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Stingless bees (Hymenoptera: Apidae) in Holocene copal and Defaunation resin from Eastern Africa indicate Recent biodiversity change

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Abstract

Although specimens in fossil to Recent resins are remarkable for their fidelity of preservation, amber is well known and studied, unlike the younger resins as Pleistocene copal (2.58 to 0.0117 Ma) and Holocene copal (0.0117 Ma to 1760 AD), or Defaunation resin, which is resin produced after 1760 AD. However, the scientific relevance of these younger resins preserving arthropods that lived in pre-Anthropocene time is often underestimated. Here, we present specimens of workers of stingless bees included in copal and Defaunation resin, from the coastal vichaka forests in Tanzania, and from northwest Madagascar, ranging in age from almost 3,000 BP years to only 80 +/- 30 BP years and from 2015, respectively. Three known species *Hypotrigona gribodoi* (Magretti, 1884), *Liotrigona bouyssoui* (Vachal, 1903), and *Liotrigona nilssoni* Michener, 1990 and two new species *Axestotrigona kitingae* **sp. nov.** and *Hypotrigona kleineri* **sp. nov.** have been discovered from thirty-six studied specimens. We also noted that the holotype of *Trigona richardsi* Darchen, 1981 is a junior subjective synonym of *Axestotrigona ferruginea ferruginea* (Lepelletier, 1841). The coastal forests in the East Africa region and in East Madagascar are now highly fragmented so that we can expect that the new species are already extinct. Here we demonstrate that the study of inclusions in copal and Defaunation resin can document losses of local biodiversity resulting, *e.g.*, from deforestation and they also bring proof of this potential anthropic defaunation.

Keywords: Hymenoptera, Apidae, Meliponini, Tanzania, Madagascar, Anthropocene

Introduction

While amber is well known for its remarkable fidelity of preservation and its inclusions are accordingly studied with great intensity for insights into ancient and extinct biotas, the organisms

preserved in copal and Defaunation resin (*sensu* Solórzano-Kraemer et al., 2020) are considered with far less scrutiny. Yet, the quality of preservation in copal and Defaunation resin is on par with that of amber and the biota included therein is of equal value for informing us about ecosystem changes, albeit of far briefer timescales. The biotas of copal and Defaunation resin exuded since the Pleistocene have the potential to document the impacts of Recent climatic events (*e.g.*, repeated Pleistocene glaciation) and even human activity, such as losses of local biodiversity resulting from habitat destruction (*e.g.*, deforestation, agricultural development, altered land uses), pollution, or climate change. Modern species no longer occurring in a region may be found to occur in copal or Defaunation resin, documenting their past persistence in a region prior to habitat changes. Similarly, species with possibly narrow niche restrictions may be found to have become recently extinct, recovered today only in copal or Defaunation resin but possibly flourishing in the recent past prior to increased human activity. Such species potentially represent “hidden” losses of the current biodiversity crisis as they may have been driven to extinction within the last century or two as their habitats were converted to farmland and before they could be discovered and described from their natural environments. It is true that many still-living species have been discovered in Defaunation resin (often reported as copal when first described). It is true that many modern species are well known from copal and Defaunation resin (*e.g.*, Olmi, 2010; Vankerkhoven et al., 2010), and several species have been first documented in Defaunation resin or Holocene copal that were later discovered to be living in the same environs today, with their copal counterparts either representing the first-known members of the species (*i.e.*, the species was hitherto unknown from the modern fauna and therefore first described from copal or Defaunation resin) or relegated to synonymy under a previously documented name (*e.g.*, Penney et al., 2005; Dunlop et al., 2016). Other species described from copal and resin remain the only known representatives of their taxon (*e.g.*, Bosselaers, 2004; Wunderlich, 2004, 2011, 2012; Bosselaers et al., 2010; Bukejsa and Legalov, 2017), either remaining to be discovered alive or having truly become recently extinct. Given that many biodiversity hotspot areas in which copal and resin are found, such as Madagascar or Colombia, have undergone or are currently undergoing dramatic anthropogenic alteration, it is not unlikely that such species in copal and resin are recently extinct. Thus, the investigation of copal and Defaunation resin biotas serve as a suitable and important source of data for exploring Recent impacts of habitat and climate change.

Here we explore the fauna of stingless bees (tribe Meliponini) preserved in Holocene copal and Defaunation resin from Tanzania and two species from Madagascar, one living species and one putative extinct species, originating from lowland coastal forests. Stingless bees are abundant, highly eusocial bees of approximately pantropical distribution, although with the majority of their nearly 520 species occurring in the Neotropics (Michener, 2007, 2013). Meliponines serve important ecological and economic roles, particularly as critical pollinators and for the production of honey or cerumen (Michener, 2013). In addition, stingless bees feature in the religious and cultural practices of indigenous populations in many tropical countries (Ayala et al., 2013; Kahono et al., 2018; Engel et al., 2019; Bhatta et al., 2020). Stingless bees are pollen generalists and therefore important to a wide variety of plants in many families. Nests are often built within cavities such as tree holes or in the ground, and sometimes are integrated into the nests of other insects such as those of termites (Roubik, 2006; Michener, 2007, 2013). Resin and resin-derived terpenes play a fundamental role in the ecology of tropical stingless bees (Leonhardt et al., 2011). As part of nest construction, workers frequently collect resin to use in

binding nest materials or as part of the production of propolis. The resin also serves to protect the colony against bacteria and fungi and to defend the colonies against predators, like *e.g.*, ants (Lehmberg et al., 2008). Given the propensity of meliponines to collect resin, it is no surprise that they are frequently represented in amber and copal (as well as Defaunation resin) (Moure and Camargo, 1978; Camargo et al., 2000; Michez et al., 2012; Engel and Michener, 2013; Barden and Engel, 2021; Engel et al., 2021), as workers assuredly collected from the trees exuding resin that would eventually become amber and copal. Thus, stingless bees have a higher probability of becoming ensnared and entombed in resin and preserved in amber (Solórzano Kraemer et al., 2018). In the Afrotropical Region there are traditionally six genera of stingless bees recognized (Michener, 2000), although differences in classification do exist (Moure, 1961; Eardley, 2004; Eardley and Urban, 2010; Rasmussen and Cameron, 2010; Rasmussen et al., 2017). About 33 species are currently known from the region, and at least nine from Tanzania (Eardley and Urban, 2010; Fabre Anguilet et al., 2015).

Specimens of *Liotrigona* Moure, 1961, *Hypotrigona* Cockerell, 1934, and *Axestotrigona* Moure, 1961 (the latter treated herein as a distinct genus *sensu* Moure, 1961, *contra* Michener, 2007, *vide infra*) have been found in Holocene copal and Defaunation resin from Tanzania and Madagascar. All three are comparatively common genera in East Africa, particularly colonies of *Hypotrigona* and *Axestotrigona*. While *Hypotrigona* is common and abundant, often represented by long series of individuals in samples, *Liotrigona* in Africa is less numerous and more infrequently sampled (Brooks and Michener, 1988). Conversely, species of *Liotrigona* are common in Madagascar where *Hypotrigona* does not occur. *Liotrigona* and *Hypotrigona* include generally smaller, even minute, bees (1.8–4.0 mm in length) found in tropical as well as more arid areas, and are particularly common in East African and Malagasy coastal forests (Koch et al., 2011; Eardley and Kwapong, 2013). Species of *Liotrigona* and *Hypotrigona* are particularly well studied but nonetheless difficult to distinguish, even for specialists. The genus *Axestotrigona* (*sensu* Moure, 1961) consists of typically small to moderate-sized bees (5–8 mm in length) that are comparatively common in Africa. There are six species in the genus, two of which occur in Tanzania, *Axestotrigona togoensis* (Stadelman, 1895) and *A. ferruginea* (Lepeletier, 1841), the latter of which is distributed widely and also occurs in the coastal forests of East Africa.

Tanzanian and Malagasy Holocene copal and Defaunation resin are well known for revealing possible recent extinctions, changes in distribution, and in some cases behaviors (Poinar, 1994; DuBois, 1998; Delclòs, et al., 2020; Solórzano-Kraemer et al., 2020). Holocene copal has an age of 0.0117 Ma to 1760 AD while Defaunation resin was defined as resin exuded after 1760 AD, which is the starting point of the Industrial Revolution (Solórzano-Kraemer et al., 2020). The ages of Tanzanian and Malagasy copal/Defaunation resin span the Holocene to the modern era (Delclòs et al., 2020; Solórzano-Kraemer et al., 2020). Holocene copal and Defaunation resin from Tanzania were produced by *Hymenaea verrucosa* Gaertner, 1791 (Angiospermae: Fabaceae), the same botanical source as Defaunation resin from Madagascar (Delclòs et al., 2020). Forests of *H. verrucosa* today occur only in some parts of East Madagascar and East Africa. *Hymenaea* resin is a diterpenic resin containing ozic acid, an enantiomer of communic acid that polymerizes fast as soon as it is exuded from the tree in contact with air and light (Cunningham et al., 1977). This property allows the resin to preserve arthropods with exquisite quality.

Copal and Defaunation resin from Tanzania come from coastal vichaka forests (*vichaka* meaning, “scrub” in Swahili), unique areas of dry lowland evergreen forest, woodland, and particularly bushland interposed between the Indian Ocean littoral mangroves and the Eastern Arc rainforests situated approximately 150 km inland (Fig. 1) (Sunseri, 2007). During the 19th century, “copal” from the Zaramo Region – from Bagamoyo in the north to Lindi in the south – was intensively traded and 95% of East African “copal” came from this area (Sunseri, 2007). Unfortunately, the East Africa forests have a long history of deforestation, from 1900 to 2000 has been deforested up to 93.0% of their surface (Aleman et al., 2017). Today, the coastal region of East Africa contains highly fragmented habitats that are of global importance and concern in relation to their biodiversity (categorized as the East African Coastal Forest biodiversity hotspot) (Ngumbau et al., 2020), with these natural areas isolated in patches and greatly susceptible to destruction and loss of species (Schipper and Burgess, 2003; Tabor et al., 2010). Indeed, according to Burgess and Muir (1994), the distinct coastal vichaka forests and bushland have a 3–5-month-long dry season and harbor a profusion of endemic plants and animals specialized to this unique environment. The biota includes species of birds, mammals, reptiles, amphibians, and innumerable invertebrates of global conservation significance (Burgess and Muir, 1994; Burgess and Clarke, 2000;), and also supports a large number of forest-obligate endemisms or threatened species with narrow geographic ranges that are often endemic to a single site or forest patch (Burgess, 2000). Among the plants, *H. verrucosa* is present in the coastal forests of Tanzania, but also more broadly is present along the African coast from Somalia to Mozambique and in the East coast of Madagascar. Trees of *Hymenaea* have also been used as a source of timber and poles for construction, and as a source of charcoal, and with about 90% of Tanzania’s energy production derived from wood and charcoal it is a vital source of income to many rural villages (Sunseri, 2014).

In Madagascar, *H. verrucosa* lives in different zones along the East coast. In the southeast it is found in the Mananjary Region; in the northeast in the Sambava Region, and in the north in the Antsiranana Region. In all these regions, copal and Defaunation resin are collected and sold in small markets and also in the international trade (Delclòs et al., 2020). Madagascar has long been recognized as one of the world’s highest priority hotspots and one of the top megadiverse countries (Mittermeier et al., 2005). As well as in the East African forest, the east coast littoral forest in Madagascar has been already deforested by over 90% with a fast rate of destruction. So fast that only in 2020, Madagascar lost 241kha of tree cover (Global Forest Watch [last access September 2021]). Accordingly, the east coast littoral forest belongs to the most threatened ecosystems of the world (Ganzhorn et al., 2001; De Gouvenain and Silander, 2003).

Here we report a new species of *Axestotrigona* in Holocene copal, and a new species of *Hypotrigona* in Holocene copal and Defaunation resin. Furthermore, we report the presence of extant species of *Hypotrigona* and *Liotrigona* in ~2800-year-old copal as well as in Defaunation resin, all these species from Tanzania. Furthermore, we report one extant species of *Liotrigona* in Defaunation resin from Madagascar. Using synchrotron-radiation microcomputed tomography (SR μ CT) as a powerful tool for the study of inclusions in resins, we highlight the importance of studying species in Holocene copal and Defaunation resin for understanding potential biodiversity changes or losses in historical times.

Material and methods

Collection

Thirty-six specimens of Meliponini bees have been studied present in Holocene copal and Defaunation resin originated in the coastal forests of Tanzania (Fig. 1). The copal and resin collection were donated to Senckenberg Research Institute and Natural History Museum Frankfurt between the years 1874 and 1901 and was labeled as “Madagascar or Zanzibar copal”. The material is housed in the amber collection at the Senckenberg Research Institute and Natural History Museum Frankfurt, Germany (SMF). A list of all pieces with the specimens presented herein and their syninclusions is presented in Table. 1.

Resin from *H. verrucosa* has been collected in Madagascar in Nosy Varika and Ambahy in 2013, and in Sacaramy (close to Antsiranana = Diego Suarez) in 2015 (Fig. 1). Permits were obtained from the Ministère de l’Environnement, de l’Écologie et des Forêts (192/13/MEF/SG/DGF/DCB.SAP/SCB and 060/15/MEEF/SG/DGF/DCB.SAP/SCB).

The holotype of *Liotrigona vetula* Moure and Camargo, 1978 N. NHMD-115579, was borrowed from the Zoological Museum of Copenhagen. It had to be polished at the SMF before revision because the surface was oxidized, covered in the typical formation of a network of cracks over the surface (“crazing”).

"[insert Figure 1.]"

Preparation and radiocarbon analyses

The pieces were cut and polished at the SMF using Phonix Beta polishing machine with grinding paper for metallography wet and dry: Grip 1200, 2500 and 4000. After polished were embedded in Epoxy resin Araldite 2020® and storage following Sadowski et al. (2021) recommendations.

We follow Solórzano-Kraemer et al. (2020) for the classification of copal and resin: Defaunation resin is younger than 1760 AD and the Holocene copal is 0.0117 Ma to 1760 AD. Determination of the age has been done with standard and rigorous methods of radiocarbon analysis (^{14}C), Beta Analytic Inc. Laboratories (www.radiocarbon.com/), before scanning with synchrotron-radiation based X-ray microtomography (SR μ CT). Results are presented in Table 1 and are reported in conventional radiocarbon age (BP), where "present" is defined as 1950 AD, calibrated radiocarbon age (cal. BP) and in calibrated calendar year (cal. AD). Calibrated ages are also included even if, in some cases, they present variability given the nature of the calibration curves. However, since we know the samples came into the museum in 1901, we eliminate the post-1950 AD and the 1876 to 1945 AD ranges given in the results from Beta Analytics Inc. Laboratories.

Table 1. Specimens with collection number, syninclusions, origin, and ^{14}C data of Holocene copal from Tanzania and Defaunation resins from Tanzania and Madagascar. Abbreviations: conventional radiocarbon age before present (BP), calibrated calendar year (cal. AD), calibrated radiocarbon age (cal. BP), before Christ (BC). Ages are presented as obtained from the

radiocarbon analysis in BP but also in cal. BC/AD/BP because for some pieces the variability between both given data can be significant.

