long as scape.

**FIGURE 5.** Habitus *Allodia keurbosensis* **sp. nov.** Holotype. Photo: Karsten Sund, Natural History Museum, University of Oslo.

**Thorax.** Antepronotum with 4 strong setae. Scutum covered with uniform small, pale setae, with strong lateral prealar and postalar setae. Discal bristles absent. Scutellum with 3 strong bristles. Laterotergite with 3 long setae. Other lateral sclerites bare.

**Legs.** Fore-, mid and hind tibiae with short setae arranged in rows. Mid tibia with 4 anterodorsal and 20 posterodorsal bristles, on distal 3/4 of segment. Hind tibia with 6 anterodorsal and 6 posterodorsal bristles.

**Wings.** Sc short, ending in R, rm almost twice as long as stem of posterior fork, base of anterior fork just opposite base of posterior fork. $R_1$ and $R_5$ setae, absent at basis of veins.

**Male terminalia** (Fig. 6). Tergite 9 medially divided, each part rounded, covered with minute trichia, with two strong bristles and three long setae. Cerci clothed with fine trichia, with some long setae apically; pseudocerci with several long setae randomly medially and apically. Dorsal lobe of gonostylus oblong, apex rounded with small, pouch-like membranous area internally, outer surface with numerous strong setae. Median lobe with straight ventral side, with row of 7 strong setae on posterior margin; 10 shorter present on posterior margin and on lobe surface; three setae on ventral side, two close to posteroventral corner, one about one third of the total distance from posterior edge. Ventral lobe slightly club-shaped with 6-7 setae apically, plus one on posterior edge. Basal

part of gonostylus with two bulbous structures of equal size, each with 4 short setae. Hypandrial lobe elongated, pointed inwards, with strong sclerotized apodemes (Fig. 6A).

**Etymology.** Named after the type locality

**Remarks.** Measurements were made on pinned specimen.

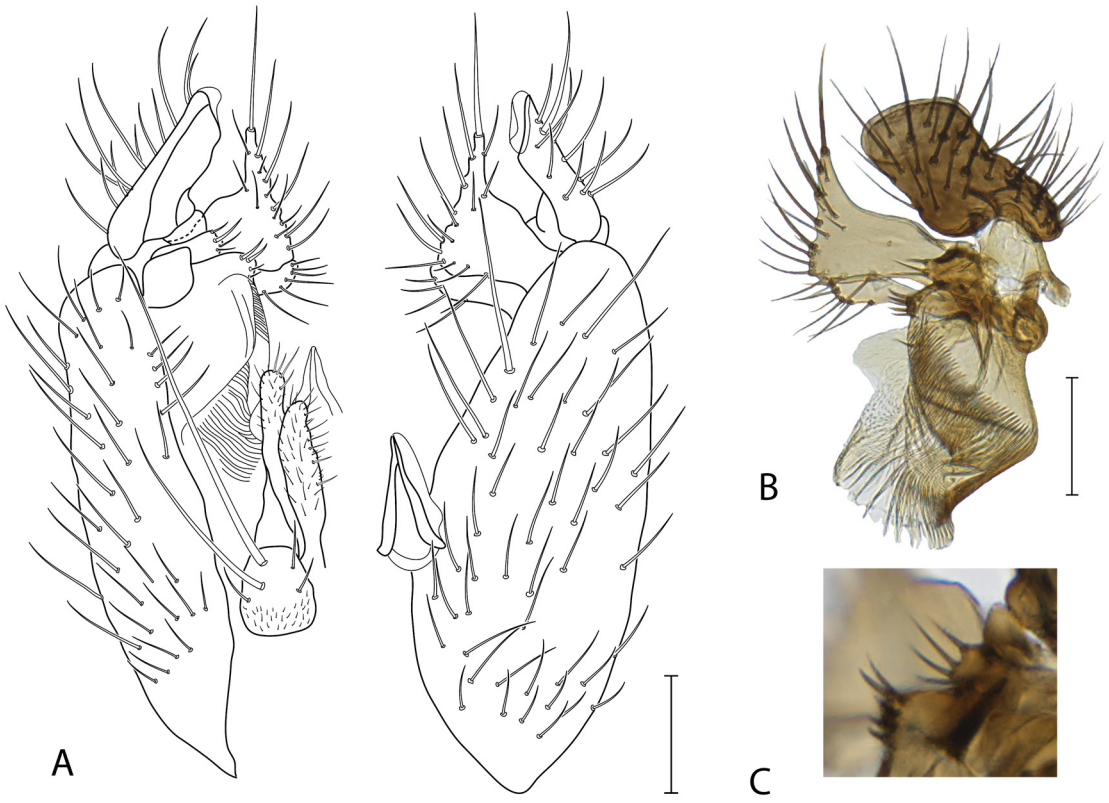


**FIGURE 6.** The male terminalia of *A. keurbosensis*. **A**, left: dorsal view, right: ventral view; **B**, Gonostylus from inner side; **C**, Basal part of gonostylus. Scale bar = 1µm.

