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A remarkable new genus of Keroplatidae (Insecta, Diptera) from the Afrotropical region, with DNA sequence data

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Abstract

A new genus and species of Keroplatidae—*Kibaleana apicospinosa* **gen. & sp. n.**—is described from Kibale National Park in Uganda. The new genus is preliminarily placed to the tribe Orfeliini and it is characterized by a unique combination of diagnostic characters: wing vein R_4 missing, vein A_1 shortened, palpi short but five-segmented, fore tibia with apical comb of setae, absence of apical comb of setae on mid and hind tibiae, and male terminalia with relatively long and narrow gonostyli, each bearing a subapical tooth. The sequences of four fragments of mitochondrial DNA (COI, CytB, 12S, 16S) are provided along with a brief discussion about taxonomic position of the new genus.

Keywords

Bibionomorpha, Sciaroidea, Orfeliini, fungus gnats, new species, taxonomy, phylogeny, Africa, mitochondrial DNA, DNA barcoding

Introduction

Considerable attention has been devoted to the systematics and phylogeny of bibionomorph flies (Insecta: Diptera) during the last decade including the use of morphological as well as molecular characters (e.g. Amorim and Rindal 2007, Ševčík et al. 2016). Despite a serious discussion about composition of taxa above family level and disagreement in placing of the so-called Sciaroidea *incertae sedis* group (*op. cit.,* Jaschhof 2011), the diagnosis and composition of most families, including Keroplatidae, has remained stable (e.g. Søli et al. 2000, Evenhuis 2006a, Vockeroth 2009). With four subfamilies—viz. Arachnocampinae, Macrocerinae, Keroplatinae and Sciarokeroplatinae—Keroplatidae are known to include almost one thousand species worldwide (Evenhuis 2006a). However, in spite of a profound study of Keroplatidae by the late Loïc Matile (1938–2000; Matile 1990), there is still a huge gap in a complete picture of understanding their diversity and phylogeny, especially as it concerns the tropics.

In the Afrotropical region, only one fourth of the estimated Keroplatidae diversity has so far been described (Kirk-Spriggs and Stuckenberg 2009). The rather fragmentary knowledge is reflected, *inter alia*, by an exceptionally high number of described monotypic keroplatid genera, viz. 33, including ten of them from the Afrotropical region. The nominal and the largest subfamily Keroplatinae, including worldwide about 70% of the described keroplatid species, is divided into two tribes: Keroplatini and Orfeliini (Evenhuis 2006a). Altogether, 136 species belonging to 24 genera of Keroplatinae are recorded from the Afrotropical region, with 15 and 121 species (8 and 16 genera) in the tribes Keroplatini and Orfeliini, respectively (Evenhuis 2006a, Ševčík et al. 2015, Blagoderov and Ševčík 2017).

Within the family limits, Keroplatinae are characterized mainly by the absence of a cerebral sclerite and the presence of a tibial comb on mid or/and hind tibia, while the number of segments of maxillary palpus and the outline of antennal segments differ between Keroplatini and Orfeliini (Matile 1990, Ševčík et al. 2015). There is no exhaustive generic key published to cover all Keroplatinae. An updated key to World Keroplatini genera has been recently published by Ševčík et al. (2015) while only regional keys are available for Orfeliini (e.g. Søli et al. 2000, Vockeroth 2009, Blagoderov and Ševčík 2017).

The current study was prompted by the discovery a series of strange looking keroplatid specimens in Malaise trap samples collected in Uganda, belonging to a single undescribed species. As it has not been possible to place it to any known genus, we decided to establish a new genus for this peculiar species.

Material and methods

All the material has been collected by a Malaise trap operated in Kibale NP in southern Uganda (Figs 1, 2, see also Kurina 2012). The material was collected initially into ethyl alcohol and sorted into selected groups of insects including that of fungus gnats in