Piece collection number	Species collection number	Syninclusions	Origin	Age
SMF Be 3503	<i>Axestotrigona kitingae</i> sp. nov. (Holotype SMF Be 3503.1a)	1 Lepidoptera	Holocene copal from Tanzania	1050 +/- 30 BP 987 - 1051 cal. AD
SMF Be 3694	<i>Hypotrigona gribodoi</i> (Magretti, 1884) SMF Be 3694.1a	8 <i>Hypotrigona gribodoi</i> (Magretti, 1884)	Defaunation resin from Tanzania	100 +/- 30 BP 1807 AD
SMF Be 3724	<i>Hypotrigona kleineri</i> sp. nov. (Holotype SMF Be 3724.1a)	1 <i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3724.2a)	Defaunation resin from Tanzania	80 +/- 30 BP 1698 to 1724 cal. AD (252 to 226 cal. BP) 1810 to 1870 cal. AD (140 to 80 cal. BP)
SMF Be 3498	<i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3498a)	3 Coleoptera: Ptilidae 1 Coleoptera: Staphylinidae: Osorinae? 4 Diptera: Cecidomyiidae 2 Diptera: Mycetophilidae 1 Diptera: Phoridae 1 Diptera: Phoridae: <i>Megaselia</i> sp. 2 Diptera: Psychodidae: Phlebotominae 5 Diptera: Sciaridae 1 Hemiptera: Auchenorrhyncha 4 Hemiptera: Sternorrhyncha 1 Hymenoptera indet. 4 Hymenoptera: Chalcidoidea: Mymaridae 1 Psocoptera	Holocene copal from Tanzania	1250 +/- 30 BP 764 - 894 cal. AD
SMF Be 3710	<i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3710.1a)	1 Blattaria 1 Coleoptera: Corylophidae 1 Diptera: Psychodidae: Phlebotominae 1 Diptera: Psychodidae: Phlebotominae rest 1 Thysanoptera rest	Holocene copal from Tanzania	2770 +/- 30 BP 937 - 807 cal. BC
SMF Be 2509	<i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 2509a)	1 Diptera: Brachycera 1 Diptera: Phoridae: <i>Syneura</i> sp. 5 <i>Hypotrigona kleineri</i> sp. nov. (Paratypes SMF Be 2509b–f)	Holocene copal or Defaunation resin from Tanzania	?
SMF Be 3525	<i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3525a)	7 <i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3525b–g) 2 Araneae 2 Coleoptera: Coccinelidae 1 Coleoptera: Curculionidae rest 4 Diptera: Brachycera 1 Diptera: Cecidomyiidae 1 Diptera: Phoridae	Holocene copal or Defaunation resin from Tanzania	?

		2 Diptera: Sciaridae 1 Diptera: Lygistorrhinidae 1 Hymenoptera: Mymaridae 1 Hymenoptera: Apidae: Meliponinae rest 1 Hemiptera: Auchenorrhyncha 2 Hemiptera: Sternorrhyncha 2 Heteroptera 6 Hymenoptera: Chalcidoidea 2 insect indet. 1 Thysanoptera Plant rest		
SMF Be 3532	<i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3532a)	1 Diptera: Psychodidae: Phlebotominae 1 Hymenoptera: Formicidae winged 1 Psocoptera rest	Holocene copal or Defaunation resin from Tanzania	?
SMF Be 3573	<i>Hypotrigona kleineri</i> sp. nov. (Paratype SMF Be 3573.1a)	4 <i>Hypotrigona kleineri</i> sp. nov. Paratypes SMF Be 3573.2a, SMF Be 3573.3a,b, SMF Be 3573.4a	Holocene copal or Defaunation resin from Tanzania	?
SMF Be 3712	<i>Liotrigona bouyssoui</i> (Vachal, 1903) SMF Be 3712a	1 Araneae: Salticidae: <i>Mogrus</i> sp. 1 Psocoptera larvae	Holocene copal from Tanzania	1000 +/- 30 BP 1024 - 1154 cal. AD
SMF Be 12431.1	<i>Liotrigona nilssoni</i> Michener, 1990 SMF Be 12431.1a	1 Hymenoptera: Chalcidoidea: Mymaridae 1 Hymenoptera: Chalcidoidea 2 insects indet. 2 <i>Liotrigona nilssoni</i> Michener, 1990	Defaunation resin from Madagascar, collected in Madagascar at Sacaramy (Antsiranana) in 2015	2015 AD

Imaging

The photographs and Z-stacks images were performed under a Nikon SMZ25 microscope, using Nikon SHR Plan Apo 0.5x and SHR Plan Apo 2x objectives with a microscope camera Nikon DS-Ri2 and the NIS-Element software (version 4.51.00 www.microscope.healthcare.nikon.com). Figures were performed using Adobe Photoshop software (CS6, version; 13.0 www.adobe.com).

SR μ CT

Specimens SMF Be 3503.1a, SMF Be 3694.1b, SMF Be 3724.2a, SMF Be 3525a, SMF Be 3710.1a, SMF Be 3498a, SMF Be 3712a, and SMF Be 12431.1a (Table 1) were analyzed using synchrotron-radiation based X-ray microtomography (SR μ CT). Due to the size of the specimen, and in order to increase the contrast, the specimen has been imaged with slight edge enhancement. The imaging was performed at the Imaging Beamline – IBL P05 - PETRA III at Deutsches Elektronen Synchrotron (DESY) in Hamburg operated by the Helmholtz-Zentrum Hereon (Greving et al., 2014; Wilde et al., 2016).

All specimens were imaged at a photon energy of 18 keV. Projections were recorded using a custom developed 20 MP CMOS camera system (Lytaev et al., 2014) with an effective pixel size

of 1.28 μm for specimen SMF Be 3525a and 0.64 μm for all the other specimens. Sample to detector distance was either 0.1 m (for specimens SMF Be 3710.1a and SMF Be 3498a) or 0.3 m (for specimens SMF Be 3503.1a, SMF Be 36941.b, SMF Be 3712a, SMF Be 3724.2a, and SMF Be 12431.1a) depending on the achieved contrast in the recorded projections. For each tomographic scan 2401 (for specimen SMF Be 3498), 3601 (for specimens SMF Be 3503.1a, SMF Be 3694.1b, SMF Be 3712a, SMF Be 3724.2a, and SMF Be 12431.1a) or 4801 (for specimen SMF Be 3498a) projections at equal intervals between 0 and π have been recorded depending on the size of the studied specimen. Tomographic reconstruction has been done by either a classical filtered back projection having some edge enhancement in the final reconstructions or by applying a transport of intensity phase retrieval approach and using the filtered back projection algorithm (FBP) implemented in a custom reconstruction pipeline (Moosmann et al., 2014) using Matlab (Math-Works) and the Astra Toolbox (Palenstijn et al., 2011; van Aarle et al., 2015; van Aarle et al., 2016). For the processing raw projections were binned for further processing two times resulting in an effective pixel size of the reconstructed volume of 2.56 μm (for sample SMF Be 3503) and 1.28 μm (for all other samples), respectively.

The specimens were segmented in three dimensions using region-growing techniques in VGStudioMax (version 3.3.1 www.volumegraphics.com/de, Volume Graphics, Heidelberg, Germany).

Results

Thirty-six specimens of Meliponini bees (Apidae: Apinae) have been studied in a collection of Holocene copal and Defaunation resin from Tanzania. The collection includes a total of 884 arthropod inclusions. ^{14}C of the analyzed material indicated a great variability of ages from 2770 \pm 30 BP to 80 \pm 30 BP (see Table 1).

We recognized two extant species, *Liotrigona bouyssoui* (Vachal, 1903) and *Hypotrigona gribodoi* (Magretti, 1884), in Holocene copal and in Defaunation resin from Tanzania, respectively. The age of the piece containing *L. bouyssoui* is 1000 \pm 30 BP and the age of the piece containing *H. gribodoi* is only 100 \pm 30 BP.

Resin from *H. verrucosa* has also been collected in the eastern part of Madagascar in 2013 (Nosy Varika region) and 2015 (Sacarami region), with a total of 1,786 inclusions in 2013 and 2,180 in 2015. From these samples only two pieces contain four specimens belonging to *Liotrigona nilssoni* Michener, 1990.

The holotype of *Liotrigona vetula* Moure and Camargo, 1978, recorded from Defaunation resin of Madagascar (Moure and Camargo, 1978), was revised and is treated herein as a separate extinct or yet-undiscovered species.

Two new species are erected herein. Morphological terminology in the systematic accounts follows that of Engel (2001), Michener (2007), and Engel et al. (2021). The first species belongs to the genus *Axestotrigona* (*sensu* Engel et al., 2021) and the holotype (SMF Be 3503.1a) has been found preserved in Holocene copal, 1050 \pm 30 BP in age according to ^{14}C analysis. The second species belongs to the genus *Hypotrigona* and the holotype (SMF Be 3724.1a) has been

found in Defaunation resin. The latter holotype has an age, as determined by ^{14}C analysis, of 80 ± 30 BP [however, the calibrated result (95% probability) indicates a broad range of time for resin production, spanning 1698 cal. AD to 1945 AD]. However, since we know the samples came into the museum in 1901 the most probable age ranges are between 1698 and 1870 cal. AD (252 to 80 cal. BP), and the paratypes in its type series reach a maximum age of 2770 +/- 30 BP.

Systematics

Key to Genera of Meliponini in Tanzanian and Malagasy Holocene Copal and Defaunation Resin

1. Smaller bees, less than 5 mm in total length; hind wing without closed cells; forewing without indications of submarginal cells; 2M terminating without bend at about position of anterior end of 1m-cu (which is, however, absent or spectral); metatibia not spoon-shaped 2
- Larger bees, greater than 5 mm in total length; hind wing with closed cells; forewing with indications of submarginal cells; 2M extending at least slightly beyond position of anterior end of 1m-cu and angulate at end of crossvein; metatibia rather spoon-shaped..... *Axestotrigona* Moure
2. Mesoscutum and mesoscutellum matt, microalveolate to imbricate; metatibia with superior distal angle broadly rounded *Hypotrigona* Cockerell
- Mesoscutum and mesoscutellum smooth and shining between fine punctures; metatibia with superior distal angle distinct and angulate *Liotrigona* Moure

Apidae Latreille, 1802
 Corbiculata Engel, 2001
 Meliponini Lepeletier, 1836
 Genus *Axestotrigona* Moure, 1961

Although *Axestotrigona* was originally established as a genus (Moure, 1961), Michener (1990, 2007) relegated the group to a subgenus of a significantly expanded *Meliponula* Moure, recasting this group to include all of those African taxa in which the metatibia was broadly spatulate in form (Fig. 2e–f and Fig. 3). Recent phylogenetic work, however, has demonstrated that Michener’s circumscription of *Meliponula* renders the group polyphyletic (Rasmussen and Cameron, 2010). Rasmussen et al. (2017) transferred *Axestotrigona* to an expanded *Meliplebeia* Moure in order to resolve the polyphyly resulting from inclusion in *Meliponula*, but such an arrangement renders the broader circumscription of *Meliplebeia* challenging. Therefore, we follow Moure’s (1961) original circumscription of the genus and as sister to a clade comprising *Apotrigona* Moure and *Meliplebeia* (Rasmussen and Cameron, 2010; Engel et al., 2021). Within the genus there are two distinct groups, here treated as subgenera (Engel et al., 2021). The Holocene copal species recorded herein belongs to the nominate subgenus and is closest to *Axestotrigona togoensis* (Stadelman). Another East African copal specimen of *Axestotrigona* was reported by Zeuner and Manning (1976); the specimen is housed at the Department of Palaeontology of the Natural History Museum, London. Zeuner and Manning (1976) attributed

their material to “*Trigona erythra*” (*A. erythra* (Schletterer, 1891)), noting that it belonged to the dark form then known as “*togoensis*”, but actually today recognized as a separate species. Their specimen is certainly not *A. erythra* as it has a dark brown metasoma (the metasoma of *A. erythra* is wholly reddish orange) and their brief account generally accords with a form of *A. togoensis* in so far as what pertinent characters are presented.

Tentative Key to Subgenera and Species of *Axestotrigona*
(workers only)

- 1. Basal area of propodeum glabrous and smooth; wing membranes darkly infumate throughout; larger bees, 7–8.5 mm in body length [subgenus *A. (Atrichotrigona)* Engel] 2
- Basal area of propodeum finely tessellate to microalveolate, sometimes faintly so and appearing nearly smooth, and laterally setose (sometimes lateral patches of setae sparse and wispy or may be difficult to discern in worn individuals or those with considerable pitch on the body); wing membranes hyaline clear to lightly infuscate (parchment-colored) or ferruginous; smaller bees, less than 6.5 mm [subgenus *A. (Axestotrigona)* Moure, *s.str.*] 3
- 2(1). Anterior terga dulled by dense and minute tessellation and with dense and minute pubescence; malar space slightly more than one half flagellar diameter *A. simpsoni* Moure
- Basal two thirds of anterior terga highly polished and shining, malar space conspicuously shorter than one half flagellar diameter *A. cameroonensis* (Friese)
- 3(1). Metasoma entirely black 4
- Metasoma largely red, or, red on anterior terga with variable dark brown to black banding apically, particularly on terga II and III, and with apical terga entirely dark brown to black 5
- 4(3). Anterior terga polished and shining; setation of lower face dense, obscuring integument; mesoscutum densely and finely tessellate, appearing micro-granulose; wings slightly infumate, like parchment *A. togoensis* (Stadelman)
- Anterior terga matte and faintly imbricate to tessellate; setation of lower face sparse; mesoscutum microalveolate; wings clear hyaline *A. kitingae* **sp. nov.**
- 5(4). Metasoma red on anterior terga, with variable black banding apically on terga II and III, remainder of apical terga dark brown to black [*A. ferruginea* (Lepeletier), *s.l.*] 6
- Metasoma entirely red, without black bands *A. erythra* (Schletterer)
- 6(5). Clypeus black *A. ferruginea ferruginea* (Lepeletier)**
- Clypeus brown *A. ferruginea gambiensis* Moure

Note that an examination of the holotype of *Trigona richardsi* Darchen in the Natural History Museum, London, reveals that it is a junior subjective synonym of *A. ferruginea ferruginea* (new synonymy**).

Axestotrigona (Axestotrigona) kitingae Engel and Solórzano-Kraemer sp. nov.
(Figs. 2–4)

"[insert Figure 2 to 4.]"

Diagnosis: Based on workers, the new species is most similar to *A. togoensis* in the wholly dark brown to black metasoma, but differs most notably in the matte and minutely, finely imbricate basal metasomal terga (versus shining and faintly tessellate to smooth in *A. togoensis*); the microalveolate mesoscutum (densely and finely tessellate, appearing granulose in *A. togoensis*); the sparse setation of the lower face (dense setation obscuring integument in *A. togoensis*); and clear wing membranes (slightly infumate in *A. togoensis*). In addition, the following combination of features are important for recognizing the new species: integument of head and mesosoma concolorous, dark brown to nearly black, without areas of yellow maculation (Fig. 2a–d); propodeal profile entirely declivitous, without defined basal surface and subvertical sloping surface; corbicula occupying complete distal half of metatibia (Fig. 3b and C); metasomal terga dull, matt, finely imbricate; retromarginal and apical margins of metatibia with stiff, simple bristles, without plumose setae, retromarginal setae pale except those near angle and along superior half of distal margin fuscous, parapenicillum dark fuscous; metatibia spoon-shaped, with superior distal angle broadly, obtusely rounded (Fig. 3); retrolateral surface of metatibia with broad keitrotrichiate field, superior border with narrow but distinct subglabrate zone separating keitrotrichiate field from retromargin; mesoscutum finely microalveolate, more strongly so on mesoscutellum and therefore appearing more like weak, contiguous, coarse punctures; metasoma matt, finely imbricate; face matte, frons with minute, contiguous, shallow punctures giving surface microscopically granulose appearance; notauli well impressed, nearly as long as medial line; mesoscutum setation exceptionally sparse, largely confined to sparse, short setae anteriorly; mesoscutellum with long, erect setae posteriorly, otherwise without setae; pleural sculpturing as on mesoscutellum; malar space slightly less than flagellar diameter.