### *Allodia jaschhofi* Magnussen sp. nov.

(Figs 7, 8)

**Diagnostic characters.** *A. jaschhofi* can best be separated from the other species described here, based on the following combination of characters: Length of rm equal to length stem of the posterior fork; the dorsal lobe of gonostylus is similar to *A. karkloofensis*, which both have a large membranous area on the internal apex of the lobe (Fig. 10); the median lobe is not distinctly separable from the other species; the basal part of the gonostylus is similar to that of *A. karkloofensis*, but with a small pointed structure present, which is absent in *A. karkloofensis* (Fig. 9). The female (Fig. 8) can be separated from *A. nyeriensis* on shape of hypogynal valves (Fig. 2).

**Type material.** Holotype ♂. South Africa: Central Drakensberg, Cathedral Peak Nature Reserve, Rainbow Gorge (28.9516°S, 29.2183°E, 1500 masl), sweep-net, old growth indigenous forest. 04 Des. 2005. TSZD-JKJ-101654. Leg. M. Jaschhof. (TMU). Paratype 1 ♀, data as for holotype. (TMU).

**Measurements.** Male: Body length 3.10 mm; wing length 2.6 mm. Female: Body length 3.65 mm; wing length 2.78 mm.

**Coloration.** Head and clypeus brown. Mouthparts and palpomeres whitish yellow. Antennae brown, with scape, pedicel and first half of first flagellomere whitish yellow. Scutum brown. Lateral sclerites brown. Wings clear without markings. Halteres whitish. Legs whitish. Abdomen brown. Terminalia whitish yellow.

**Head.** Two ocelli, located close to the eye margin. Head covered with fine trichia, except for row of about 5 short setae near eye margin, above ocellus. Antennae in male about twice as long as thorax, in female slightly longer than thorax (1.22 mm/0.74 mm, respectively). Scape and pedicel with several setae on distal third. Flagellomeres cylindrical, densely clothed with fine trichia. First flagellomere twice as long as scape.

**Thorax.** Antepronotum with 5 strong setae. Scutum covered with uniform small, pale setae, with strong lateral prealar and postalar setae. Discal bristles absent. Scutellum with 2 strong bristles. Laterotergite with 6 long setae. Other lateral sclerites bare.

**Legs.** Fore-, mid and hind tibiae with short setae arranged in rows. Mid tibia with 8 anterodorsal and 25 posterodorsal bristles, on distal 3/4 of segment. Hind tibia with 7 anterodorsal and 8 posterodorsal bristles.

**Wings.** Sc short, ending in R, length of rm equal to length of stem of posterior fork, base of anterior fork distinctly before base of posterior fork. $R_1$ and $R_5$ with setae, absent at basis of veins.

**Male terminalia** (Fig. 7). Tergite 9 medially divided, each part rounded, covered with minute trichia, with two strong bristles and four small setae. Cerci clothed with fine trichia, with long setae apically; pseudocerci with several long setae medially and apically. Dorsal lobe of gonostylus oblong, apex prominently rounded, with large, round membranous area internally; outer surface with numerous strong setae. Median lobe with slightly curved ventral side, but without heel-shaped posteroventral corner; 8 strong and 3 shorter setae on posterior margin; two small setae on dorsal edge; one long seta on posterior edge, with long distance from ventral margin (less than half of the total distance from basis). Ventral lobe slightly club-shaped, with 5 long and three short setae at apex. Basal part of gonostylus with three structures; dorsal structure small and pointed with 1-2 small setae, middle structure round with one long seta and ventral structure large, rounded with several short setae (Fig. 7C).