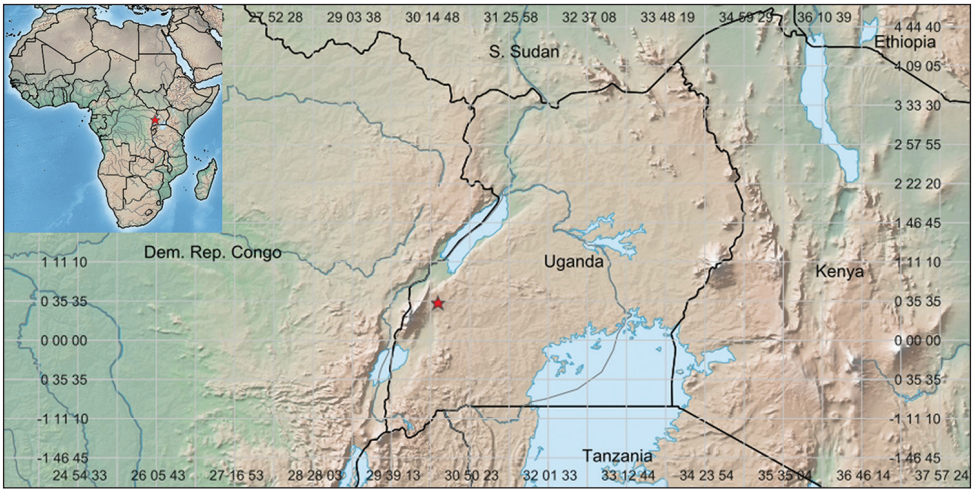

Figure 1. The sampling locality of *Kibaleana apicospinosa* sp. n. in southern Uganda.

Figure 2. Malaise trapping at Kibale National Park in southern Uganda (Photo by O. Kurina).

laboratory conditions under a stereomicroscope. The material is now stored at a temperature of -20°C, allowing appropriate study in the future. For preparing permanent slides for detailed morphological study of the new species, the wings were detached and mounted under a separate coverslip in Euparal medium. The rest of the body including terminalia was macerated in warm concentrated potassium hydroxide (KOH), thereafter washed in distilled water and dehydrated in stages of increasing concentration

of ethyl alcohol. Thereafter, parts of the body were mounted separately between two coverslips in Euparal medium and attached to the same slide using stripes of adhesive tape (see also Hippa and Kurina 2012). The slide-mounting was made under a Leica S8APO stereomicroscope. The photos of general habitus, head, thorax and terminalia were combined using the software LAS V.4.1.0. from multiple gradually focused images taken by a Leica DFC 450 camera attached to a Leica 205C stereomicroscope or Leica DM 6000 B compound microscope, respectively (see also Kurina et al. 2015). Adobe Photoshop CS5 was used for editing the figures and compiling the plates. The morphological terminology follows that of Matile (1990) and Søli et al. (2000).

The material is deposited in the following collections: IZBE – Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences (formerly Institute of Zoology and Botany), Tartu, Estonia; JSL-UOC – the reference collection of the Ševčík Lab, University of Ostrava, Czech Republic.

Molecular techniques used in this study were principally described in Ševčík et al. (2016). The mitochondrial DNA sequences of COI (barcode region, 658 bp), CytB (433 bp), 12S (356 bp), and 16S (341 bp) regions are deposited in the GenBank database.

Taxonomy

***Kibaleana* gen. n.**
http://zoobank.org/32C65548-8F31-4F31-ADFB-5A9815D1C1CF

Type species. *Kibaleana apicospinosa* sp. n.

Diagnosis. A medium-sized keroplatid fungus gnat with unmarked wings. Three ocelli in a triangular position. Median ocellus distinctly smaller, rudimentary. Antennae relatively long, with cylindrical flagellomeres, each flagellomere at least twice as long as broad. Palpus with five segments but proximal two very short and inconspicuous. Wing transparent, brownish, without markings. Vein R_4 absent. Sc ending in C, rather short, not reaching to Rs. A_1 short and weak. Fore tibia with apical comb of setae. All tibiae with one apical spur. Male terminalia with gonostylus about 5 times as long as wide, bearing a single strong subapical megaseta.

Etymology. The generic name is derived from the type locality – Kibale National Park in southern Uganda.

Gender. Feminine.

***Kibaleana apicospinosa* sp. n.**
http://zoobank.org/2A466C44-0E1C-43F2-9D26-CA35E1A05C64
Figs 3–7

Type material. *Holotype.* Male, UGANDA, Kibale NP, Kanyawara Biological Station, 00°33,901667'N 32°21,468333'E, 1504 m a.s.l., Malaise trap (No 1), 11–18.iv.2010,