Description: Worker. Total body length 5.82 mm; forewing length 4.97 mm. Head broader than long (Fig. 4); upper interorbital distance greater than lower interorbital distance; scape slightly shorter than torulocellar distance; malar space 0.22 mm, slightly less than flagellar diameter; flagellomeres each longer than wide.

Integument of clypeus imbricate and impunctate; parocular area below tangent of antennal toruli imbricate blending above to minute, contiguous, shallow punctures; supraclypeal area between antennal toruli and frons with minute, contiguous, shallow punctures giving integument microscopically granulose appearance; integument blending to imbricate in ocellocular area; vertex and gena imbricate and impunctate. Mesoscutum finely microalveolate; mesoscutellum microalveolate; mesepisternum dorsal-anteriorly finely microalveolate, gradually blending to strongly imbricate ventrally and posteriorly; propodeum imbricate; metasoma matt and finely imbricate.

Integumental coloration poorly preserved but clearly lacking areas of yellow maculation; apparently dark brown to black on head and mesosoma; metasoma lighter brown; legs apparently dark brown, except lighter on tarsi.

Pubescence of face sparse, vertex with scattered, short (less than ocellar diameter in length), erect, simple setae. Mesoscutum with exceptionally sparse setae, setae largely confined to sparse, short (1 ocellar diameter or less), erect, simple setae anteriorly; mesoscutellum with long, erect, simple setae posteriorly, otherwise without setae. Metatibia with superior and apical margins with stiff, long, simple bristles, without plumose setae, retromarginal setae pale except those near angle and along superior half of apical margin fuscous, parapenicillum dark fuscous. Metasoma with sparse, short, simple setae on terga; sterna with areas of longer, erect to suberect, simple setae.

Queen. Unknown.

Drone. Unknown.

Material: Worker holotype SMF Be 3503.1a, preserved in Tanzanian Holocene copal and housed at SMF.

Remarks: The ancient resin where the specimen is preserved has been dated by Beta Analytic testing laboratory with ^{14}C and ^{13}C at an age of 1050 \pm 30 BP.

Etymology: The specific epithet is a matronym honoring Tanzanian gender activist Judith Kitinga.

ICZN ZooBank registration: urn:lsid:zoobank.org:act:8B7CAD02-D6F9-43B5-AEE4-D3650B099CA7.

Genus *Hypotrigona* Cockerell, 1934

The genus *Hypotrigona* includes particularly small bees and, as noted, it can be challenging to distinguish species, although morphometrics, nest architecture, chemical signatures, and molecular tools have significantly aided the recognition of what are otherwise sometimes nearly cryptic taxa (Ndungu et al., 2018a, 2018b, 2019). In East Africa, three species of *Hypotrigona* may be found, *H. gribodoi*, *H. araujo* (Michener, 1959), and *H. ruspolii* (Magretti, 1898) while a fourth species *H. squamuligera* (Benoist, 1973) is largely distributed further to the west (Ndungu et al., 2018a, 2019). Two species have been identified in copal and Defaunation resin, with one representing a fairly common species, *H. gribodoi*.

Hypotrigona gribodoi (Magretti, 1884)
(Fig. 5 and Supplementary Fig. 1)

"[insert Figure 5.]"

Brief description: Based on eight workers; body size small, the sizes of one of the specimens is as follow: 2.59 mm, intertegular distance 0.04 mm, forewing length 2.25 mm. Face, mesoscutum, and mesoscutellum without abundant, appressed, squamose setae (such setae abundant in *H. squamuligera*); face with setae not extending to ocelli (Fig. 5e and f); metasoma uniformly colored (lacking the distinctly lighter apical margins of *H. ruspolii*); parapenicillum at superior distal angle of metatibia (Fig. 5g).

Material: 8 workers with the numbers SMF Be 3694.1a,b and SMF Be 3694.2a–f, preserved in a unique piece of Tanzanian Defaunation resin (100 \pm 30 BP) and housed at SMF.

Hypotrigona kleineri Engel and Solórzano-Kraemer sp. nov.
(Figs. 6–7 and Supplementary Figs. 2–4)

"[insert Figure 6 to 7.]"

Diagnosis: Based on workers; face, mesoscutum, and mesoscutellum without abundant, appressed, squamose setae (Fig. 6–7) (such setae abundant in *H. squamuligera*); metasoma uniformly colored; parapenicillum at posterior apical angle of metatibia; mesoscutum and mesoscutellum matt, microalveolate, except blending to coarsely imbricate on mesoscutellum; notauli prominent (Fig. 7d and supplementary Fig. 2), nearly as long as medial line and about as well impressed; propodeum tessellate (Fig. 7f); mesoscutum and mesoscutellum exceedingly

sparsely setose, with some moderate-length to long, erect setae along borders; mesoscutellum with erect, elongate setae mostly posteriorly; pleura imbricate; metasoma largely smooth and shining except becoming imbricate posteriorly on each disc, and on apical margins of terga. Larger than *H. gribodoi* (*vide supra*), *H. araujoi*, or *H. ruspolii*.

Description: In addition to those characters of diagnosis, the following metrics (from the holotype): Total body length 3.75 mm (paratypes from 3.47 mm to 4.08 mm), head length 1.02 mm, head width 1.33 mm, upper interorbital distance 0.85 mm, lower interorbital distance 0.73 mm, intertegular distance 0.06 mm, forewing length 3.02 mm, metatibia length 0.99 mm, metatibia width 0.42 mm.

Queen. Unknown.

Drone. Unknown.

Material: Worker in the piece holotype SMF Be 3724.1a (Age: 80 +/- 30 BP, 1698 to 1724 cal. AD, 252 to 226 cal. BP or 1810 to 1870 cal. AD, 140 to 80 cal. BP). Workers in the pieces paratypes SMF Be 3724.2a, and SMF Be 3710.1a (Age: 2770 +/- 30 BP), SMF Be 2509.3a–f, SMF Be 3532a, SMF Be 3498a (Age: 1250 +/- 30 BP), SMF Be 3525a–g, SMF Be 3573.1a, SMF Be 3573.2a, SMF Be 3573.3a,b, SMF Be 3573.4a, preserved in Tanzanian Holocene copal and in Tanzanian Defaunation resin. All housed at SMF.

Etymology: The specific epithet *kleineri* is dedicated to Prof. Matthias Kleiner, who was President of the Leibniz Association from 2014 to 2022.

ICZN ZooBank registration: urn:lsid:zoobank.org:act:9D394DDF-92E9-45BC-8059-8CCBB868E50F.

Genus *Liotrigona* Moure, 1961

Species of *Liotrigona* are particularly abundant and diverse across Madagascar, with eight species currently recorded (Pauly et al., 2001). Colonies are particularly abundant in the more arid southwest (Brooks and Michener, 1988), but although less frequently collected in the northern or eastern parts of the island, five of the species have been recorded in those areas (Brooks and Michener, 1988; Pauly et al., 2001). A putatively extinct species, *L. vetula*, has also been recorded from Defaunation resin of Madagascar (Moure and Camargo, 1978), perhaps from the Sambava-Antalaha Region from where most of the resin originates (Delclòs et al., 2020). The original type material, located in the Zoological Museum of Copenhagen (Moure and Camargo, 1978), has also been examined to determine whether or not it is conspecific with *L. nilssoni*, which is found in Defaunation resin from Madagascar. We can here confirm that is not. However, to determine whether or not it is conspecific with the living species proposed in the last 30 years and a truly remains evidence of an additional species once occurring in the near past of Madagascar, a revision of the living material and a PC morphometric plot for all of the species is needed. Certainly, given the extensive habitat destruction occurring throughout Madagascar, the probability remains high for it to represent a species driven to extinction by human activity. In continental Africa, *Liotrigona* is less diverse, with four species distributed across tropical and southern Africa, although only two (*L. bottegoi* (Magretti, 1895) and *L. baleensis* Pauly and Fabre Anguilet, 2013) extend into East Africa while a third (*L. parvula* Darchen, 1971) closely approximates the area and may eventually be discovered there (Eardley, 2004).

Liotrigona bouyssoui (Vachal, 1903)
(Fig. 8a–b and Supplementary Fig. 5)

"[insert Figure 8.]"

Brief description: Mesoscutum smooth and shining (Fig. 8a and b), with minute, fine, shallow punctures separated by 2–4 times a puncture width over central disc, becoming sparser toward lateral and posterior borders; mesoscutal setation exceedingly sparse, virtually lacking except some exceedingly minute setae anteriorly intermixed with long, erect setae anterolaterally; notauli inconspicuous; mesoscutellum as on mesoscutum except virtually glabrous, with minute punctures only at bases of elongate setae, mostly on posterior border with a few on disc in posterior half; basal area of propodeum glabrous, smooth, shining, without faint tessellation (tessellation present in *L. vetula*); metatibial superior distal angle slightly projecting (unlike *L. bottegoi*) (Supplementary Fig. 5f and i); legs apparently slightly lighter in coloration relative to darker head and mesosoma (as in *L. bottegoi*).

Material: 1 worker in the piece number SMF Be 3712a, preserved in Tanzanian Holocene copal (1000 +/- 30 BP) and housed at SMF.

Liotrigona vetula Moure and Camargo, 1978
(Fig. 9)

"[insert Figure 9.]"

Brief description: Mesoscutum smooth and shining (Fig. 9c), with minute, fine, shallow setiferous punctures separated by more than a puncture width over central disc; mesoscutal setation exceedingly sparse, virtually lacking except some exceedingly minute setae anteriorly intermixed with longer, erect setae anterolaterally; notauli inconspicuous; mesoscutellum as on mesoscutum; basal area of propodeum glabrous (Fig. 9d), shining, with faint tessellation; metatibial superior distal angle not projecting; legs light brown (Fig. 9a), lighter than dark brown head and mesosoma (as in *L. bottegoi*).

Material: 1 worker in the piece number NHMD-115579, preserved in Holocene copal or Defaunation resin of Madagascar, perhaps from the Sambava-Antalaha Region. It is housed at the Zoological Museum of Copenhagen.

Liotrigona nilssoni Michener, 1990
(Figs. 8c and 10)

"[insert Figure 10.]"

Brief description: Mesoscutum with longest setae on disc about as long as longest setae of vertex, and about as long as ocellar diameter (Fig. 10), thus more conspicuous mesoscutal setation than in preceding species (about 12 such mesoscutal setae are in focus in a single profile view); underside of scape with numerous minute setae (Michener, 1990).

Material: 3 workers in the piece number SMF Be 12431.1, preserved in Malagasy Defaunation resin collected from *H. verrucosa* in May 2015 in the Sacaramy Region. Coordinates: South 12°25'621'', East 049°18'985'', and height 278 m.a.s.l. Housed at SMF.

Discussion

Stingless bees are notorious for their many cryptically similar species, differing in minute details of integumental sculpturing, morphometrics, terminalia, and/or behavioral traits such as nest architecture (Michener, 2007). It is therefore easy to confuse a complex of several species as a single, widespread species in the absence of finer data. This is rendered all the more difficult when attempting to compare with copal- or resin-included specimens. Nonetheless, the potential for species to be first recognized from copal or Defaunation resin is great given the propensity for misidentification among the living fauna. Moreover, meliponines can be easily disturbed by changes in habitat, particularly the surrounding flora upon which they rely for pollen, nectar, and resin. Given this reality alongside the extensive human-induced habitat degradation of the last 150 years throughout eastern Africa, it is equally likely that copals and resins have sampled species that have become recently extinct as a result of human activity. While some of the species identified here from copal and resin are clearly among those still active in well-maintained habitats in the region today, others clearly differ in typical species-specific traits and represent either, 1, recently extinct species, or 2, species present in the modern fauna but yet to be recognized either owing to the cryptic nature of meliponine species or owing to a general scarcity in the current Afrotropical fauna. Unlike *Liotrigona* and *Hypotrigona*, both of which include minute species, the genus *Axestotrigona* comprises larger bees that should be more easily recognized, suggesting that this is a species that has become recently extinct. Extensive efforts should be made in preserved coastal forests to look for individuals of this taxon to assess whether this is the case.

The specimens in Defaunation resin from Madagascar collected in 2015 can be classified within the genus *Liotrigona* (Fig. 8c). The species of *Liotrigona* are most abundant in the dry regions of the south and west in Madagascar (Koch et al., 2011), however, they are also collected in the northern or eastern parts of the island, which is the region where the *H. verrucosa* resiniferous trees are growing. The genus can be recognized by the integument of the mesoscutum shining with extremely minute, well separated punctures; malar area little shorter to longer than the diameter of the third flagellomere; metatibia with distal posterior angle angulate; corbicula shorter, not attaining middle of metatibia; mesoscutellum not projecting, ending at same level as metanotum, which is hidden from above; and dorsal surface of propodeum finely strigose to nearly smooth. These characters differentiate *Liotrigona* from *Hypotrigona*. The two genera can also be differentiated by the male terminalia and worker sting sclerites, as they differ quite considerably between the two lineages (Brooks and Michener, 1988). However, these structures require delicate dissection in such minute bees, and we have only workers in resin that cannot be dissected (and the sclerites of the sting apparatus are minute, beyond the resolution of μ CT scans, and partially membranous). The most common species in Malagasy resin is *L. nilssoni* Michener, 1990.

The Holocene copal and Defaunation resin obtained in countries on both sides of the Mozambique Channel (mainly Tanzania, Mozambique, and Madagascar) has been regularly traded as “East African copal” or “Zanzibar copal”. Unfortunately, copal and resin from Tanzania and Madagascar were both sold under the name of “Zanzibar copal” through the beginning of the 20th century (Delclòs et al., 2020). There are several historical samples of “Zanzibar copal” from Tanzania in German institutions, a fact that is not surprising given that in

1884 German traders based in Zanzibar took the lead in asserting a permanent presence on the mainland. At that time, the German East Africa Corporation (DOAG) undertook a series of expeditions to obtain treaties that ultimately resulted in an established German colony (Sunseri, 2014).

Inclusions in Tanzanian copal and Defaunation resin are abundant and well preserved, yet few bioinclusions have been adequately documented or described in fewer than 30 publications. Stroiński (2007) described a putatively extinct species of planthopper (Ricaniidae), *Pochazoides szwedoi* Stroiński, 2007, while Abdullah (1964) described a new species of darkling beetle (Tenebrionidae: Lagriinae) as *Sora (Sora) zanzibariensis* Abdullah, 1964. The origin of the latter piece of copal is unclear and the genus *Sora* is broadly distributed across East Africa as well as in Madagascar. Some other examples are mentioned by Bouju and Perrichot (2020). Conversely, in copal and Malagasy Defaunation resin, the amount of described species is much more significant involving about 100 to 120 species, in about 80 publications (Delclòs et al., 2020). One of those descriptions include the stingless bee *L. vetula*.