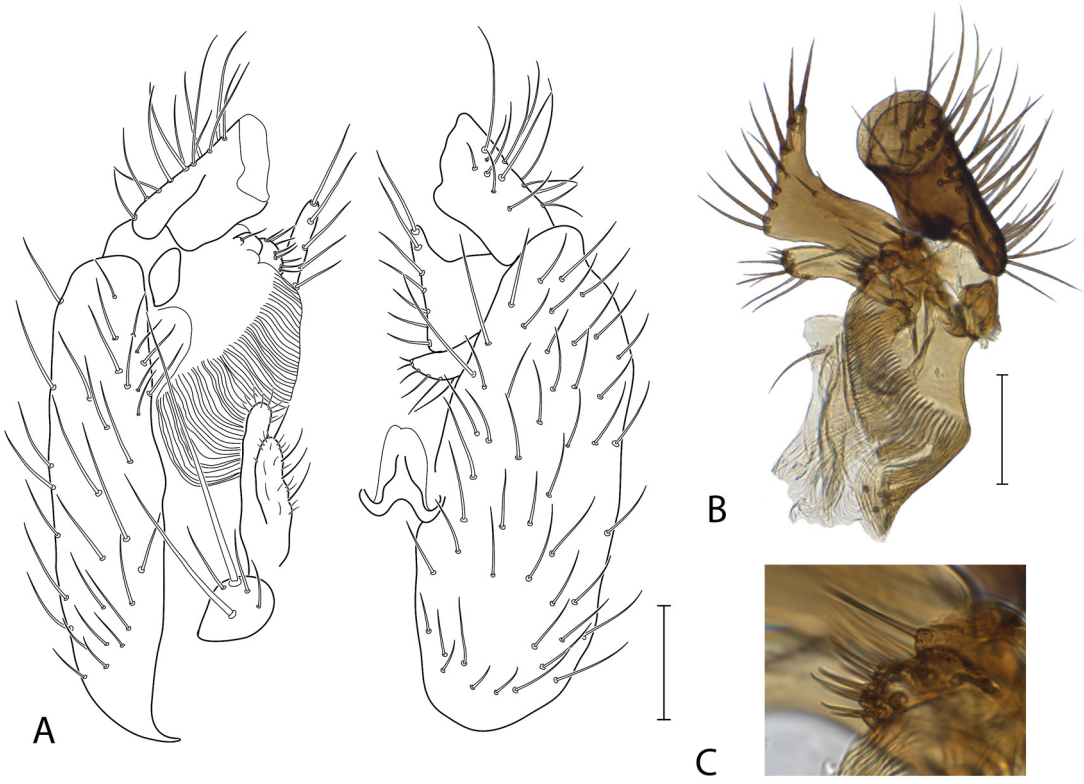


**FIGURE 7.** The male terminalia of *A. jaschhofi*. **A**, left: dorsal view, right: ventral view; **B**, Gonostylus from inner side; **C**, Basal part of gonostylus. Scale bar = 1µm.

**Female terminalia** (Fig. 8). Tergite 8 short. Cerci two-segmented; first segment oblong, slightly arcuate, fused at basis; second segment egg-shaped, narrower than first segment, with several long setae (Figs 8A, 8B). Gonapophyses fused, membranous, tongue-shaped with small setae around edge. Hypogynal valves elongated, triangular shaped, blunt, slightly curved towards apex; one long seta on tip; labia membranous (Fig. 8C).

**Etymology.** Named after the collector.

**Remarks.** The specimen has become pale after years of storage in ethanol. Measurements were made on specimen in ethanol.

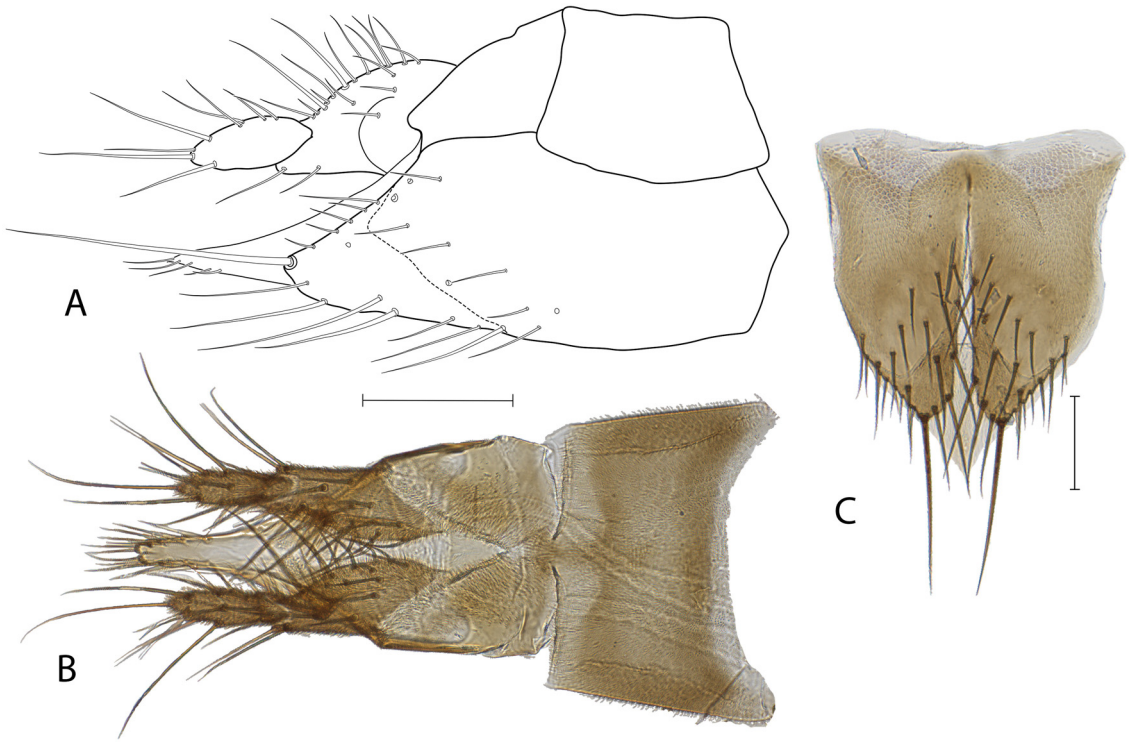


**FIGURE 8.** The female terminalia of *A. jaschhofi*. **A**, Lateral view; **B**, Dorsal view; **C**, Hypogynium, ventral view. Scale bar = 1µm.