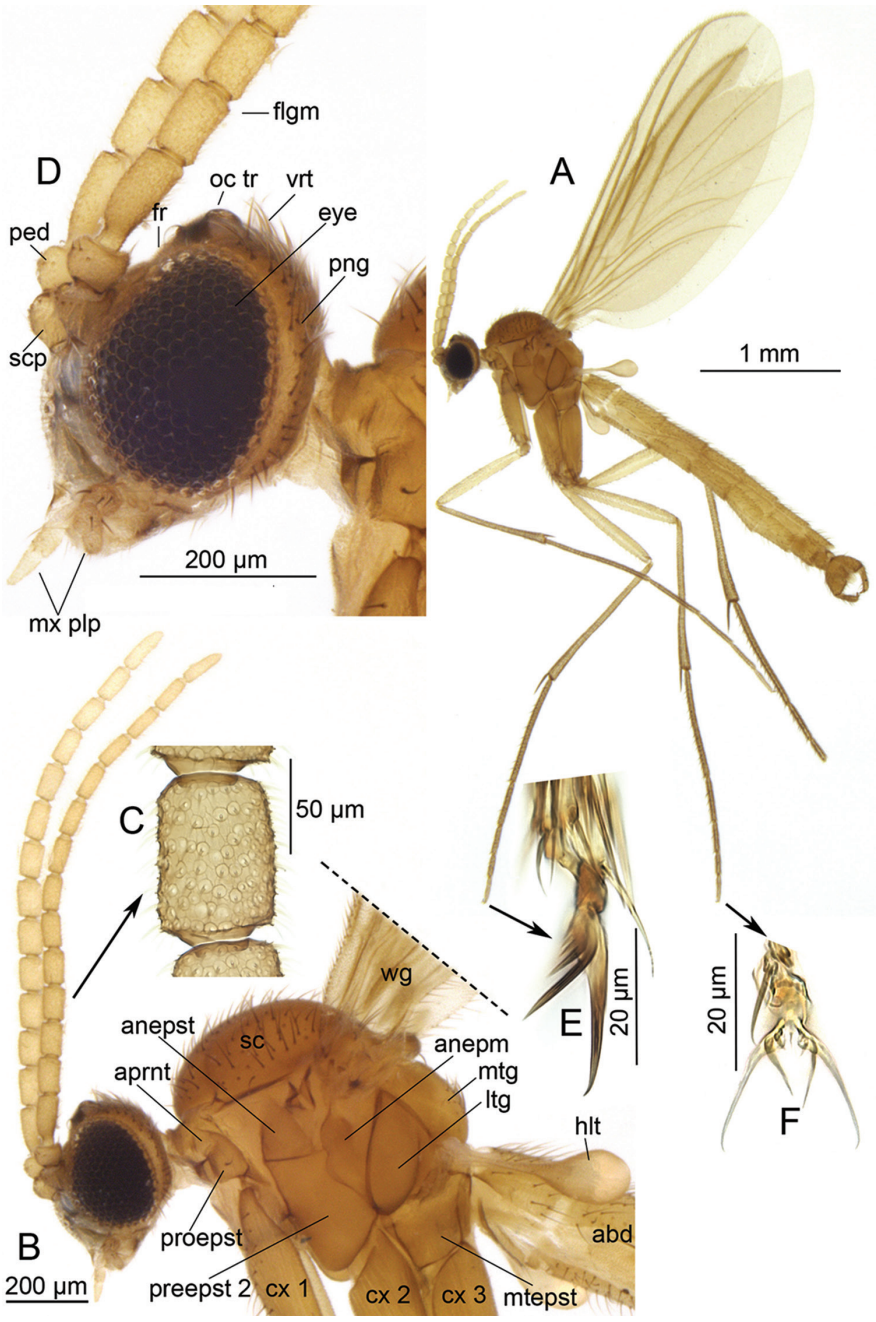

Figure 3. *Kibaleana apicospinosa* sp. n., male. **A** General habitus, lateral view **B** Head and thorax, lateral view **C** Fourth segment of flagellum, lateral view **D** Head, lateral view **E** Claw, lateral view **F** Claw, dorsal view. *Abbreviations:* abd = abdomen; anepst = anepisternum; anepm = anepimeron; aprnt = antepronotum; cx = coxa; eye = compound eye; flgm = flagellar segment; fr = frons; hlt = halter; ltg = laterotergite; mtepst = metepisternum; mtg = mediotergite; mx plp = maxillary palpus; oc tr = ocellar triangle; ped = pedicel; png = postgena; preepst 2 = preepisternum 2; sc = scutum; scp = scape; vrt = vertex; wg = wing.

S. Katusabe et al. leg. (in alcohol, IZBE). *Paratypes*. Male, same as holotype (in alcohol, IZBE), 7♂♂, same as holotype except 28.iii–4.iv.2010 (3♂♂ on slides, IZBE; 2♂♂ in alcohol, IZBE; 2♂♂ used for DNA analysis and subsequently stored in a pinned microvial with glycerol, JSL-UOC).

Male. Body length 3.3–3.5 mm.