Because *Hypotrigena* is not present in Madagascar (Brooks and Michener, 1988) but abundant in East Africa, its occurrence within a particular piece of Defaunation resin or copal could be used as an indicator of a piece's provenance. Other indicator taxa are found only in Holocene copal and Defaunation resin from Tanzania, and include: *Chaetastus* Nunberg, 1953 (Coleoptera: Curculionidae: Platypodinae) and *Dorylus* Fabricius, 1793 (Hymenoptera: Formicidae: Dorylinae) (Fig. 11), both absent in Madagascar but present in Holocene copal and Defaunation resin from Tanzania.

"[insert Figure 11.]"

The resin and copal studied herein most likely originated from the vichaka forest, since during the nineteenth century, resin and copal from the Zaramo Region — from Bagamoyo in the north to Lindi in the south (Fig. 1) — were intensively traded and 95% of the East African copal comes from this one area (Sunseri, 2007). This forest is now classified as a dry lowland forest, and local studies in East Africa indicate that over the last 3,000 years the climatic and ecological conditions were comparatively stable (Haberyan, 1987). The coastal forest started to become fragmented during the last 10 million years, and increasingly arid, although with periodic episodes of wetter climatic regimes during the late Miocene and Pliocene, and with severe climatic fluctuations during the Holocene, suggesting that the forests today are fragmented relicts of a once widespread flora (Burgess et al., 1998). *Hymenaea verrucosa* trees are well adapted to this ecosystem and are distributed throughout East Africa, Madagascar, and Zanzibar (Fig. 1). The oldest piece of copal we document here is 2770 +/- 30 BP. Apparently, the forest at that time was growing under a relatively similar climate as today, although human disturbance has been increasing with agricultural activities and increased incidence of fire. Both factors resulted in major forest fragmentation with a concomitant change in species' distribution patterns (Clarke and Karoma, 2000). This explains why most forests are now located in areas with poorer soils, and on remote and more hilly positions, and that endemic species are becoming increasingly constrained, representing mere vestiges of their former occurrence and abundance, with their populations scattered and isolated within the remaining forest pockets, with considerable potential to eventually become extinct (Burgess et al., 1998). This is especially important

because isolation of small local populations tends toward the loss of genetic diversity and the extinction of local populations due to low population number. This situation is exacerbated in stingless bees as they are highly eusocial, meaning their effective population size (N_e) is reduced relative to solitary insects. This reduction in N_e means their populations have decreased amounts of intraspecific genome-wide genetic polymorphism (*e.g.*, Bromham and Leys, 2005; Romiguier et al., 2014), with effective population sizes more similar to vertebrates than most solitary insects (Romiguier et al., 2014). This reduced genetic variation makes such isolated populations particularly susceptible to episodes of crisis (*e.g.*, climate change and fire) that can lead to localized extermination of the population. Furthermore, continually decreasing fragment sizes put greater and greater strain on small social insect populations, eventually reaching a critical point where the capacity of the fragment to support the population is no longer tenable, even in the absence of any abiotic or biotic episode of stress, and these distances between fragments need not be considerable. For example, in the Neotropical species *Melipona compressipes* (Fabricius, 1804) and *M. quadrifasciata* (Lepeletier, 1836), their populations would be effectively isolated if forest fragments were greater than 2 km apart, while in a minute species like *Plebeia droryana* (Holmberg, 1903), the bees would be effectively isolated if inter-fragment distances were merely greater than 600 m (Araújo et al., 2004).

Bees preserved in fossilized *Hymenaea* spp. resins are well documented from the Miocene of the northern Dominican Republic and southern Mexico, both of which were exuded by extinct species of *Hymenaea*. In fact, stingless bees in particular are among the most frequently encountered inclusions in these amber deposits. Four species of the genus *Proplebeia* Wille and Chandler, 1964 are documented from Dominican amber (Michener, 1982; Camargo et al., 2000; Greco et al., 2011), while until now only a single species of the same genus is known from Mexican amber (Camargo and Pedro, 2007; Ayala et al., 2013; Engel and Michener, 2013), although additional taxa are known (Solórzano Kraemer, pers. obs.). Aside from meliponines other bee tribes are also documented from both of these fossiliferous resins. Mexican amber includes at least one orchid bee (Engel, 2014) and will likely be found to include additional bee lineages as exploration continues. In Dominican amber there are seven tribes of bees in addition to that of the stingless bees, and some, like those of the Halictinae and Xeromelissinae are represented by more than one species and sometimes by multiple genera (*e.g.*, Engel, 1995, 1996, 1997, 1999a, 1999b, 1999c, 2009; Rozen, 1996; Engel and Rightmyer, 2000; Engel et al., 2012; Engel and Breitkreuz, 2013). While the stingless bees frequently collect resins, accounting for their particular abundance in fossil resins (Engel et al., 2021), many of the other groups in amber are not known to collect such materials and several are of groups that otherwise live in the ground rather than in arboreal cavities or hollowed twigs. Such occurrences demonstrate that resins are capable of sampling the fauna associated with the resin-producing trees, and taxa using the surrounding area around the trees (Solórzano-Kraemer et al., 2018). In the current samples of Holocene copal and Defaunation resin we only have resin-collecting bees present, and a resin bee of the genus *Heriades* Spinola, 1808 was identified by Engel (2001) from East African copal in the Oxford University Museum. Future studies should hunt for additional bee lineages, particularly those that do not collect resin or nest in trees.

Studying unsorted collections of Miocene amber, Holocene copal, and Defaunation resin we noticed a decrease in the abundance of stingless bees, although not in all collections. The unsorted Mexican amber collections treated by Solórzano Kraemer et al. (2015) had a total of

about 15% Meliponini. Though the abundance is variable between the collections, it is convincing to say that they are one of the most abundant inclusion in this amber. In Dominican amber Poinar and Poinar (1999) reported an amount of about 5% Meliponini in an unsorted amber sample. However, the study of an unsorted private collection of Dominican amber revealed only 13% of all the hymenopterans and only 2.4 % of the total of inclusions (Table 2). The Meliponini from Tanzania here studied, come from a collection housed in Senckenberg Research Institute and Natural History Museum Frankfurt for the last 120 years, and it is therefore not possible to know if the collection is biased or not towards some taxonomic groups and/or sizes of the bioinclusions. All of these pieces needed polishing and preparation in order to observe all of the inclusions owing to years of accumulated damaged the surface of the pieces (*e.g.*, crazing). From this collection, 4 % belong to Meliponini. Finally, from the resin collected in 2013 and 2015 in Madagascar only a 0.1% belong to Meliponini from a number of specimens larger than that of the aforementioned Mexican amber collections (Table 2). It should be noted that all of these are resins from *Hymenaea* spp. trees. Naturally, these are not entirely comparable figures as those of amber represent the continual sampling of the biota over potentially tens of thousands of years, if not longer, while those of the more modern copal and resins represent considerably shorter episodes of time for bees to become ensnared. Furthermore, all of these sources represent different habitats, and Meliponini are today naturally far less diverse and abundant in Afrotropical and Palearctic realms than they are in the Neotropics (Michener, 2007), and this has probably been true for the tribe during at least the last 50 million years. Nonetheless, the amount of material studied in each deposit is similar, and the resin-producing tree and overall environmental conditions are somewhat similar. It is well known that the more active organisms living in or in closer relation to the resin-producing tree have a higher likelihood of becoming entombed in resin (Solórzano Kraemer et al., 2018). This is certainly the case for Meliponini, regardless of geographical location, particularly as they are well known to collect resins of *Hymenaea* spp. and other plants for nest construction and defence (Grüter, 2020). Forest fragmentation and loss of forest in tropical ecosystems impacts stingless bee abundance and diversity (Meléndez Ramírez et al., 2013). Thus, a tendency toward fewer captured stingless bees from the Miocene to present in resiniferous forests, if found to be more than a time-for-sampling bias, might be expected as lowland forests around the world become increasingly fragmented and relict due to high human impact. Within merely a couple of centuries these relicts are highly threatened by human activity, and likely to be extirpated within the next century or less.

Table 2. Amount of Meliponini specimens in different amber, Holocene copal and Defaunation resin collections. Acronyms: IHNFG = Museo de Paleontología “Eliseo Palacios Aguilera”, Secretaría de Medio Ambiente e Historia Natural, Tuxtla Gutiérrez, Chiapas, Mexico; SMF = Senckenberg Research Institute and Natural History Museum Frankfurt, Germany; SMNS = Staatliches Museum für Naturkunde, Stuttgart, Germany; UCMP = University of California, Museum of Paleontology, Berkeley, California, USA.

Deposit	Deposition of the Collections	% of Meliponini	Total of specimens
Miocene Mexican amber	SMNS, IHNFG, UCMP	15.0	2819
Miocene Dominican amber	Poinar and Poinar (1999) unsorted private collection	5.0	2904

Miocene Dominican amber	J. Caridad unsorted private collection	2.4	3715
Holocene copal and Defaunation resin from Tanzania	SMF	4.7	785
Defaunation resin from Madagascar	SMF	0.1	3966

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Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Correspondence and material related to this paper may be requested from Mónica M. Solórzano Kraemer (monica.solorzano-kraemer@senckenberg.de). Nomenclatural acts established herein are registered in ZooBank (www.zoobank.org), as required by the International Code of Zoological Nomenclature, and under the LSID: urn:lsid:zoobank.org:pub:E3EBFBCC-0717-4DB7-90FB-BEB077B2D181.

Author contributions

M.M.S.K., and M.S.E. designed the project and wrote the original draft, M.S.E. described the specimens, M.M.S.K., J.U.H., and R.K. scanned the specimens at DESY, R.K. segmented the scans and take pictures, M.M.S.K. prepared the figures, M.M.S.K., X.D., and E.P. collected the specimens in Madagascar, M.M.S.K., R.K., J.U.H., E.P., X.D., and M.S.E. wrote the manuscript and contributed to the discussion. M.M.S.K, J.U.H., and X.D. acquired the funding.

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Figure captions

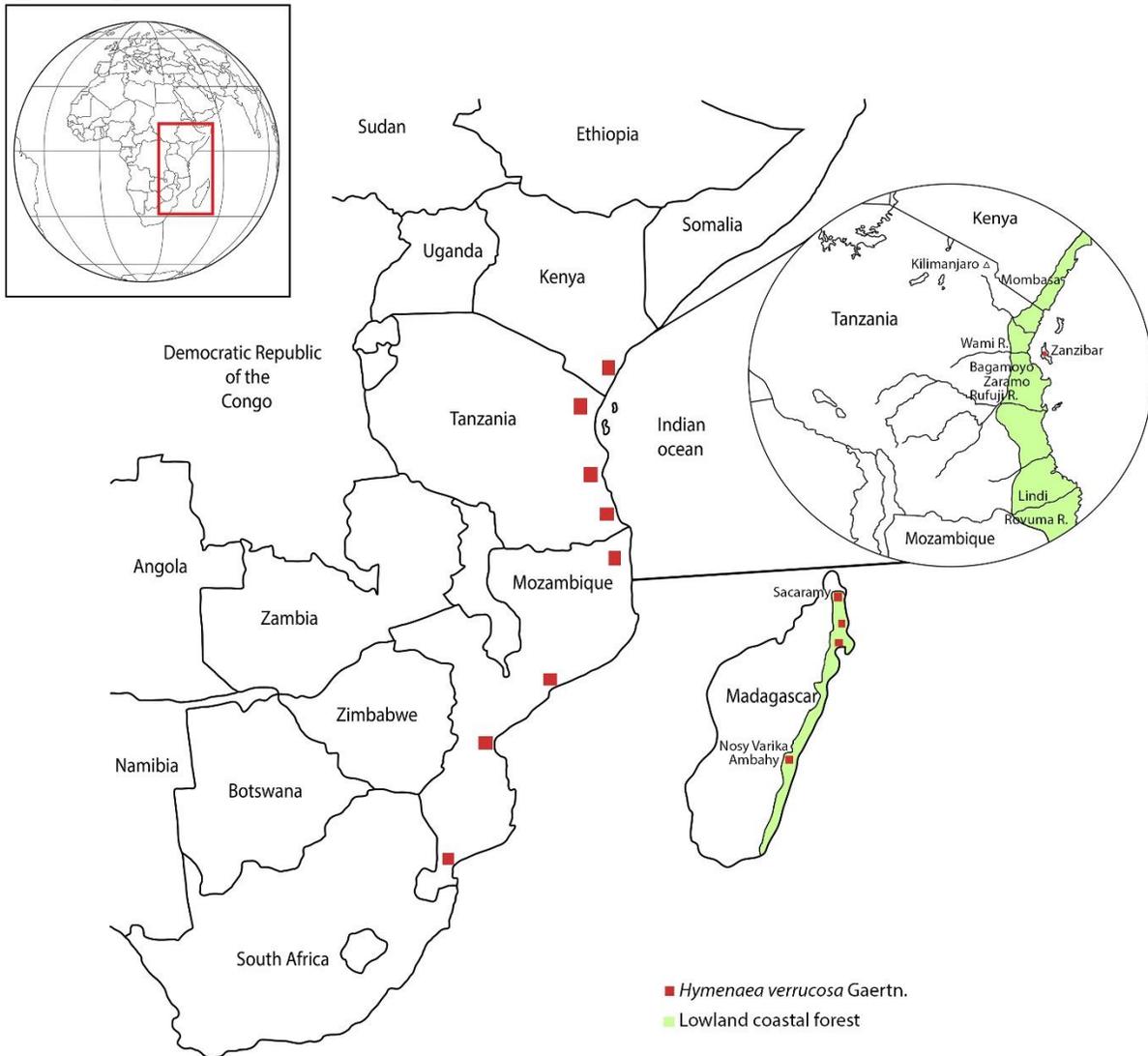


Fig. 1. Map showing the countries with occurrence of *Hymenaea verrucosa* trees across the East African coastal forest and Madagascar (red squares), with expanded detail of the coastal forest in Tanzania, from which the Holocene copal and Defaunation resin originate.