***Allodia karkloofensis* Magnussen sp. nov.**
(Fig. 9)

**Diagnostic characters.** *A. karkloofensis* can best be separated from the other species described here based on the following combination of characters: Length of rm is equal to the stem of posterior fork; the dorsal lobe of gonostylus is similar to *A. jaschhofi*, which both have a large membranous area on the internal apex of the lobe; the shape of the median lobe is distinct, having a very rounded posteroventral corner, with one seta at the distal part of the ventral edge (Fig. 10); the basal part of the gonostylus (Fig. 9C) is similar to that of *A. jaschhofi* (Fig. 7C), but without a small pointed structure.

**Type material.** Holotype ♂. South Africa: KwaZulu-Natal national park, Howik district, Karkloof Range, Geekies Farm (29.1600°S, 030.2100°E), malaise trap. 29 Feb. – 09 Mar. 2000. TSZD-JKJ-101653. Leg. W. Brakemeyer. (TMU).

**Measurements.** Male: Body length 4.35 mm; wing length 3.04 mm.

**Coloration.** Head and clypeus brown. Mouthparts and palpomeres whitish yellow. Antennae brown, with scape, pedicel and first half of first flagellomere whitish yellow. Scutum brown. Lateral sclerites brown. Wings clear without markings. Halteres whitish. Legs whitish. Abdomen brown, with slightly paler area close to lateral edge of tergites. Terminalia whitish yellow.

**Head.** Two ocelli, located close to the eye margin. Head covered with fine trichia, except for row of about 8 short setae near eye margin, above ocellus. Antennae about twice as long as thorax. Scape and pedicel with several setae on distal third. Flagellomeres cylindrical, densely clothed with fine trichia. First flagellomere twice as long as scape.

**Thorax.** Antepronotum with 5 strong setae. Scutum covered with uniform small, pale setae, with strong lateral prealar and postalar setae. Discal bristles absent. Scutellum with 2 strong bristles. Laterotergite with 4 long setae and three shorter. Other lateral sclerites bare.

**Legs.** Fore-, mid and hind tibiae with short setae arranged in rows. Mid tibia with 9 anterodorsal and 30 posterodorsal bristles, on distal 3/4 of segment. Hind tibia with 8 anterodorsal and 8 posterodorsal bristles.

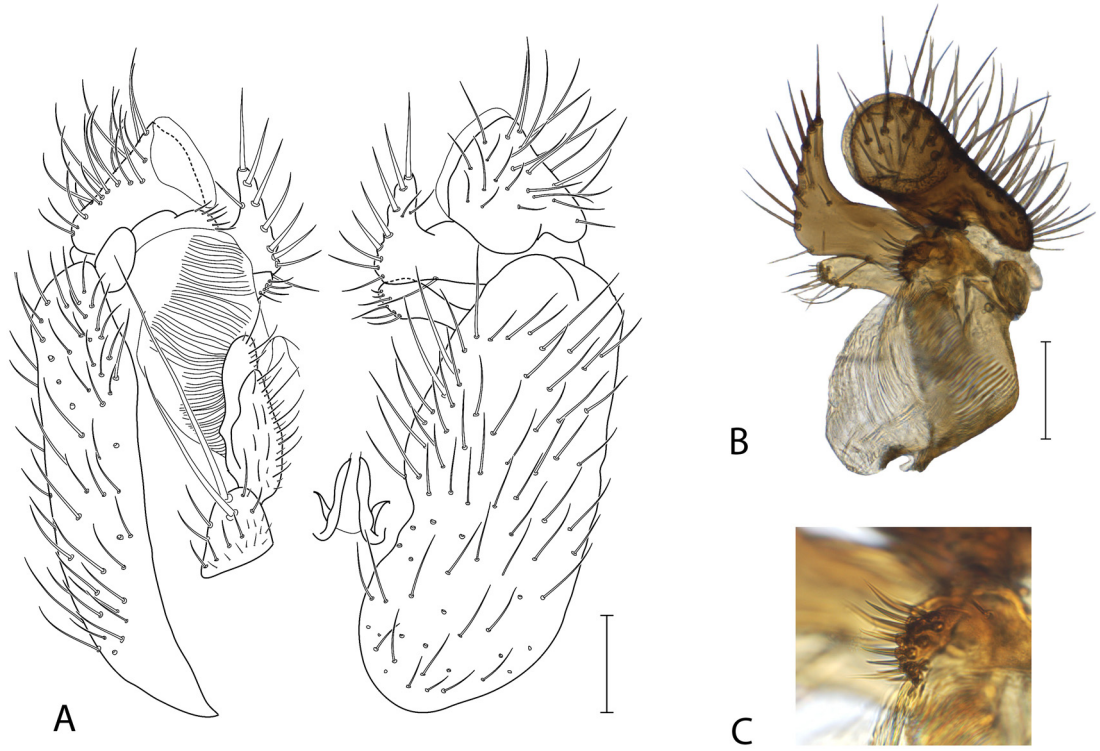


**FIGURE 9.** The male terminalia of *A. karkloofensis*. **A**: left: dorsal view, right: ventral view, **B**: Gonostylus from inner side, **C**: Basal part of gonostylus. Scale bar = 1µm.