Head (Figs 3D, 4). Round, light brown. Three ocelli in an equilateral triangular arrangement, laterals about twice as large as medial. Ocellar triangle prominent, and darker than vertex and frons, with a medial furrow bifurcating towards medial ocellus. Compound eye almost round with shallow invagination laterally from antenna; about 1.3 times as high as broad in lateral view; ommatidia surrounded by 5–7 setulae. Vertex and postgena with short dark setae, frons non-setose. Face non-setose, rectangular, with centro-vertical furrow and darker anterior margin. Clypeus obovate, non-setose, with lateral portions darker. Antenna entirely brownish yellow, lighter than vertex and scutum. Scape cylindrical, 0.67 times as long as wide. Pedicel almost spherical, about 0.75 times as long as wide. Flagellum apically slightly tapering, with 14 segments, first segment elongated, 2.7 times as long as wide; fourth segment 1.57 times as long as wide; preapical segment 2.4 times as long as wide; apical segment conical, 3 times as long as wide basally. Scape and pedicel, both with incomplete preapical circle of short dark setae. Flagellum with pubescence about 1/3 of segment's width. Mouthparts yellowish. Palpus with 5 segments: first two segments extremely short and discernible only from posterior view; third segment cylindrical almost 2 times as long as wide, with sparse dark setae; fourth segment apically widening, about as long as wide apically, with sparse dark setae; fifth segment tapering, about 3 times as long as wide basally, with 3–4 short apical and 5–6 subapical setae; length ratio of palpal segments 3:4=1.25 and 4:5=0.67.

Thorax (Figs 3B, 5B). Light brown with areas along setae lighter. Scutum with distinct dorsocentral, acrostichal and lateral setae. Acrostichal setae present as a simple row; two rows of dorsocentrals, both consist of two close lines of subequal setae; lateral setae numerous, not divided to pre- and postalars. Scutellum with 8 short setae along its margin. Proepisternum with one seta, mediotergite with ca 20 setae on posterior part. Remaining thoracic parts non-setose.

Wing (Fig. 5A). Length 2.6 mm, width 0.93 mm, ratio of length to width 2.8. Hyaline, without any darker markings. Membrane covered with light brown microtrichia, without macrotrichia. Veins light brown, costal and radial veins thicker and slightly darker, R_1 and apical 2/3 of R_5 with macrotrichia dorsally. Costa produced beyond R_5 to about 2/3 of the distance of the tips of R_5 and M_1. Sc ending in C at level of Rs, Sc_2 absent. Rs oblique, as long as r-m. tb pale but discernible, m-cu about three times as long as tb. R_5 slightly sinusoid. Cu_1 not reaching wing margin. Cu_2 fold like, in basal 2/3 distinct, in apical 1/3 pale, apically approaching Cu_1 and not reaching wing margin. A_1 short, indistinct. Haltere pale, stem with anterodorsal row of dark setae, knob with few sparse weak setae.

Legs (Figs 3A, 6A–D). Yellowish to light brown, coxae darker but lighter than thorax. Tibiae and tarsi seem darker because of dense setae. Fore coxa with dark ante-

