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Evolution

Microallopatric speciation in the relict dung beetle genus *Grebennikovius* (Coleoptera: Scarabaeidae) in the Eastern Arc Mountains

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The Eastern Arc Mountains (EAMs) in Tanzania and Kenya are renowned for their exceptional species endemism often restricted to isolated mountain blocks. Forest fragmentation during the Pliocene–Pleistocene played a significant role in shaping the EAMs' biodiversity by facilitating allopatric speciation between different mountains. However, only a few studies focused on species diversification within the same mountain block. In this article, we investigated the taxonomy and evolution of the dung beetle genus *Grebennikovius*, endemic to the Uluguru Mountains. Our goal was to unravel factors promoting allopatric speciation within a confined geographic area like the Ulugurus. We used an integrative taxonomic approach, combining phenotypic data and COI barcodes to delimit species and reconstructed a timetree of the genus. Molecular and morphological evidence consistently recovered 4 distinct *Grebennikovius* species, of which 3 are new to science: *G. armiger* n. sp., *G. basilewskyi* (Balthasar, 1960), *G. lupanganus* n. sp., and *G. pafelo* n. sp. *Grebennikovius* represents a rare case of microallopatry, with all species occurring in close proximity within different regions of the limited Uluguru forest (~230 km²). We infer that speciation was driven by the interplay between climate fluctuations during the Miocene and mid-Pleistocene, Uluguru topography, and the stability of rainforest ecological conditions. The latter factor probably favored the loss of wings and a specialization for Uluguru microhabitats, reinforcing population isolation and divergence. This study highlights a unique instance of diversification resulting from small-scale speciation events, providing valuable insights into the evolution of Eastern African rainforest arthropods and underscoring the importance of microhabitats conservation.

Key words: East Africa, molecular species delimitation, new species, rainforest, Uluguru Mountains

Introduction

The Eastern Arc Mountains (EAMs) of Tanzania and Kenya are widely recognized as one of the major biodiversity hotspots on Earth (Myers et al. 2000). This mountain chain is composed of an archipelago of 13 crystalline blocks, whose isolated rainforests host a disproportionate amount of endemic animals and plants (Burgess et al. 2007). EAMs vertebrates have been the subject of several faunistic, phylogeographic, and taxonomic studies (e.g., Cordeiro et al. 2005; Doggart et al. 2006; Rovero et al. 2014; Menegon et al. 2022; Kilwanila et al. 2023), whereas their highly endemic invertebrate fauna remains poorly known (Grebennikov 2015; Grebennikov and Heiss 2018).

The exceptional biodiversity of the EAMs is largely attributable to the antiquity of their rainforests. During Paleocene and Eocene (65.5–33.9 Mya), a pan-African rainforest extended from the West to the East of Africa. In Oligocene (33.9–23.0 Mya) and Plio-Pleistocene (5.3–0.01 Mya), such biota experienced cyclic contractions and expansions triggered by climatic fluctuations (deMenocal 2004;

Couvreur et al. 2021). However, the environmental stability of the EAMs favored a constant vegetation cover possibly since 30 Mya (Lovett et al. 2005; Mumbi et al. 2008; Finch et al. 2009), making them a unique mosaic of refugia for rainforest-specialized taxa (Lovett and Wasser 2008; Skarbek 2008). These mountains are therefore considered "alive museums," preserving relict lineages of different ages (Fjeldså and Lovett 1997; Grebennikov 2021). Additionally, forest fragmentation shaped the current pattern of endemism by favoring allopatric speciations in forests isolated on different EAMs blocks. Evidence for this has been found in a variety of taxa, highlighting the role of Plio–Pleistocene climatic cycles in the evolution of EAMs endemic lineages (Burgess et al. 2007; Bryja et al. 2014; Ceccarelli et al. 2014; Hemp et al. 2015; Grebennikov 2017; Dimitrov et al. 2012) as well as other organisms throughout Africa (see Couvreur et al. (2021) and references therein).