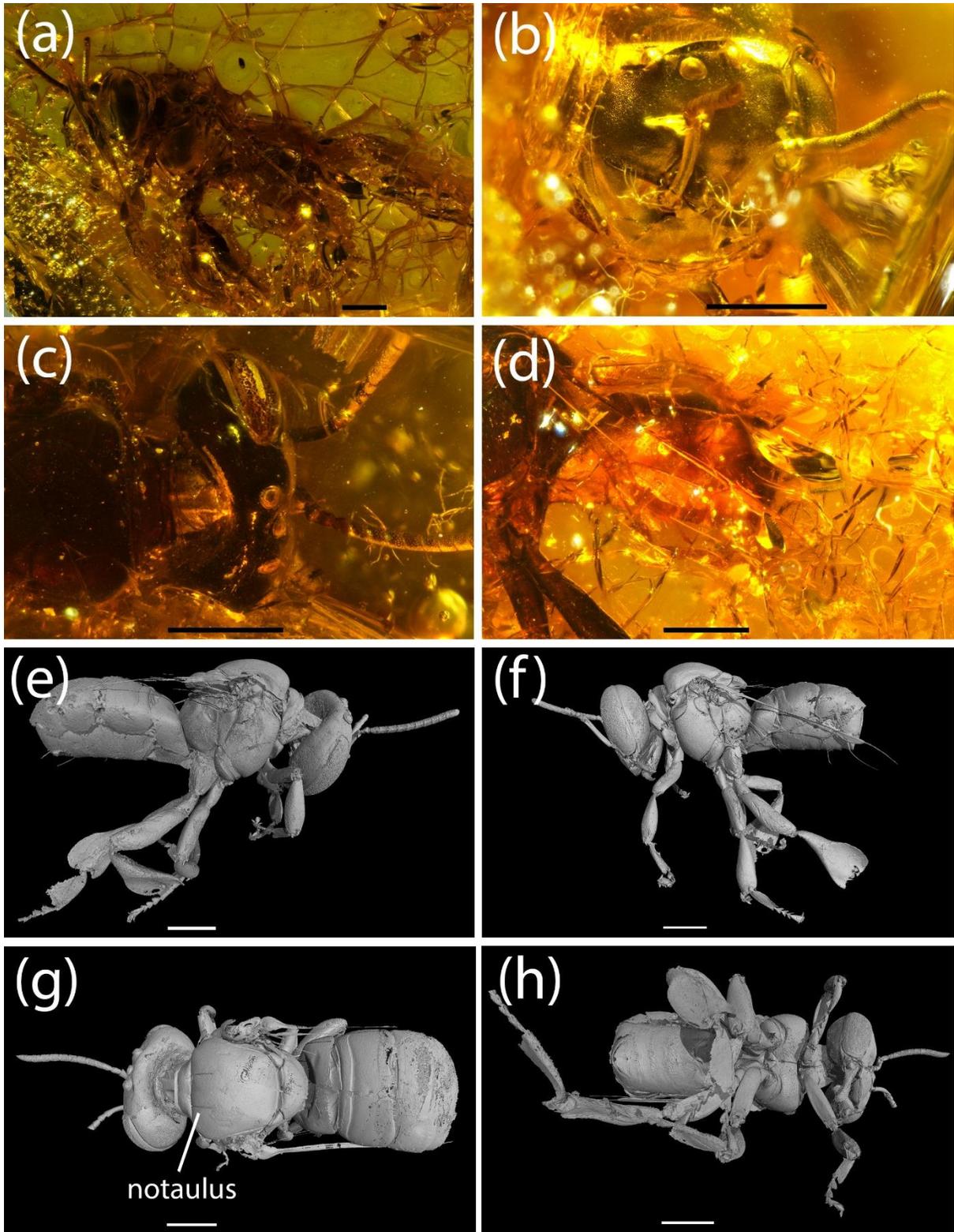


Fig. 2. Worker of *Axestotrigona kitingae* sp. nov. in Tanzanian Holocene copal. Holotype: piece number SMF Be 3503.1a. (a) Habitus in lateral view, (b) Head in antero-dorsal view, (c) Head in dorsal view, (d) Left wing, (e–h) Virtual representation of the holotype, (e, f) in lateral views, (g) in dorsal view and (h) in ventral view. Scale bars 1 mm.

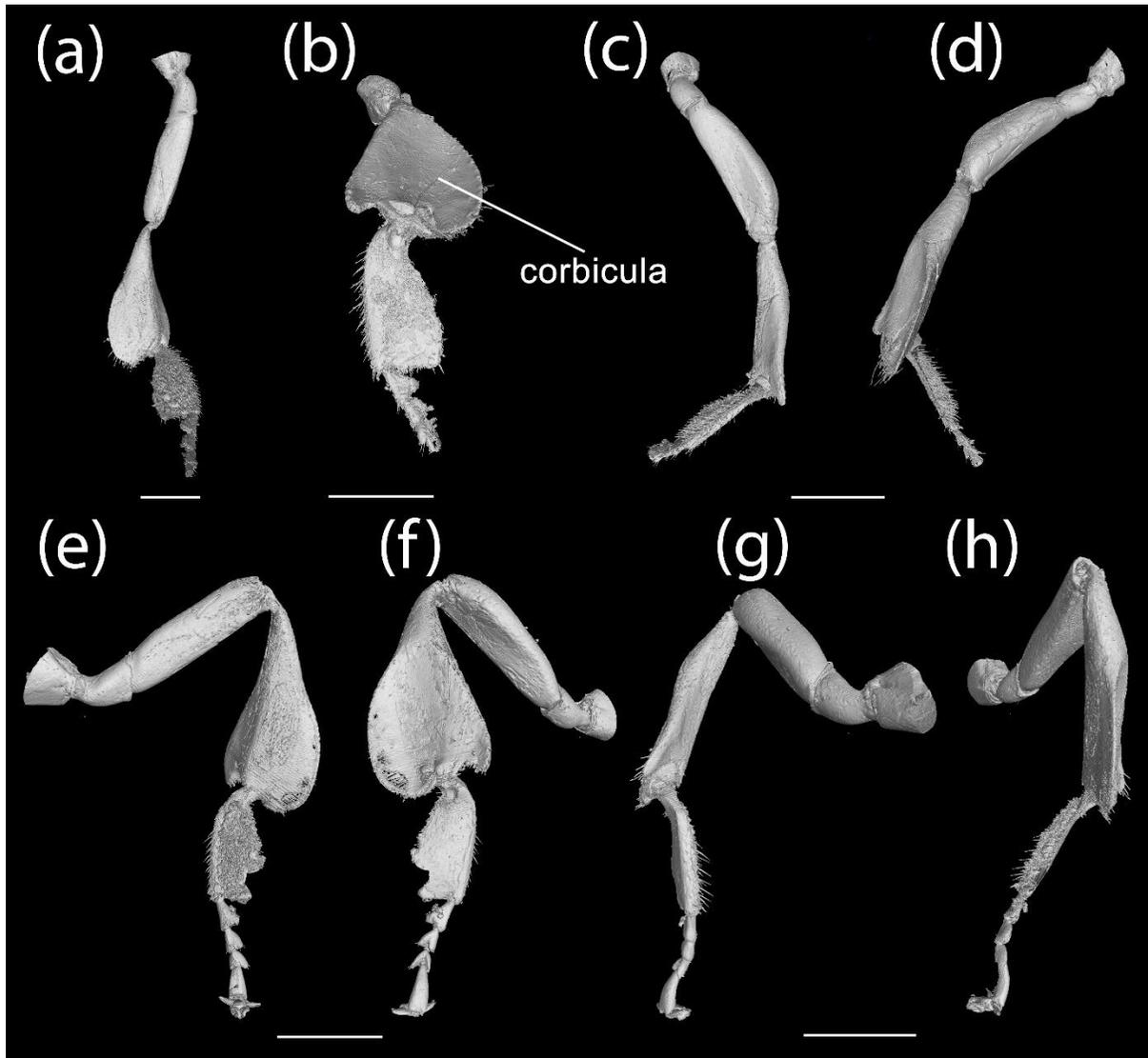


Fig. 3. Virtual representation of the hind legs of *Axestotrigona kitingae* sp. nov. in Tanzanian Holocene copal. Holotype: piece number SMF Be 3503.1a. (a–d) Left hind leg in different views, (e–h) Right hind leg in different views. Scale bars 1 mm.

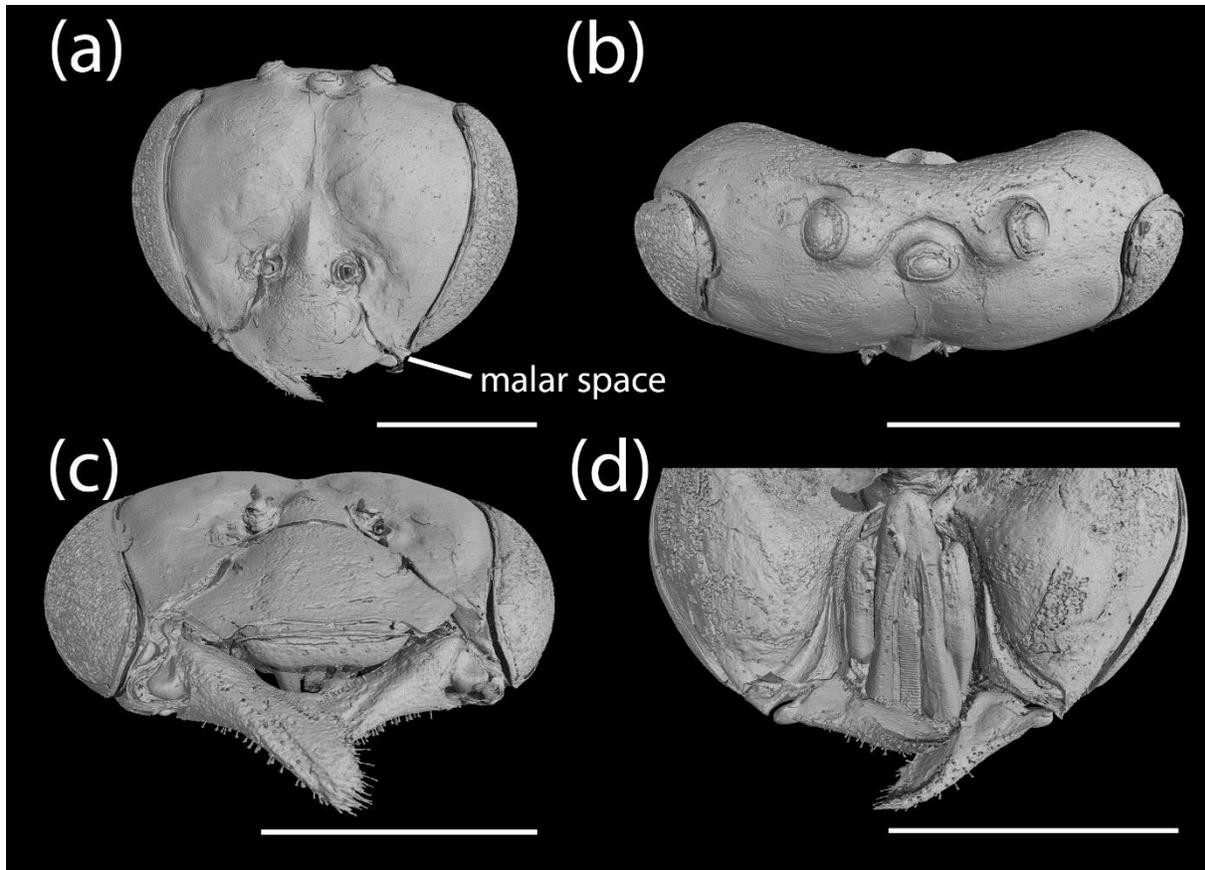


Fig. 4. Virtual representation of the head of *Axestotrigona kitingae* sp. nov. in Tanzanian Holocene copal. Holotype: piece number SMF Be 3503.1a. (a) Anterior view, (b) Dorsal view, (c) Anteroventral view showing the mandibles, (d) Posterior view. Scale bars 1 mm.

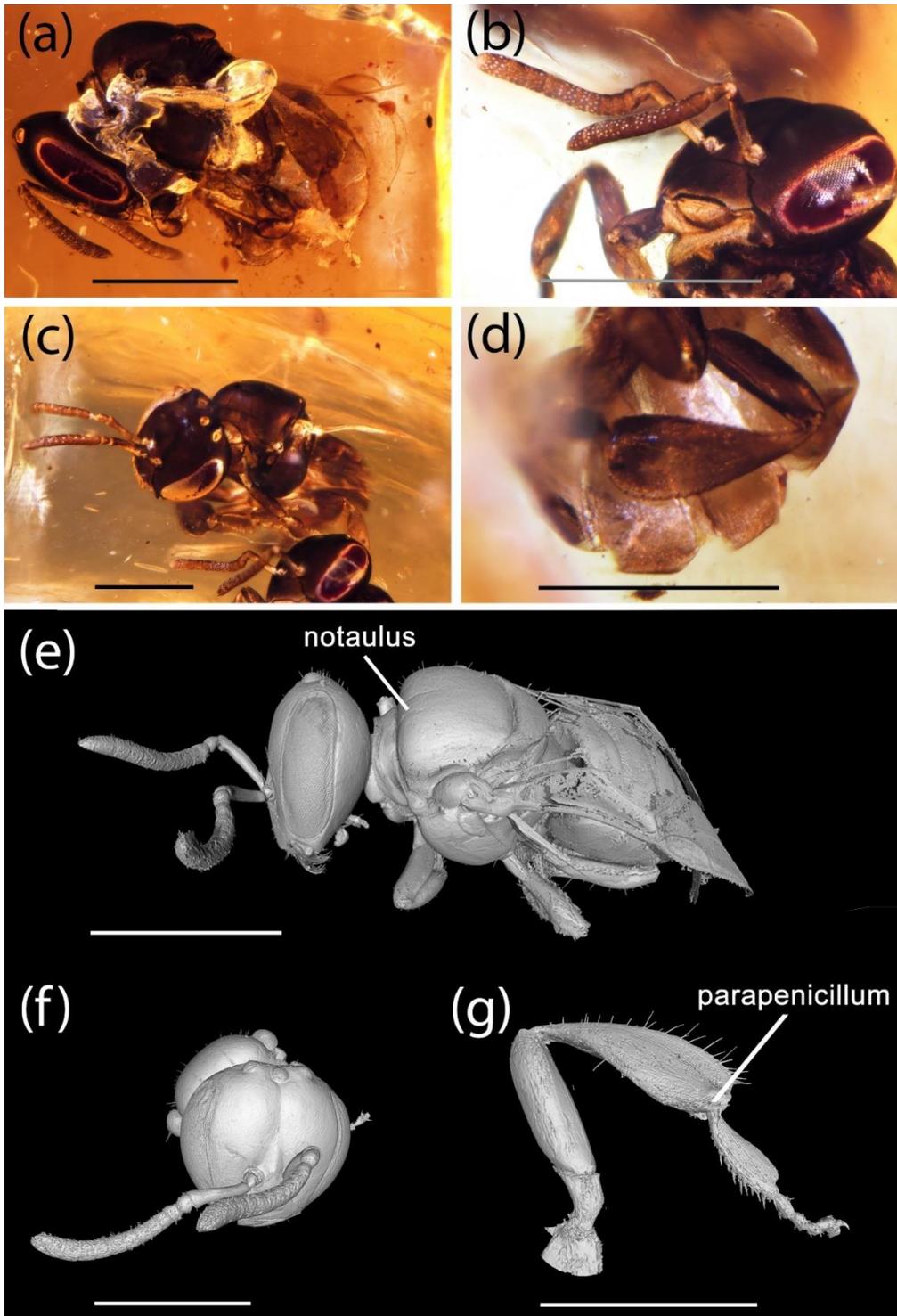


Fig. 5. Worker of *Hypotrigona gribodoi* in Tanzanian Defaunation resin (100 +/- 30 BP). (a) Piece number SMF Be 3694.1a, (b, d–g) Piece number SMF Be 3694.1b (c) Piece number SMF Be 3694.2a,b. (a) Habitus in lateral view, (b) Head close-up, (c) Heads of two specimens in anterolateral views, (d) Left metatibia, (e) Virtual representation of the habitus in laterodorsal view, (f) Virtual representation of the head in anterodorsal view, (g) Virtual representation right hind leg. Scale bars 1 mm.