**Wings.** Sc short, ending in R, length of rm equal to length of stem of posterior fork, base of anterior fork slightly before base of posterior fork. R1 and R5 with tapering setae.

**Terminalia male** (Fig. 9). Tergite 9 medially divided, each part rounded, covered with minute trichia, with two strong bristles and 8 small setae. Cerci clothed with fine trichia, with long setae apically; pseudocerci with several long setae medially and apically. Dorsal lobe of gonostylus oblong, apex prominently rounded, with large, round membranous area internally; outer surface with numerous strong setae. Median lobe with indistinct posteroventral corner, with 7 strong and 4 shorter setae on posterior margin; one small setae on surface of lobe; two long setae on ventral edge, one at basis, and one just before posteroventral corner. Ventral lobe slightly club-shaped, with about nine long setae at apex. Basal part of gonostylus with one large round structure, covered with many stout setae. Hypandrial lobe blunt, weakly sclerotized at apex (Fig. 9A).

**Etymology.** Named after the type locality.

**Remarks.** Measurements were made on specimen in ethanol.

**TABLE 2.** Between group mean distance. Interspecific p-distances for CO1 calculated in Mega 7. Distances between Afrotropical species highlighted in bold.

| | Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *A. embla* | | | | | | | | | |
| 2 | *A. lugens* | 0.095 | | | | | | | | |
| 3 | *A. ornaticollis* | 0.105 | 0.088 | | | | | | | |
| 4 | *A. pyxidiiformis* | 0.107 | 0.102 | 0.106 | | | | | | |
| 5 | *A. truncata* | 0.119 | 0.106 | 0.119 | 0.123 | | | | | |
| 6 | *A. tuomikoskii* | 0.106 | 0.109 | 0.098 | 0.103 | 0.130 | | | | |
| 7 | *A. keurbosensis* | 0.106 | 0.102 | 0.134 | 0.123 | 0.136 | 0.130 | | | |
| 8 | *A. nyeriensis* | 0.113 | 0.113 | 0.121 | 0.126 | 0.128 | 0.125 | **0.098** | | |
| 9 | *A. drakensbergensis* | 0.110 | 0.109 | 0.141 | 0.135 | 0.133 | 0.141 | **0.056** | **0.110** | |
| 10 | *A. jaschhofi* | 0.109 | 0.103 | 0.115 | 0.124 | 0.127 | 0.115 | **0.100** | **0.078** | **0.102** |