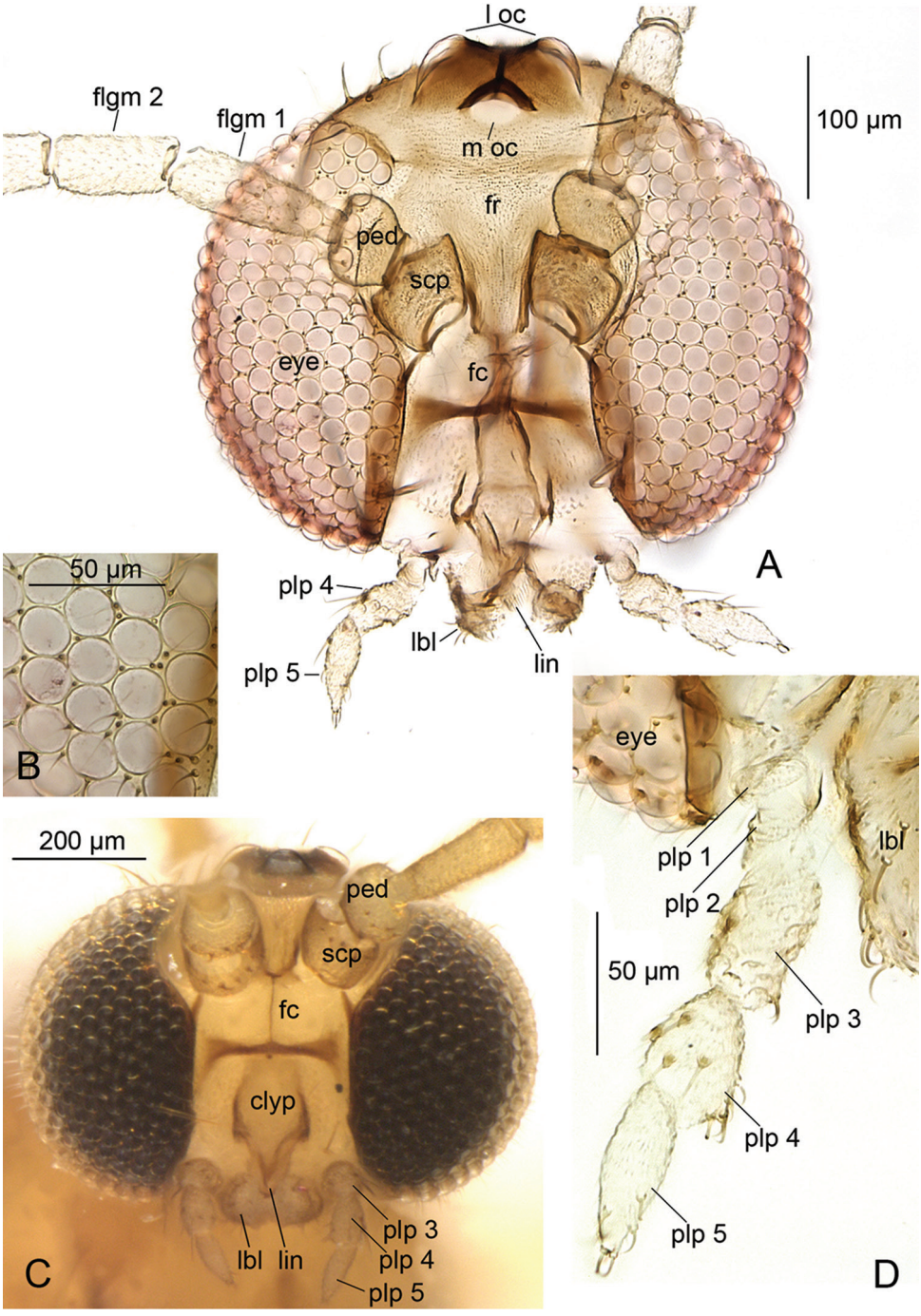

Figure 4. *Kibaleana apicospinosa* sp. n., male. **A** Head, frontal view **B** Compound eye, closer view **C** Head, frontal view **D** Maxillary palpus, posterior view. *Abbreviations:* clyp = clypeus; eye = compound eye; fc = face; flgm = flagellomeres; fr = frons; lbl = labellum; lin = lingua; l oc = lateral ocellus; m oc = medial ocellus; ped = pedicel; plp = segments of maxillary palpus; scp = scape.