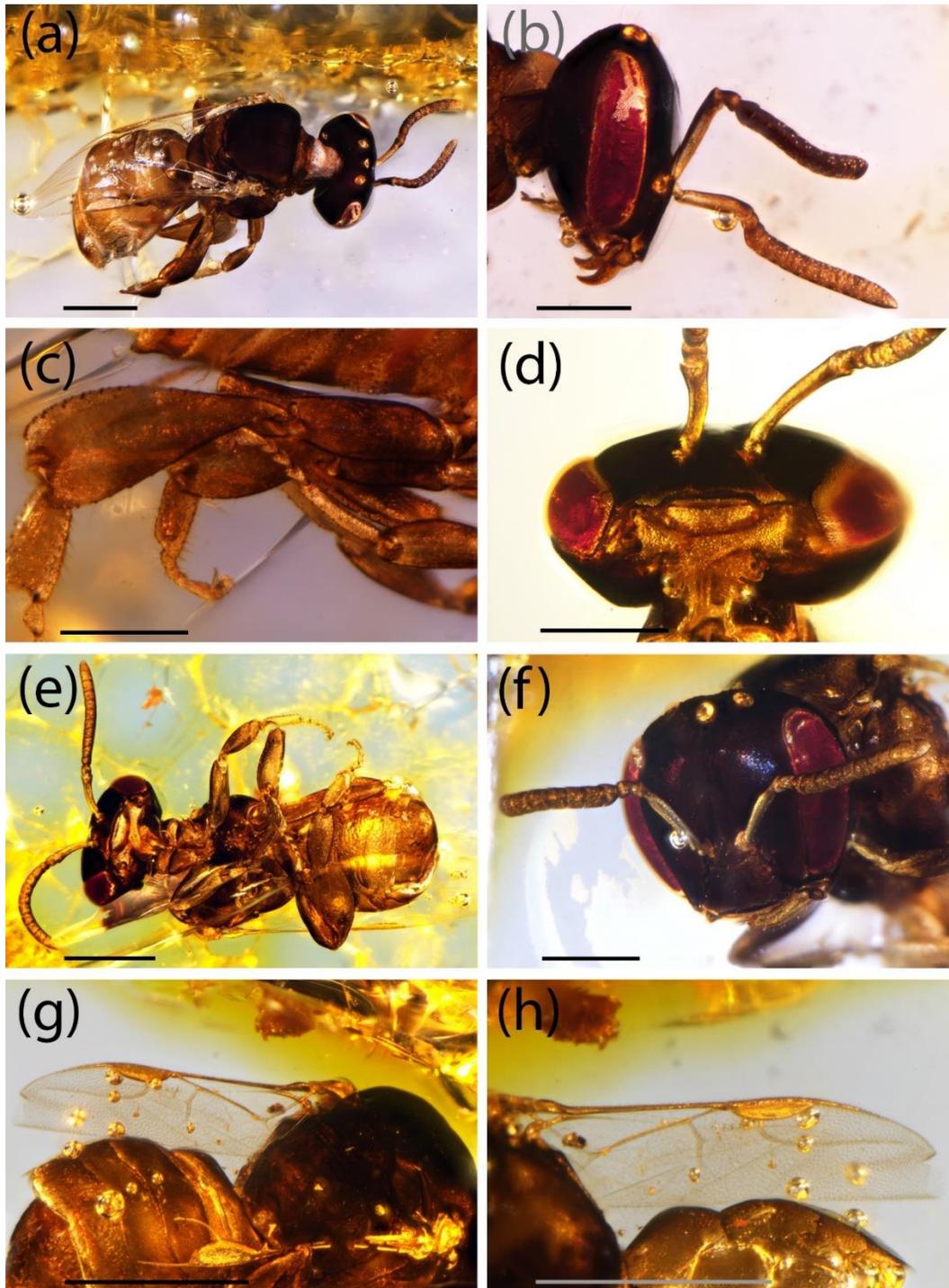


Fig. 6. Workers of *Hypotrigona kleineri* sp. nov. in Tanzanian Defaunation resin (80 +/- 30 BP). Piece numbers Holotype SMF Be 3724.1a and Paratype SMF Be 3724.2a. (a) Habitus in dorsal view of SMF Be 3724.2a, (b) Head in lateral view of SMF Be 3724.1a, (c) Metatibia of SMF Be 3724.2a, (d) Mandible close-up of SMF Be 3724.2a, (e) Habitus in ventral view of SMF Be 3724.1a, (f) Head in anterior view of SMF Be 3724.1a, (g) Left forewing SMF Be 3724.2a, (h) Right forewing SMF Be 3724.2a. Scale bars 1 mm.

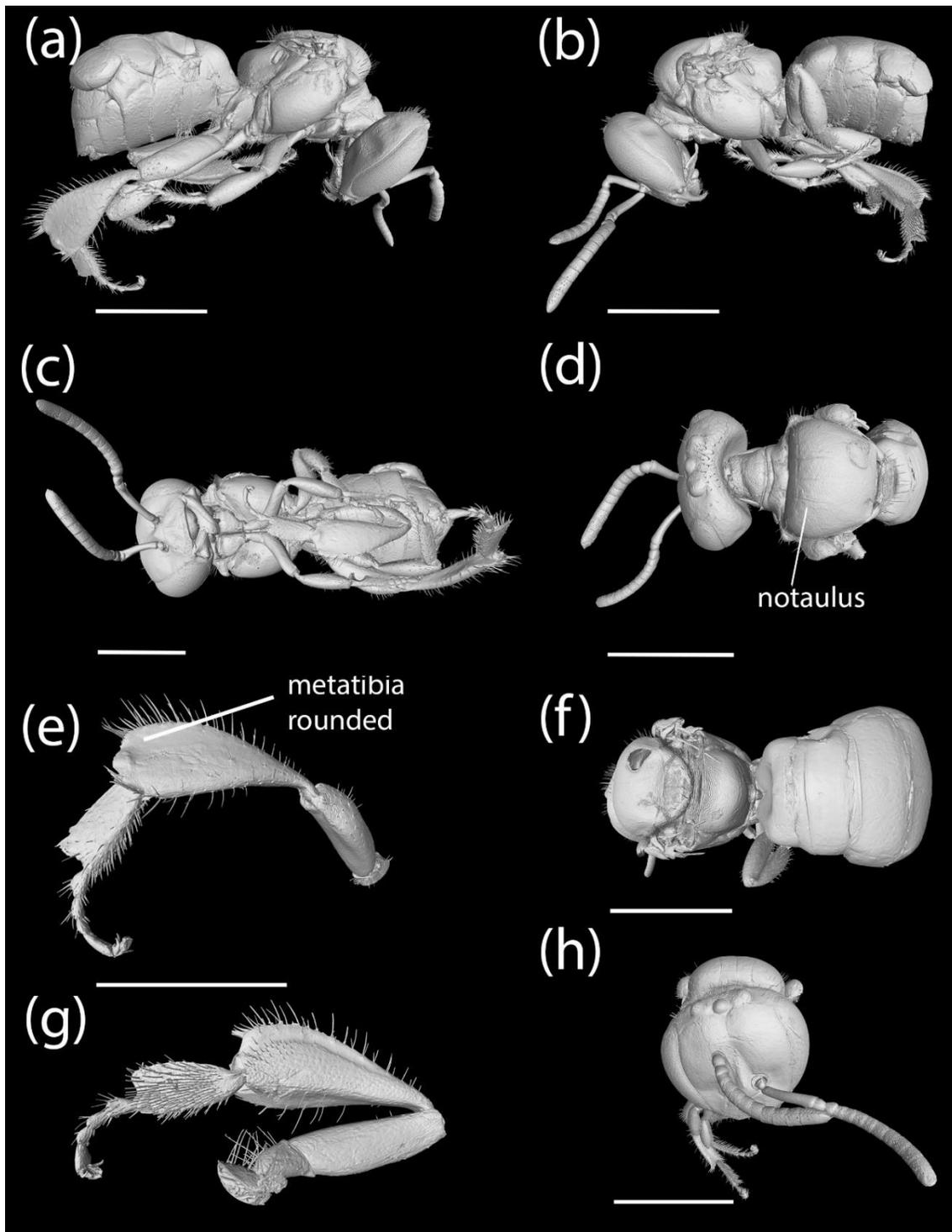


Fig. 7. Virtual representation of a worker of *Hypotrigona kleineri* sp. nov. in Tanzanian Defaunation resin (80 +/- 30 BP). Piece number SMF Be 3724.2a. (a) Habitus in right lateral view, (b) Habitus in left lateral view, (c) Habitus in ventral view, (d) Dorsal view of head and mesoscutum, (e) Right hind leg, (f) Dorsal view of metanotum and dorsal part of the propodeum, (g) Left hind leg, (h) Head in anterodorsal view. Scale bars 1 mm.

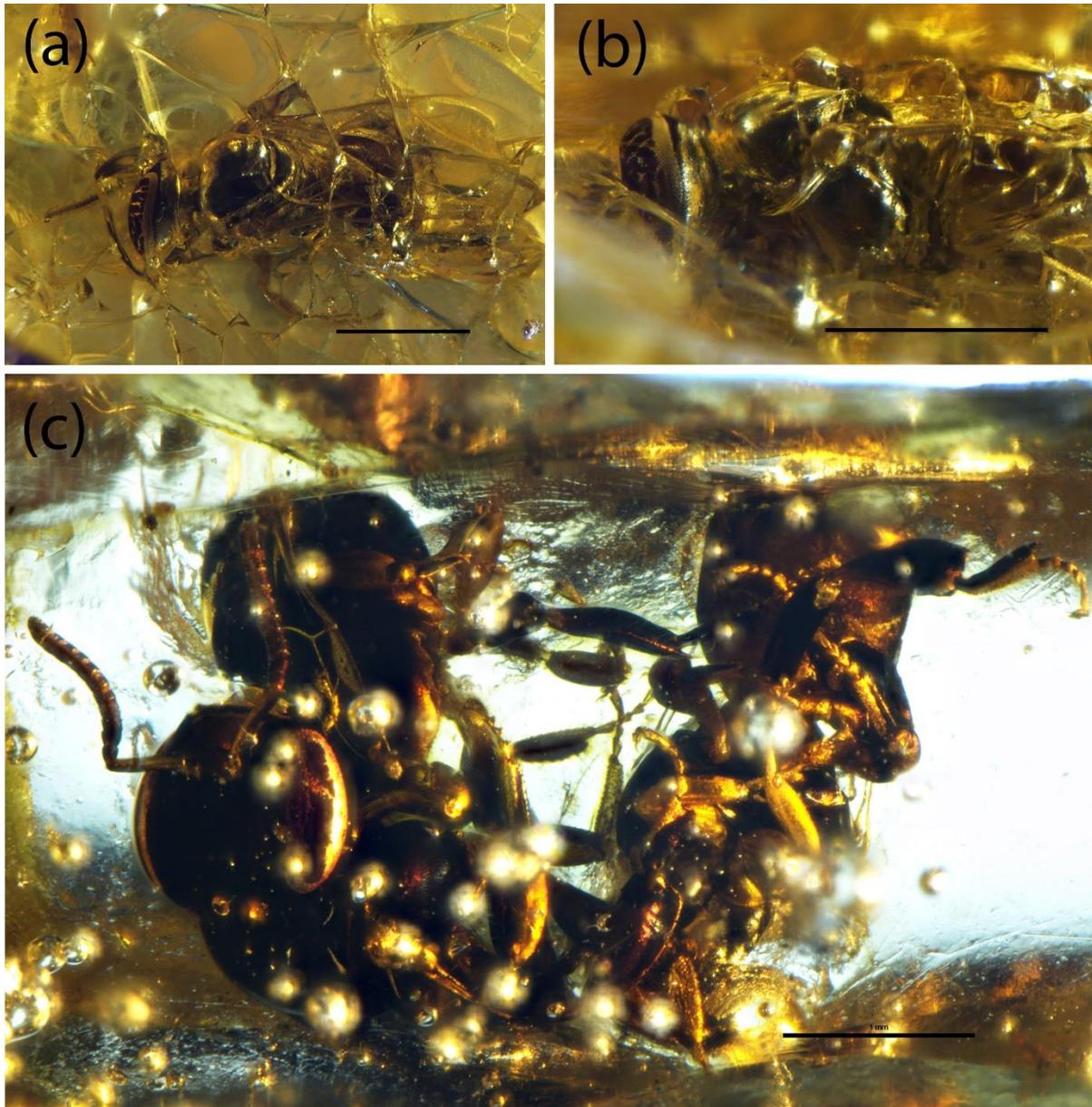


Fig. 8. Workers of *Liotrigona* in (a–b) Holocene copal from Tanzania and (c) Defaunation resin from Madagascar. (a) *L. bouyssoui* in Tanzanian Holocene copal (1000 +/- 30 BP) in dorsal view (Piece number SMF Be 3712a), (b) *L. bouyssoui* in laterodorsal view, (Piece number SMF Be 3712a), (c) Three workers of *L. nilssoni* collected in 2015 as bioinclusion in *Hymenaea verrucosa* resin from Sacaramy (close to Antsiranana), Madagascar (Piece number SMF Be 12431.1). Scale bars 1 mm.

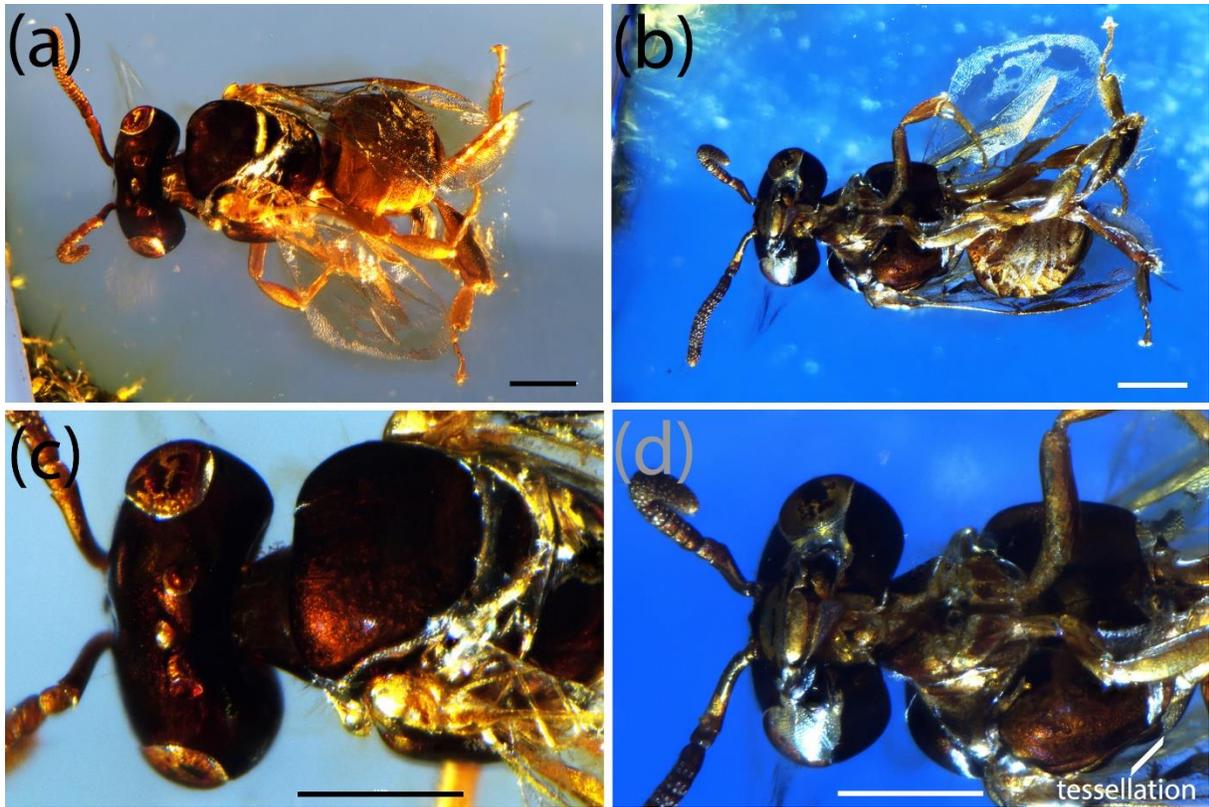


Fig. 9. Worker of *Liotrigona vetula* (Holotype NHMD-115579) in Defaunation resin from Madagascar. (a) Habitus in dorsal view, (b) Habitus in ventral view, (c) Head and mesoscutum in dorsal view, (d) Head and mesoscutum in ventral view. Scale bars 1 mm.