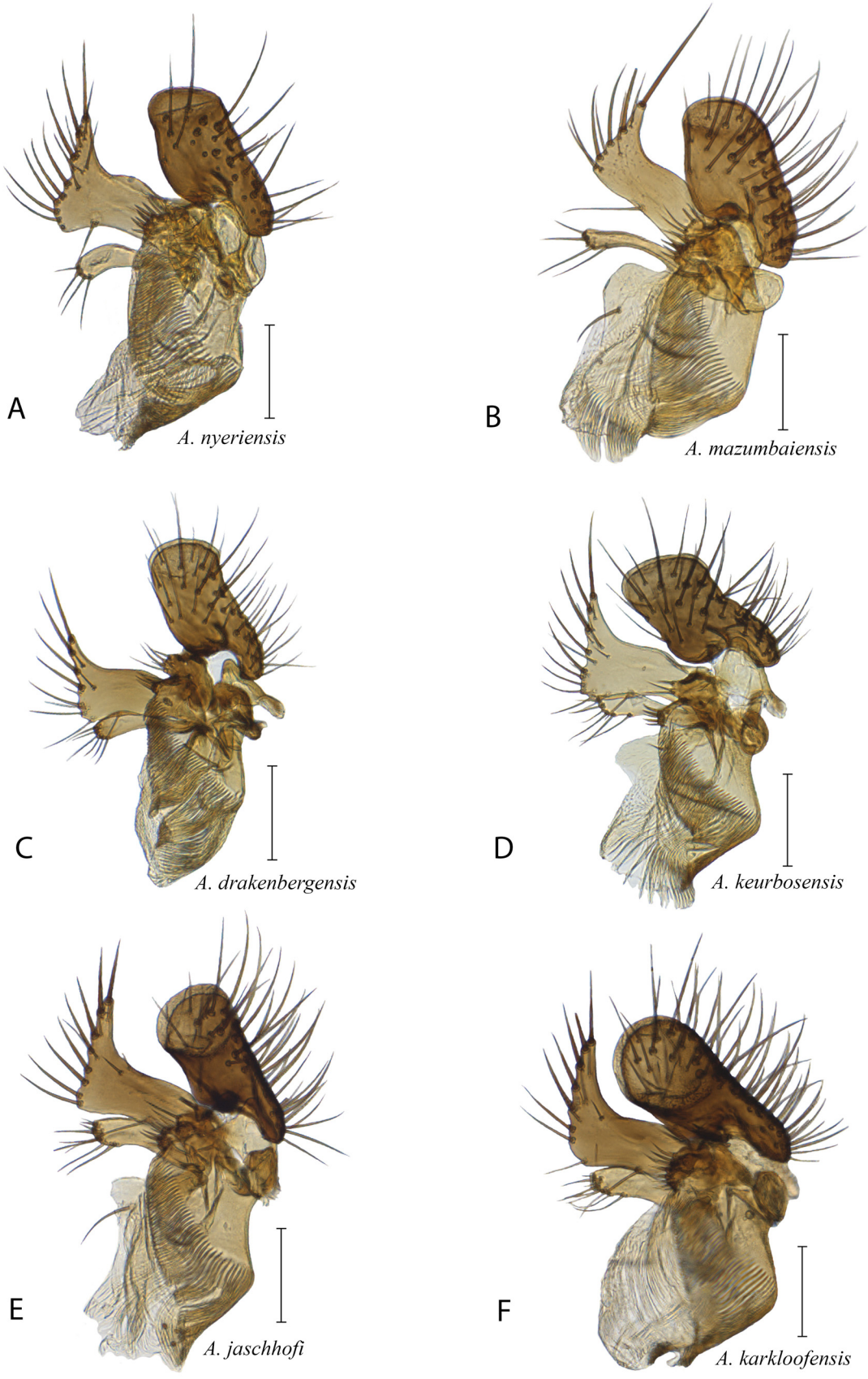


**FIGURE 10.** Variation between Afrotropical *Allodia*, gonostylus from inner side. **A**, *A. nyeriensis*; **B**, *A. mazumbaiensis*; **C**, *A. drakensbergensis*; **D**, *A. keurbosensis*; **E**, *A. jaschhofi*; **F**, *A. karkloofensis*. Scale bar = 1µm.

**CO1 sequence data.** Full length, bidirectional sequences of the CO1 barcode region were successfully obtained for four of the six new species, *A. nyeriensis*, *A. jaschhofi*, *A. keurbosensis* and *A. drakensbergensis*. We were unable to obtain CO1 sequences from the two remaining new species; *A. karkloofensis* and *A. mazumbaiensis*. Interspecific p-distances among the Afrotropical species ranged between 5.6% and 11% (Table 2). The genetic distance between the Afrotropical species and the six outgroup taxa ranged between 10.2% and 14.1%. All the Afrotropical species grouped together with respect to the outgroup taxa.