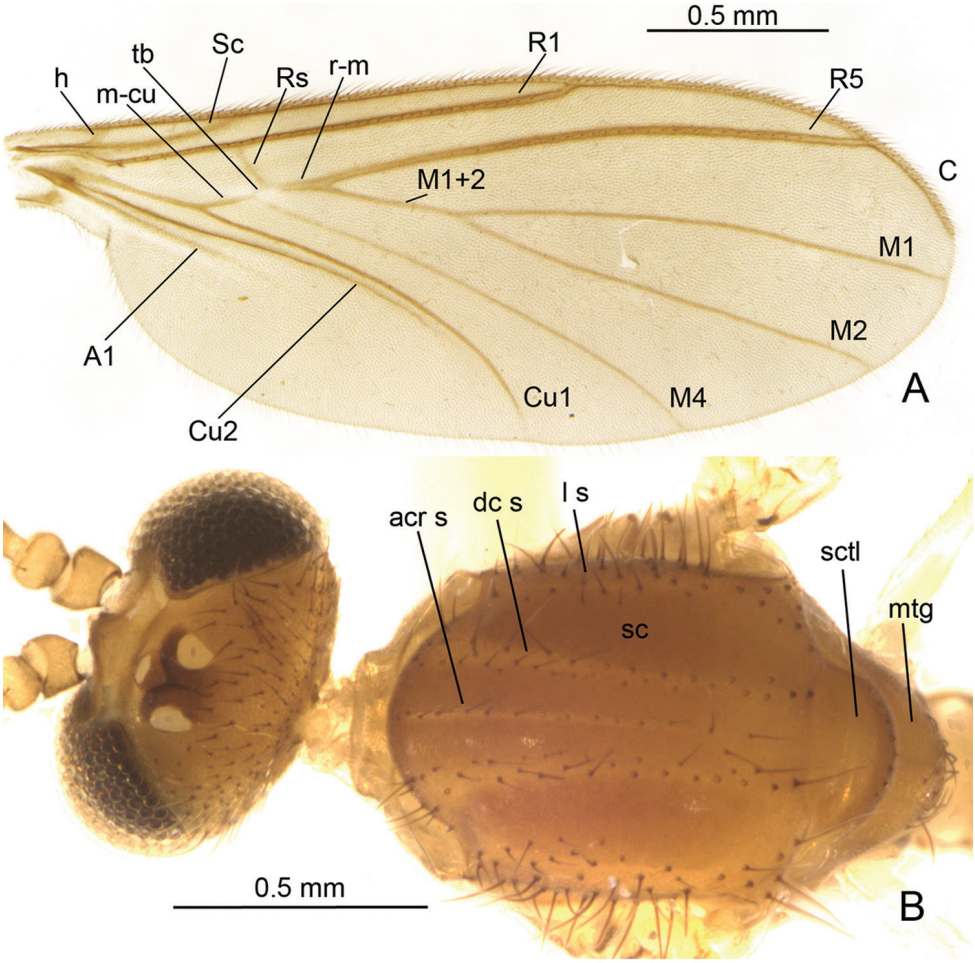

Figure 5. *Kibaleana apicospinosa* sp. n., male. **A** Wing, dorsal view **B** Head and thorax, dorsal view. *Abbreviations:* A = anal vein; acr s = acrostichal setae; C = costal vein; Cu = branches of cubitus; dc s = dorsocentral setae; h = humeral vein; l s = lateral setae; M = branches of media; m-cu = medio-cibital crossvein; mtg = mediotergite; R = branches of radius; r-m = radio-medial crossvein; Sc = subcostal vein; sc = scutum; sctl = scutellum; tb = basal transversal.

rior setae on its entire length and with 2–3 apico-medial setae. Mid coxa with anterior setae on apical half. Hind coxa with row of posterior setae on apical half. Femora entirely covered with short dark setae which are more scattered ventrally. Tibiae apically slightly widening, with setae not arranged to distinct rows. All tibiae with one apical setose spur. Spurs 1.4, 2.6 and 3.3 times as long as apical width of fore-, mid- and hind tibia, respectively. Fore tibia with a distinct apical comb of setae. Mid and hind tibiae without distinct apical combs of setae; with 3–4 apical setae somewhat deviating from other setosity (Fig. 6). Tarsal claws small, with basoventral comb of teeth, the most apical one about half as long as the claw itself. Ratio of femur to tibia for fore-, mid- and

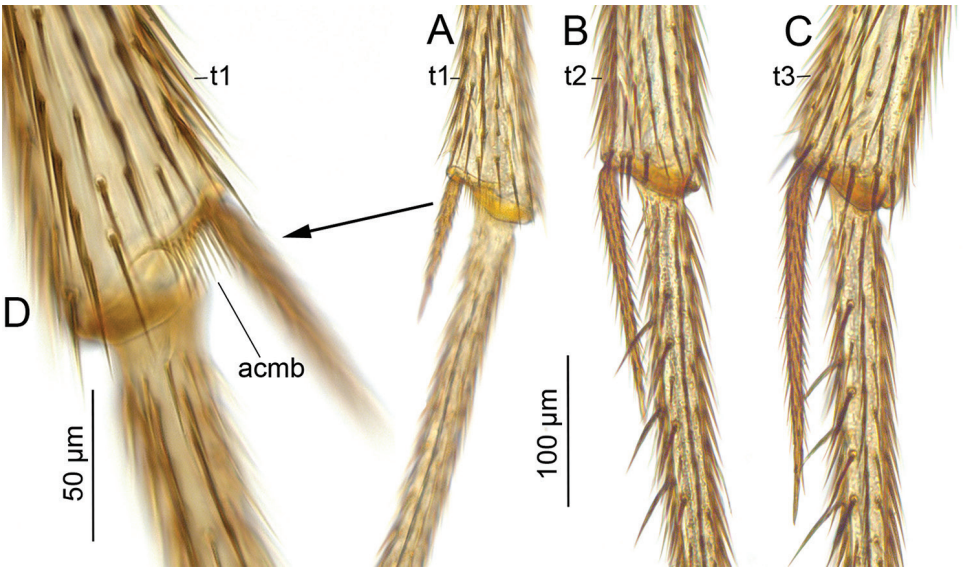

Figure 6. *Kibaleana apicospinosa* sp. n., apical part of tibia and basal part of first tarsal segment, male. **A** Fore leg **B** Mid leg **C** Hind leg **D** Fore leg, closer view. *Abbreviations:* acmb = apical comb of setae; t = fore, mid and hind tibiae.

hind legs: 0.85; 0.72; 0.96. Ratio of tibia to basitarsus for fore-, mid- and hind legs: 1.04; 1.33; 1.4.

Abdomen. Yellowish, apically somewhat darker.