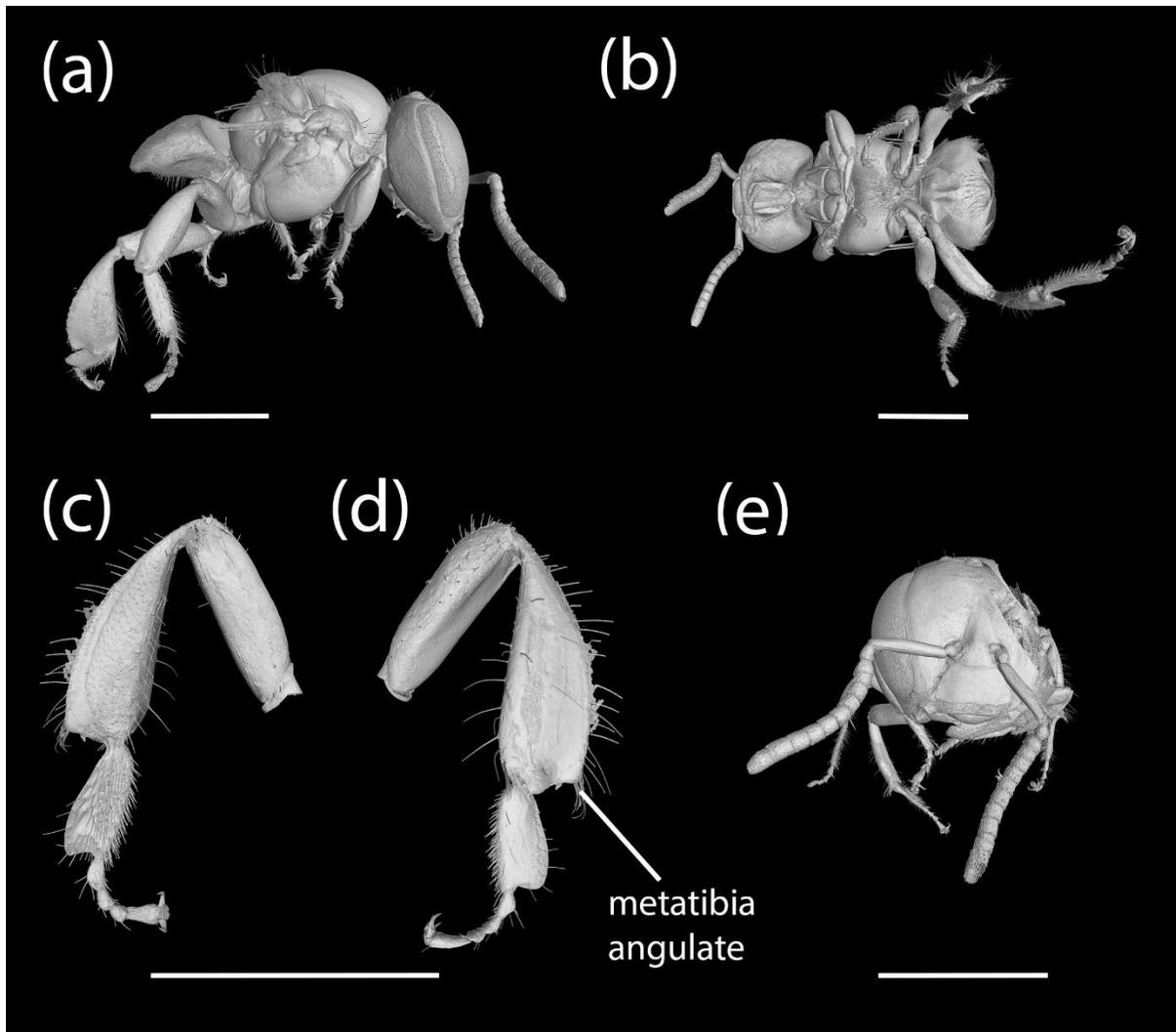


Fig. 10. Virtual representation of *Liotrigona nilssoni* in Defaunation resin from Madagascar. Piece number SMF Be 12431.1a collected in 2015 as bioinclusion in *Hymenaea verrucosa* resin from Sacaramy (close to Antsiranana), Madagascar. (a) Habitus in left lateral view, (b) Habitus in ventral view, (c, d) Right hind leg in ventral and dorsal views, (e) Head close-up. Scale bars 1 mm.

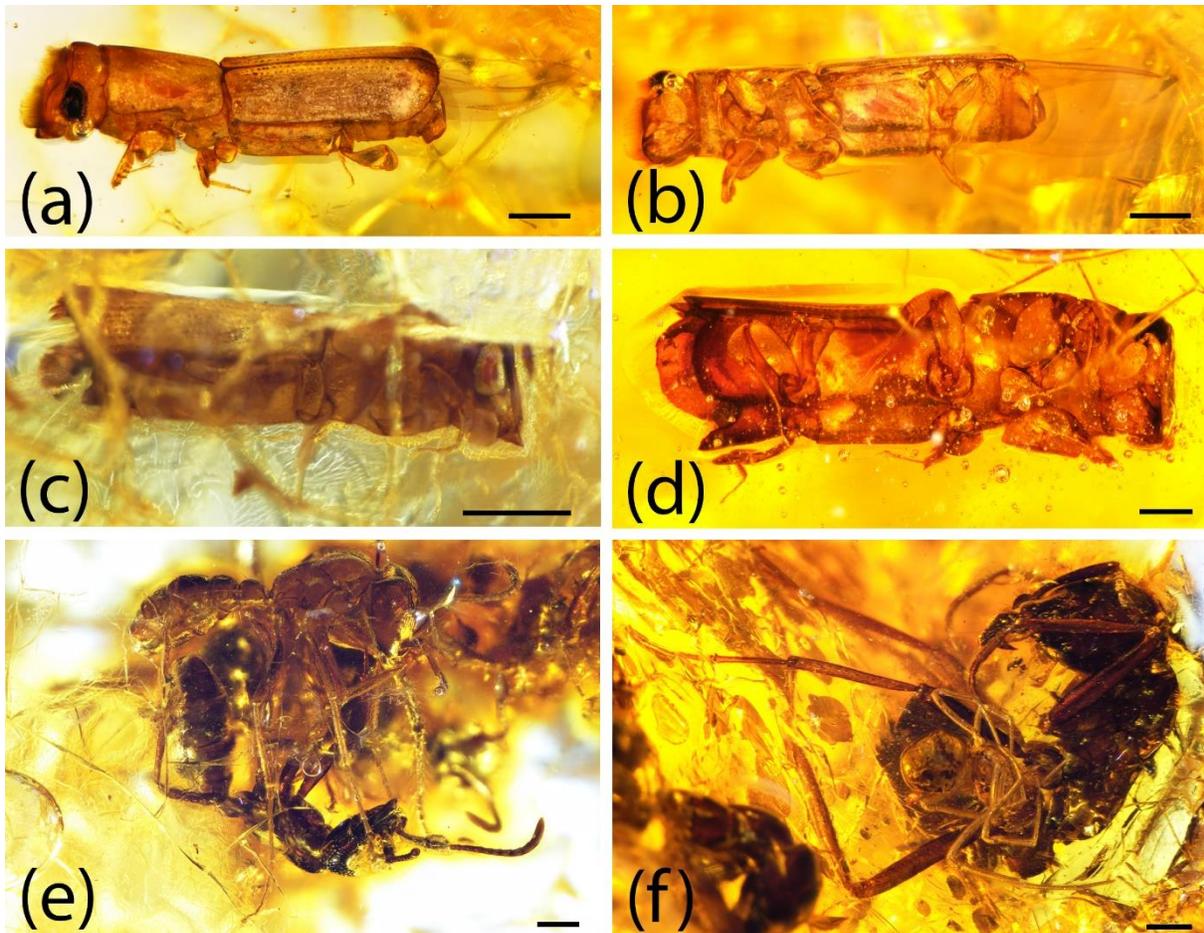


Fig. 11. Indicator taxa in Holocene copal or Defaunation resin from Tanzania (age unknown). (a) Female of *Chaetastus* sp. (Coleoptera: Curculionidae: Platypodinae) in lateral view (piece number SMF Be 3504a), (b) Female of *Chaetastus* sp. in ventral view (piece number SMF Be 3504b), (c) Male of *Chaetastus* sp. in lateral view (piece number SMF Be 3706a), (d) Male of *Chaetastus* sp. in ventral view (piece number SMF Be 3504c), (e–f) Exemplars of *Dorylus* sp. (Hymenoptera: Formicidae: Dorylinae) in lateral view (piece numbers SMF Be 3558ab and SMF Be 3739a). Scale bars 0.5 mm.

Supplementary Information for

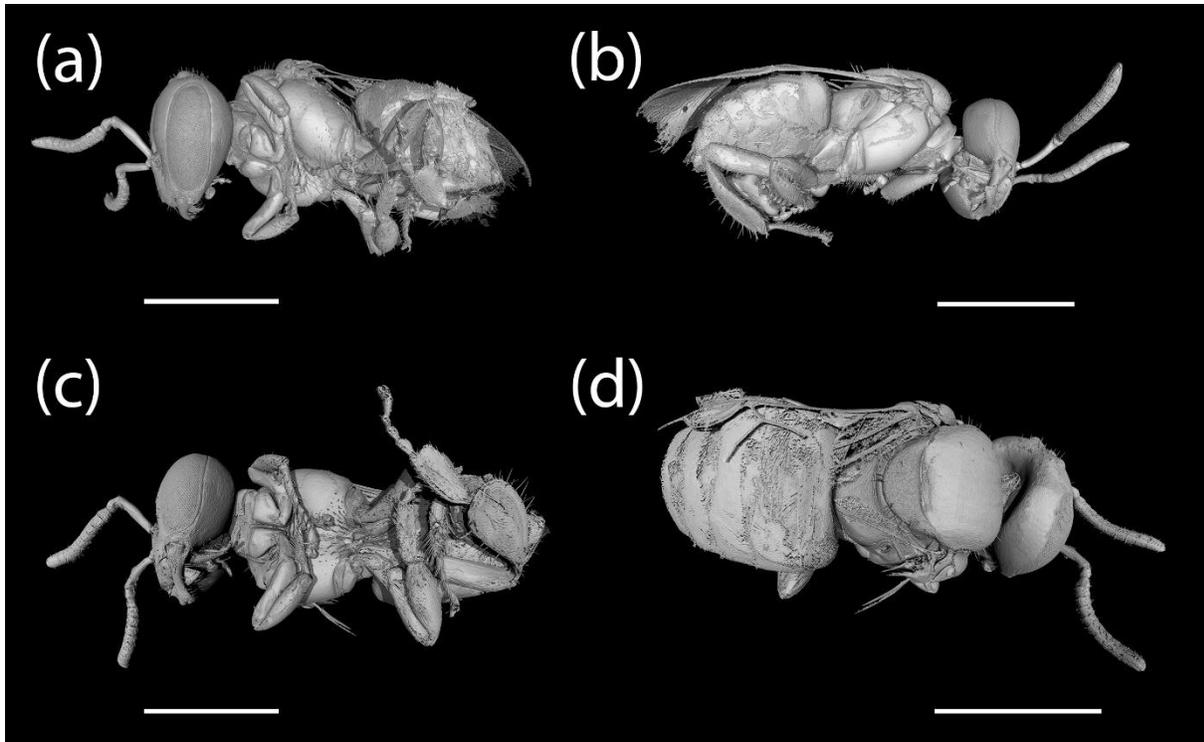
Stingless bees (Hymenoptera: Apidae) in Holocene copal and Defaunation resin from Eastern Africa indicate Recent biodiversity change