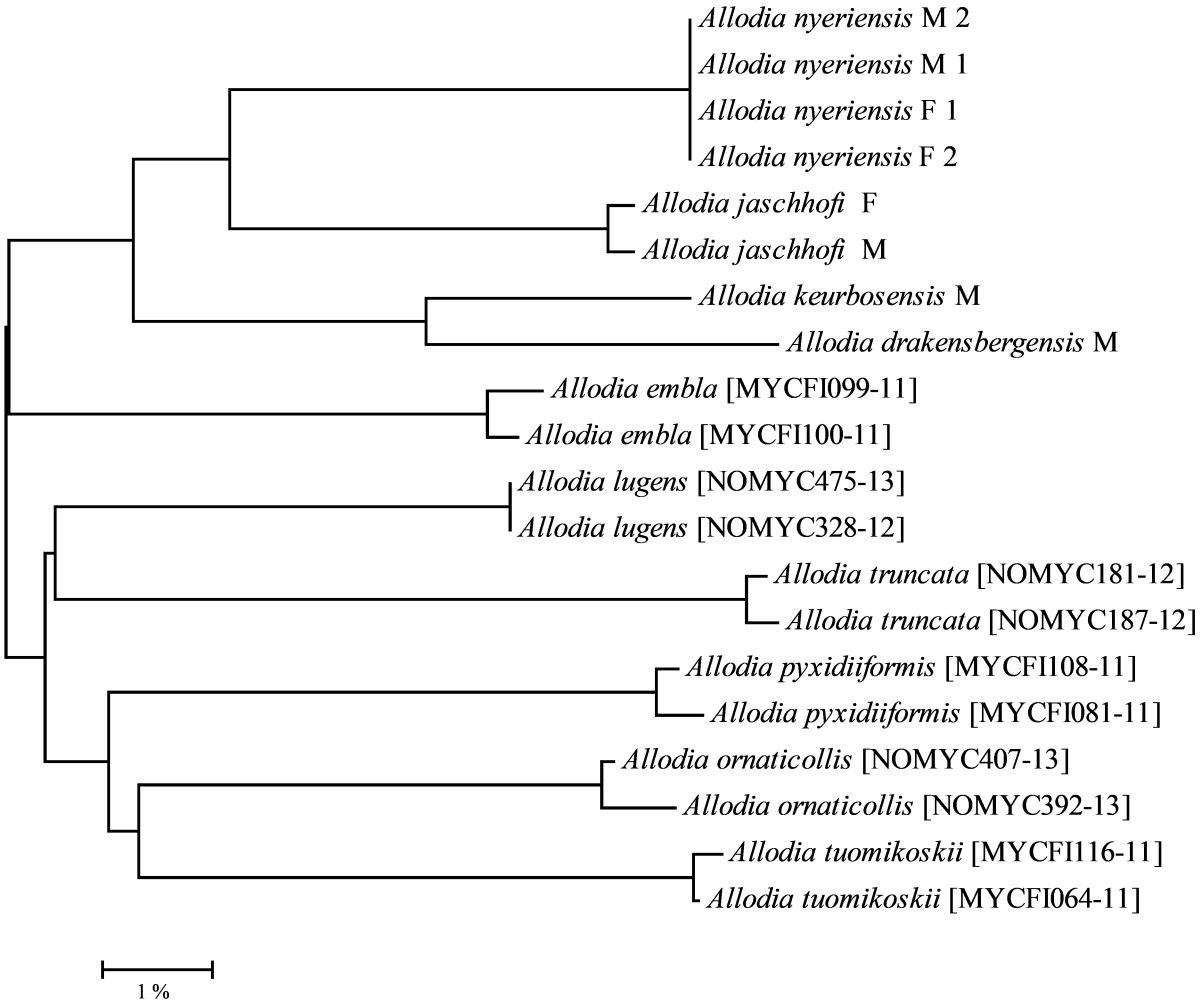


**FIGURE 11.** Identification tree. Neighbor-Joining analysis involving 20 CO1 sequences. Evolutionary distances were calculated using the p-distance model. Abbreviations: M = male, F = female. Sequences retrieved from BOLD (Ratasingham & Hebert 2007) are labelled with Process ID (see Appendix 1). See methods for details.

## Discussion

Traditionally, due to intraspecific stability, genital characters have been used to separate species in the genus *Allodia*, as for Mycetophilidae in general (Søli 1997; Zaitzev 2003). In the subgenus *Brachycampta,* the composition and structure of the male genitalia is highly diverse, compared to what is observed in *Allodia s.s*. The male genitalia of *Allodia s.s.* all have a similar morphology, with clear stability in the structures. This is also the case in the present study, where small but consistent variations in the outline and chaetotaxy of the male genitalia have been found between the newly described species. In the keys to the Holarctic species of *Allodia* (Zaitzev 1983; 1984), detailed illustrations showing the outline of the dorsal, medial and ventral lobe are given and used as the primary source for the identification of species in both subgenera. For Palaearctic *Allodia*, the characters found in the male terminalia are distinctive and considered to be substantial between species (Zaitzev 1983; 2003). However, cases exist where the differences are more subtle: the characters used by Kurina (1997) to separate *A.*

*zaitzevi* Kurina, 1997 and *A. pyxidiiformis* Zaitzev, 1983 are similar to those used in the present study to separate between Afrotropical species.

Based on the Palaearctic fauna of *Allodia* available for comparison, the male terminalia of the Afrotropical species most closely resembles those of *A. lugens* Wiedemann, 1817. This widely distributed species has the same shape of the median and ventral lobe, but a more elongate and tapered dorsal lobe. Interestingly, the outline of the apex of the dorsal lobe in the Afrotropical species is quite complex and unlike that observed in any other known species, and may represent a synapomorphy for the Afrotropical species group. The female terminalia are generally considered to have smaller interspecific differences, compared to the male terminalia (e.g. McAlpine 1981). The two females described here, still have clear differences in characters of the hypogynium.

The descriptions of the new species are based on a limited number of individuals, with those of *A. karkloofensis*, *A. drakensbergensis*, *A. mazumbaiensis* and *A. keurbosensis* all being based on only one specimen. This is not optimal, but material from the region is scarce, and the group seems to be rare, which complicates the study of *Allodia* in this region. This means that the variation within each of these species is unknown, as well as their distribution. As more material becomes available, this will allow for a more detailed account of these variables. The problem of rarity and singletons is common in taxonomy in general, and especially in tropical areas (see Lim *et al*. 2012).

Complete CO1 sequences were obtained for *A. nyeriensis*, *A. jaschhofi*, *A. keurbosensis* and *A. drakensbergensis*. The genetic difference between members of the different species ranged from 5.6% to 10.1%, which supports the morphological species delimitation. Our species delimitation based on genetic distances is in accordance with what is seen in other closely related species of Mycetophilidae (Jürgenstein *et al*. 2015; Kurina *et al*. 2015). Due to small sample size of *A. jaschhofi*, *A. keurbosensis* and *A. drakensbergensis*, and that all individuals of *A. nyeriensis* were collected at the same time at the same locality, the intraspecific distances could not be calculated. The smallest genetic distance, found between *A. drakensbergensis* and *A. keurbosensis* (5.6%), is also reflected in their morphological similarity and geographical proximity.