Terminalia (Fig. 7). Yellowish brown. Tergite IX quadrangular; about as long as wide; with wide anterior excavation; posteriorly with shallow concavity; posterior half with setae similar to these on gonocoxa ventrally. Cercus oval, about 0.6 times as long as tergite IX. Hypoproct tapering posteriad, posteriorly blunt, with two stronger setae at posterolateral corner of both sides. Gonocoxa ventrally with membranous medial part that is posteriorly drawn into wide, apically slightly concave process. Gonocoxa dorsally simple with posterior setae more prominent and dense. Height of aedeagal complex less than that of gonocoxa. Gonocoxal and parameral apodemes dark brown, well discernible from dorsal view. Ejaculatory apodeme simple. Aedeagus membranous, apically widening. Gonostylus simple, about 5 times as long as wide; apically more sclerotized and pointed, with preapical additional slerotized spine on ventral side; basal third with dorsomedial flange. Setae similar to these on gonocoxite except two more prominent ones on medial side; pointed apical part of gonostylus non-setose. Gonostylus with small hook-shaped process basally.

Female. Unknown.

Etymology. The specific name is Latin, composed of the prefix *apico-* [apical] and word *spinosa* [spiny or thorny], referring to the preapical spine on the gonostylus ventrally.

DNA sequences. Partial DNA sequences of four mitochondrial markers (COI, CytB, 12S and 16S) are deposited in the GenBank database (the accession numbers are as follows: KY963422, KY963423, KY963420, and KY963421).