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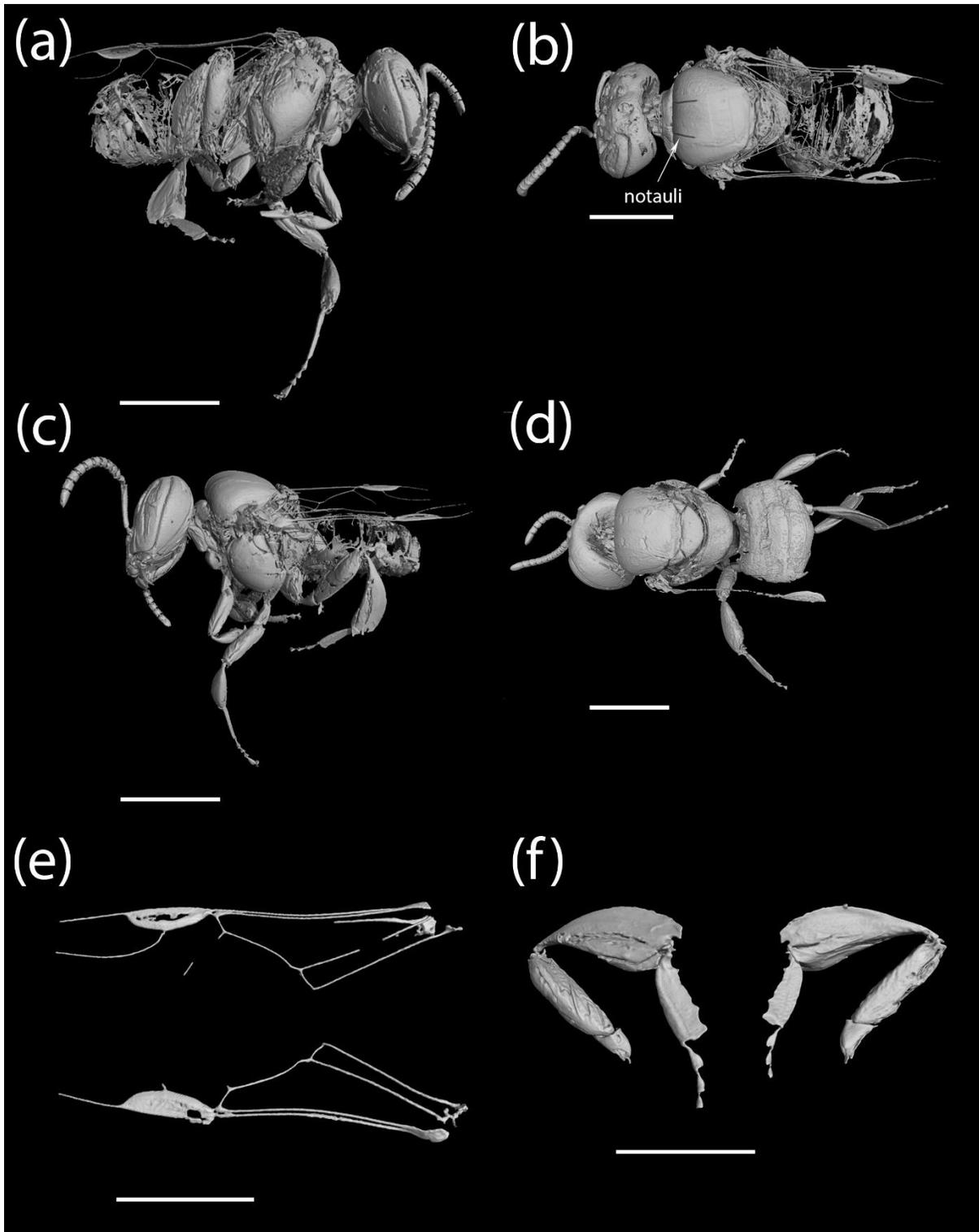
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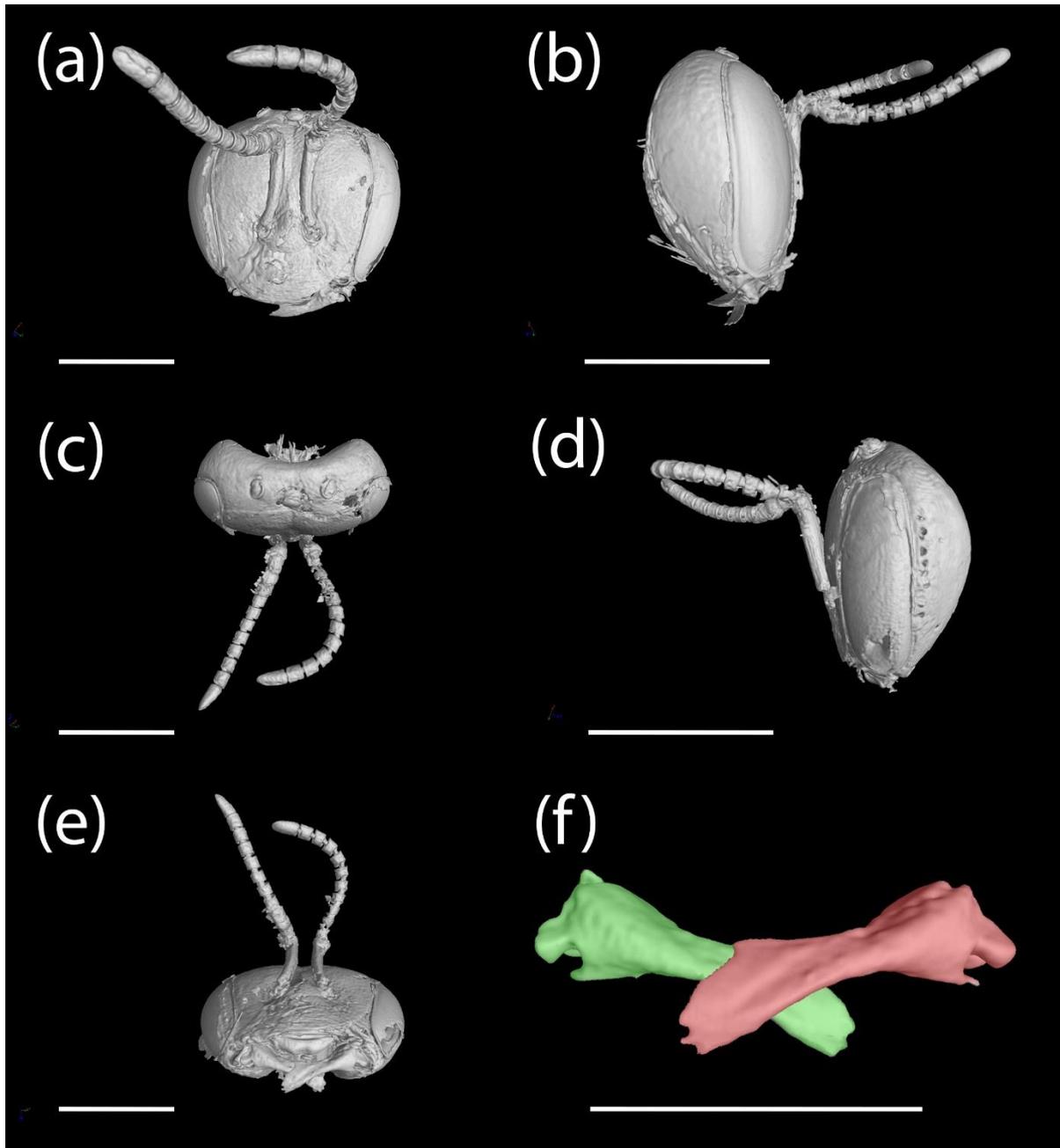
Supplementary Figures



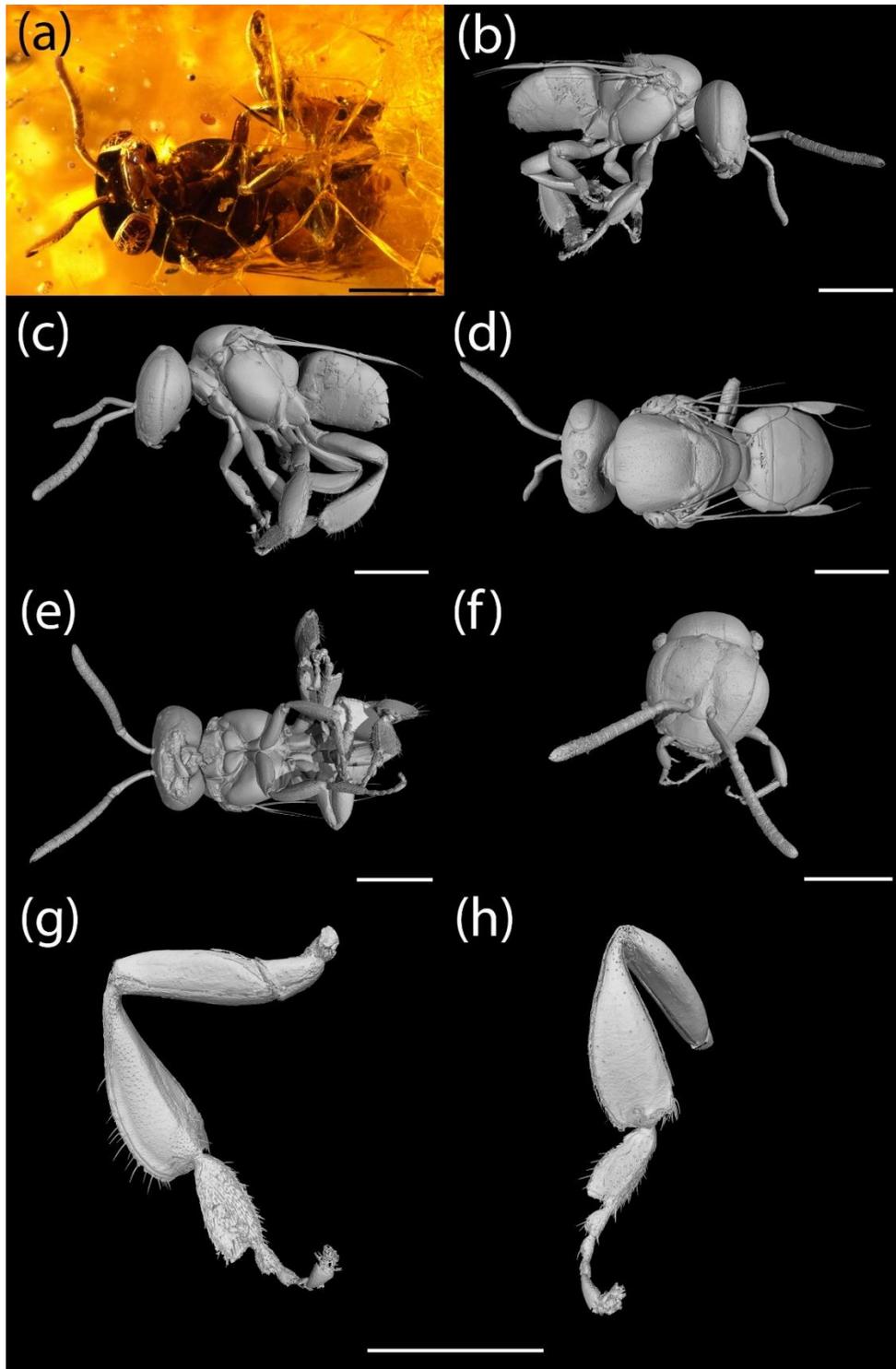
Supplementary Fig. 1. Virtual representation of *Hypotrigena gribodoi* in Tanzanian Defaunation resin (100 +/- 30 BP). Piece number SMF Be 3694.1b. (a) Habitus in left lateral view, (b) Habitus in right lateral view, (c) Habitus in ventral view, (d) Habitus in dorsal view. Scale bars 1 mm.



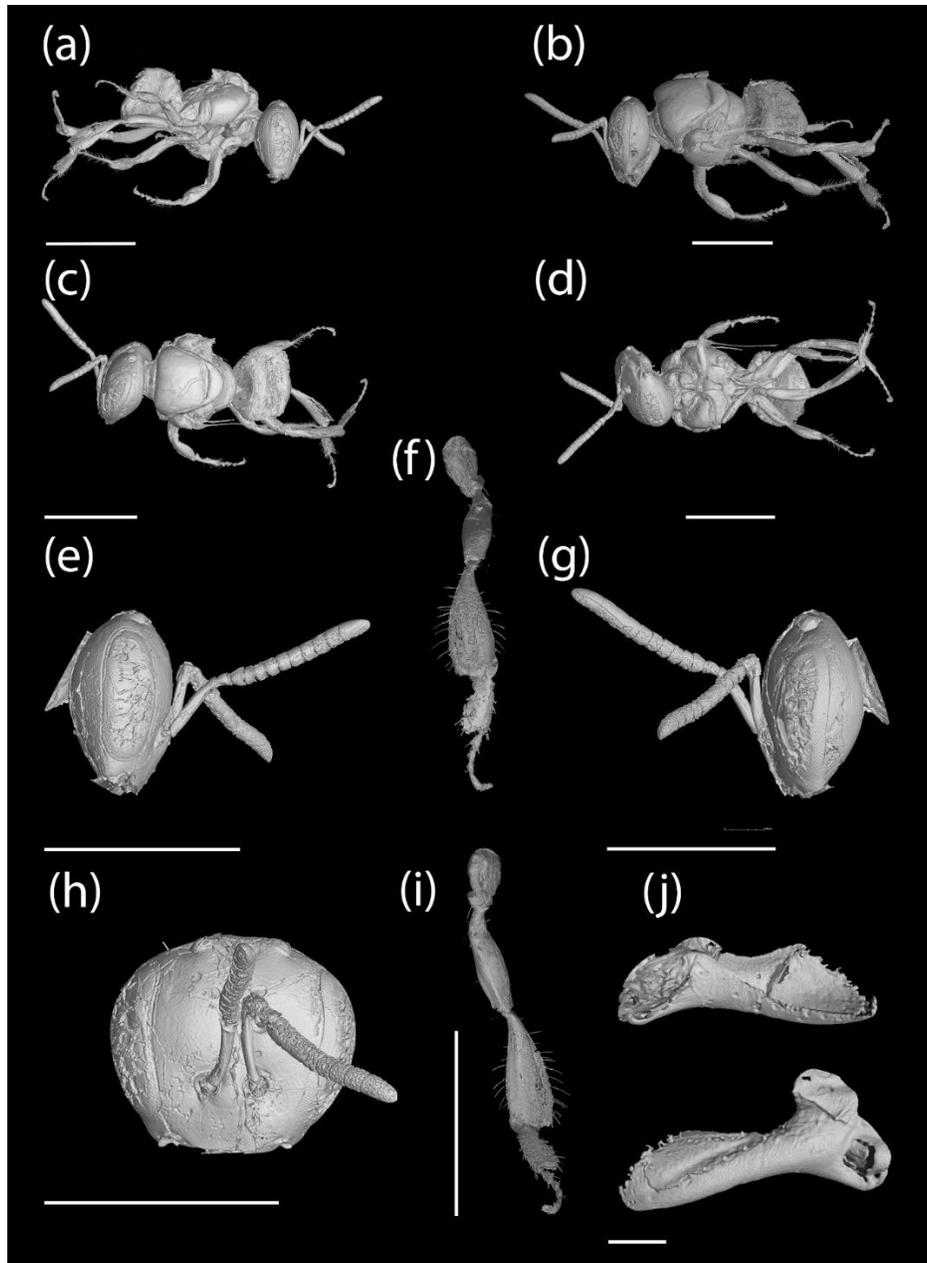
Supplementary Fig. 2. Virtual representation of two workers of *Hypotrigona kleineri* n. sp. in Tanzanian Holocene copal or Defaunation resin. Paratypes piece number SMF Be 3525a and SMF Be 3525b (age unknown). (a–b) Habitus in right lateral and dorsal views, (c) Habitus in left lateral view, (d) Habitus in dorsal view, (e) Forewings, (f) Hind legs. Scale bars 1 mm.



Supplementary Fig. 3. Virtual representation of the head of *Hypotrigona kleineri* n. sp. in Tanzanian Holocene copal or Defaunation resin. Paratype piece number SMF Be 3525a (age unknown). (a) Anterior view, (b) Right lateral view, (c) Dorsal view, (d) Left lateral view, (e) Anteroventral view, (f) Mandibles. Scale bar 1 mm.



Supplementary Fig. 4. Habitus in amber (a) and virtual representation of *Hypotrigona kleineri* n. sp. in Tanzanian Holocene copal. Piece number SMF Be 3498a (age 1250 +/- 30 BP). (a) Habitus in ventral view, (b) Habitus in lateral right view, (c) Habitus in lateral left view, (d) Habitus in dorsal view, (e) Habitus in ventral view, (f) Head in frontal view, (g) Left hind leg, (h) Right hind leg. Scale bars 1 mm.



Supplementary Fig. 5. Virtual representation of *Liotrigona bouyssoui* in Tanzanian Holocene copal. Piece number SMF Be 3712a (age 1000 +/- 30 BP). (a) Habitus in right lateral view, (b) Habitus in left lateral view, (c) Habitus in laterodorsal view, (d) Habitus in ventral view, (e) Head in right lateral view, (f) Extern side of left hind leg, (g) Head in left lateral view, (h) Head in frontal view, (i) Internal side of left hind leg, (j) Mandibles. Scale bars in (a–i) 1 mm, in (j) 0.05 mm.