We were unable to obtain sequences from *A. karkloofensis* and *A. mazumbaiensis*, probably due to low DNA concentration. Age, storage medium and/or collecting procedure, are possible factors that can explain the negative result of the DNA extraction of the holotypes. Despite the deficiency of sequence data for *A. karkloofensis* and *A. mazumbaiensis*, we consider the morphological differences to be adequate to delimit these two species. The diagnostic morphological characters in these two species differ to the same degree as between the Afrotropical species also delimited with CO1 sequence data.

The Afrotropical region is vast and homogeneous, but also contains several centres of endemism, with species restricted to a certain habitat or area (Kingdon 1990). Large and frequent shifts in climate during the Pleistocene have led to isolated populations restricted to forested areas in different mountainous regions. Such shifts can in turn lead to speciation, which is suggested to have happened in several insect taxa (see for example Lachaise & Chassagnard 2001; Fattorini 2007). The tribe Exechiini is considered to be a relatively young group of fungus gnats, which has gone through an extensive and rapid radiation (Rindal *et al*. 2007). All of the studied species are collected in high altitude forests, ranging from 500 to 3050 masl. A rough estimate of the age of the species can be made by applying the method of Brower (1994), with a constant mutation rate of 2.3% per million years. This gives age estimates for the species described here ranging from 2.3 to 4.7 million years. The estimated age coincides with the highly variable environment caused by climate fluctuations in the region during the Pleistocene (for an overview, see e.g. Fjeldså and Lovett (1997) and references therein). This suggests that the *Allodia* species described here can be traced back to a single evolutionary origin, and that the recent isolation of populations has led to speciation in allopatry. We hope that this contribution to our understanding of the diversity and distribution of *Allodia* will initiate further studies of fungus gnats in this highly diverse and interesting biological region.

**Acknowledgements**

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**APPENDIX 1.** Outgroup taxa and specimens retrieved from BOLD.

| Species | Process ID | Specimen information |
|---|---|---|
| *Allodia (Allodia) embla* Hackman, 1971 | MYCFI099-11 | Norway, Finnmark, Sør-Varanger, Samentijohka ved Sameti (69.4011°N, 29.7192°E). 19 Jun. 2010. Leg. Finnmarksprosjektet/G. Søli. (NHMO) |
| *A. (A.) embla* | MYCFI100-11 | Norway, Finnmark, Sør-Varanger, Samentijohka ved Sameti (69.4011°N, 29.7192°E). 19 Jun. 2010. Leg. Finnmarksprosjektet/G. Søli. (NHMO) |
| *A. (A.) lugens* Wiedemann, 1817 | NOMYC475-13 | Norway, Oslo, Østmarka, Skullerud (59.862°N, 10.842°E). 12 Jun. 2013. Leg. G. Søli. (NHMO) |
| *A.(A.) lugens* | NOMYC328-12 | Norway, Oslo, Østmarka, Rundtjern (59.904°N, 10.87°E). 22 May 2010. Leg. G. Søli. (NHMO) |
| *A. (A.) truncata* Edwards, 1921 | NOMYC181-12 | Norway, Oslo, Østmarka, Rundtjern (59.904°N, 10.87°E). 22 May 2010. Leg. G. Søli. (NHMO) |
| *A. (A.) truncata* | NOMYC187-12 | Norway, Oslo, Østmarka, Rundtjern (59.904°N, 10.87°E). 22 May 2010. Leg. G. Søli. (NHMO) |
| *A. (A.) pyxidiiformis* Zaitzev, 1983 | MYCFI108-11 | Norway, Finnmark, Alta, Gargia (69.8053°N, 23.4894°E). 26 Jun. 2010. Leg. Finnmarksprosjektet. (NHMO) |
| *A. (A.) pyxidiiformis* | MYCFI081-11 | Norway, Finnmark, Alta, Gargia (69.8053°N, 23.4894°E). 10 Jul. 2010. Leg. Finnmarksprosjektet. (NHMO) |
| *A. (A.) ornaticollis* Meigen, 1818 | NOMYC407-13 | Norway, Oslo, Østmarka, Lutdalen (59.897°N, 10.836°E). 18 Jul. 2012. Leg. G. Søli. (NHMO) |
| *A. (A.) ornaticollis* | NOMYC392-13 | Norway, Oslo, Østensjø, Bogerudmyra (59.879°N, 10.836°E). 19 May 2012. Leg. G. Søli. (NHMO) |
| *A. (A.) tuomikoskii* Hackman, 1971 | MYCFI116-11 | Norway, Finnmark, Alta Gåppa river (70.0279°N, 23.3948°E). 13 Jun. 2010. Leg. Finnmarksprosjektet/G. Søli. (NHMO) |
| *A. (A.) tuomikoskii* | MYCFI064-11 | Norway, Finnmark, Alta, Gargia (69.8053°N, 23.4894°E). 10 Jul. 2010. Leg. Finnmarksprosjektet. (NHMO) |