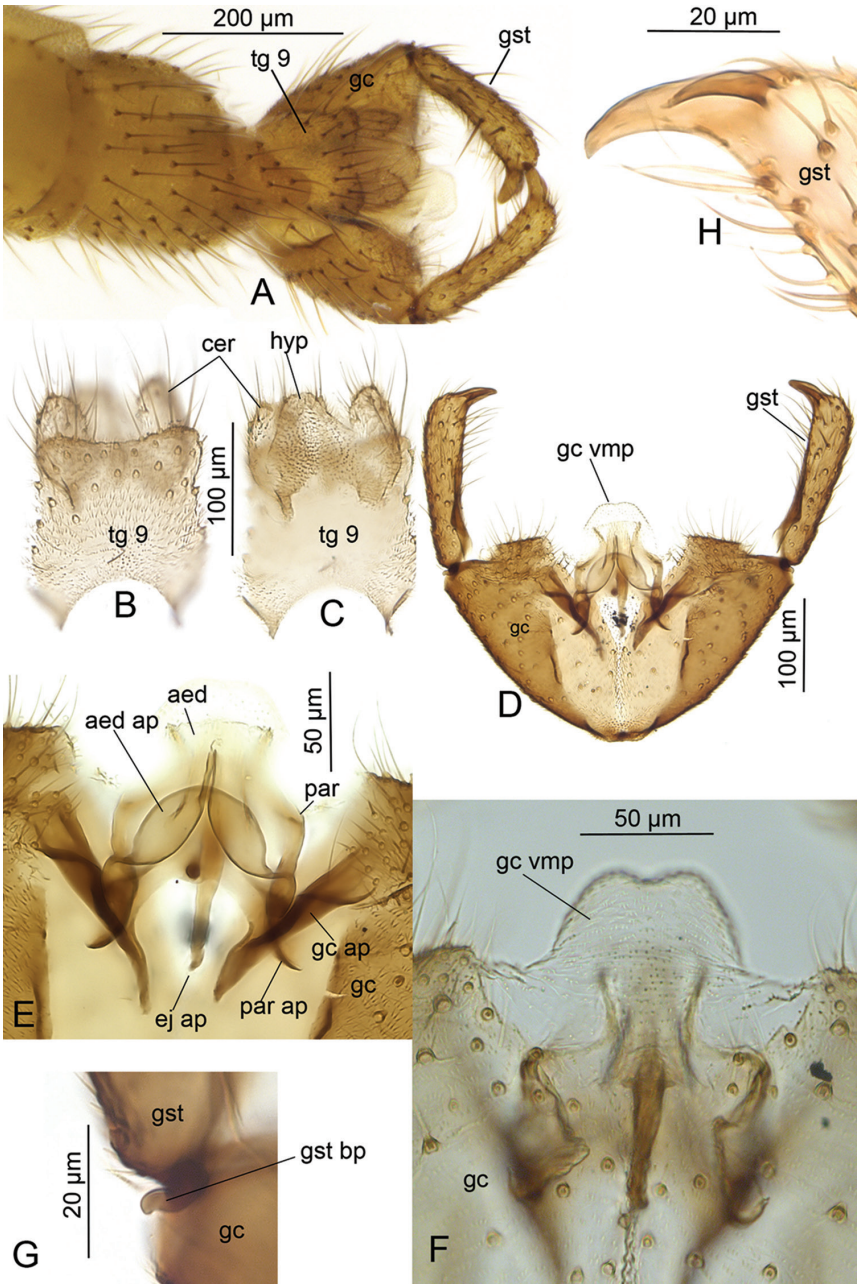

Figure 7. *Kibaleana apicospinosa* sp. n., male terminalia. **A** Dorsal view **B** Tergite IX and cerci, dorsal view **C** Tergite IX, cerci and hypoproct, ventral view **D** Male terminalia, dorsal view, tergite IX detached **E** Aedeagal complex, dorsal view **F** Medial part of gonocoxite, ventral view **G** Basal part of gonostylus, dorsal view **H** Apical part of gonostylus, ventral view. *Abbreviations:* aed = aedeagus; aed ap = aedeagal apodeme; cer = cercus; ej ap = ejaculatory apodeme; gc = gonocoxite; gc ap = gonocoxal apodeme; gc vmp = ventromedial process of gonocoxite; gst = gonostylus; gst bp = basal process of gonostylus; hyp = hypoproct; par = paramere; par ap = parameral apodeme; tg 9 = tergite IX.

Discussion

If we use the key to the genera of Keroplatidae in the Manual of Afrotropical Diptera (Blagoderov & Ševčík 2017), the new genus would run either to Macrocerinae, but it has no distinct cerebral sclerite and also its flagellomeres are not so much elongated, or to the Keroplatinae tribe Orfeliini, if we ignore the absence of apical combs on mid and hind tibia. The overall habitus of *Kibaleana* gen. n. suggests that it should be assigned rather to the tribe Orfeliini, at least tentatively. Most genera of Macrocerinae (except *Micromacrocera* Papp, 2008 and *Robsonomyia* Matile & Vockeroth, 1980) also have the basal part of M distinct or traceable, which is not the case of *Kibaleana* gen. n.

On the other hand, according to its wing venation, the new genus falls into the distinct group of genera in the tribe Keroplatini, with vein R_4 absent and A_1 reduced. This group currently includes the following mainly tropical genera: *Asiokeroplatus* Ševčík, Mantič & Blagoderov, 2015, *Chetoneura* Colless, 1962, *Microkeroplatus* Ševčík & Papp, 2009 and *Pseudochetoneura* Ševčík, 2012. However, all these four genera have reduced mouthparts and more or less shortened, flattened or otherwise modified antennae (cf. Ševčík 2012, Ševčík and Papp 2009, Ševčík et al. 2015), allowing them to be classified into the tribe Keroplatini. The number of palpal segments in *Kibaleana* gen. n. is five but actually only the apical three are clearly discernible (Fig. 4) while the proximal two are very small, so that they may well have been overlooked in the case of other genera. The male terminalia of *Kibaleana* gen. n. are quite different from both *Asiokeroplatus* and *Pseudochetoneura*, as well as from the other genera of Keroplatini, but also from most Macrocerinae.

The wing venation including absence of vein R_4, arrangement of setae on the scutum and the general facies puts the new genus close to *Trigemma* Hardy, 1960 – an Orfeliini genus, known by two endemic species from the Hawaiian Islands (Hardy 1960, Evenhuis 2006b). Both genera have each tibia with one apical spur: *Trigemma* has the spur on fore tibia small but distinct while the spurs on the mid and hind tibiae are very large (Evenhuis pers. comm.), while the spurs' length of *Kibaleana* gen. n. is at least 1.4 times of the apical width of respective tibia (see Fig. 6). However, the new genus has only fore tibia with an apical comb of setae but in *Trigemma*, there is a small apical comb on the apex of the fore tibia and very dark and conspicuous combs on the mid and hid tibiae (Evenhuis pers. comm.). In contrast to only three clearly discernible palpal segment in *Kibaleana* gen. n., the species of *Trigemma* have four well visible segments with the apical segment very long and thin, pencil like, and length of the remaining segments about 1.5–2 times of their width (Evenhuis pers. comm.). In addition, the new genus has the anepisternum non-setose (with setae in *Trigemma*) and medial flagellar segments 1.5 times as long as wide (about rectangular in *Trigemma*). Male terminalia of both genera correspond to the simple ground plan of Keroplatidae but the gonostylus of *Kibaleana* gen. n. is prolonged with a subapical tooth (more wide and with a single or two pronglike projection apically in *Trigemma*) and tergite IX quadrangular (prolongedly ovoid in *Trigemma*).

The problems related to the placement of the new genus among the subfamilies and tribes within Keroplatidae represent a further confirmation of the opinion expressed

by Ševčík et al. (2015), that the traditional delimitation of the tribes Keroplatini and Orfeliini, as well as of many other supraspecific keroplatid taxa, is getting more and more questionable and requires further study, especially by molecular methods.

Molecular phylogeny of the families Keroplatidae and Lygistorrhinidae based on multiple nuclear and mitochondrial markers will be presented elsewhere (Mantič et al. in prep.) and it will definitely shed more light on the phylogenetic position of not only *Kibaleana* gen. n. but also of many other enigmatic taxa.

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