However, only a few studies have examined species diversification and the factors influencing speciation at small scales within the same mountain block (Measey and Tolley 2011). Our study focuses on the dung beetle genus *Grebennikovius* (Coleoptera: Scarabaeinae), which represents a case of allopatric speciation occurring within a single block of the EAMs. The aim of this study is thus to identify the factors that can promote species divergence on a small geographical scale.

Dung beetles account for several poorly studied taxa found in the EAMs, including 2 endemic genera: *Grebennikovius* Mlambo, Scholtz & Deschodt, 2019 and *Tanzanolus* Scholtz & Howden, 1987 (Scholtz and Howden 1987; Davis et al. 2008; Mlambo et al. 2019). A third genus, *Janssensantus* Paulian, 1976, was also considered to be exclusive to the EAMs, until it was recently found in Zambia (Josso 2022) and Mozambique (Daniel et al. 2023). The monotypic genus *Grebennikovius* was established by Mlambo et al. (2019) for a flightless, forest-dwelling species, *Madaphacosoma basilewskyi* (Balthasar, 1960) (currently *Grebennikovius basilewskyi*), found in the Uluguru Mountains (Balthasar 1960). Recently, Rossini et al. (2022) provided an intriguing biogeographic scenario for *Grebennikovius* and allied taxa. In their work, as previously highlighted in other studies (Monaghan et al. 2007; Wirta and Montreuil 2008; Mlambo et al. 2014; Tarasov and Dimitrov 2016), *Grebennikovius* and its Malagasy sister genus *Epactoides* Olsoufieff, 1947 form a sister clade to the Oriental *Ochicanthon* Vaz-de-Mello, 2003. For these 3 genera, the new tribe Epactoidini Rossini, Grebennikov, Merrien, Miraldo, Viljanen & Tarasov, 2022 was proposed. According to the biogeographic reconstruction, Epactoidini originated in Africa around 46 Mya and subsequently dispersed to the Oriental region with *Ochicanthon* (~50 species), and to Madagascar and Réunion island with *Epactoides* (~40 species). Surprisingly, the ancestral Epactoidini seem to have gone almost entirely extinct in continental Africa, possibly due to periodic fragmentations of the Pan-African rainforest in which these beetles evolved (Rossini et al. 2022). The sole African locality where they are known to persist is the forest of Uluguru Mountains in the East Central EAMs, where to date only one species has been identified (*G. basilewskyi*).

The Ulugurus (Figs. 1c and 3b and c) occupy a relatively limited territory, stretching for ~45 km from North to South and hosting a forest cover that currently does not exceed ~230 km² (Fjeldså and Hansen 1995; Kitula et al. 2012). Nevertheless, the diverse topography of these mountains, which comprises several peaks, gorges, and a 2,600 m high plateau, makes the Ulugurus one of the 3 most important spots for wildlife conservation in the EAMs (Burgess et al. 2002).

Recent fieldwork conducted by one of us (V.V.G.) in the Uluguru Mountains yielded an unprecedented number of *Grebennikovius* specimens, forming the primary material for the present study. We focus on exploring *Grebennikovius* species diversity through an integrative taxonomic approach that blends various data sources, allowing us to formulate robust species hypotheses (De Queiroz 2007; Yeates et al. 2011; Ranasinghe et al. 2022). Specifically, our approach combines several species delimitation methods: (a) morphological examination based on distinctive phenotypic traits commonly used in dung beetle species separation and (b) 3 molecular species delimitation methods relying on COI barcodes. Furthermore, to reconstruct the evolutionary history of *Grebennikovius*, we inferred a timetree and assessed it in a biogeographical and ecological context.

Our results reveal the existence of at least 4 distinct species of *Grebennikovius*, which we describe herein and accompany with an identification key. Notably, *Grebennikovius* exemplifies a single-mountain microallopatric speciation, a phenomenon rarely observed in dung beetles and the fauna of the EAMs. Below, we discuss the evolutionary history of *Grebennikovius* in relation to historical forest fragmentation, the current Uluguru Mountains environment, and the flightlessness of these beetles.

Materials and Methods

Specimens Collection

We studied 64 specimens of *Grebennikovius*, most of which were recently collected by V.V.G. in several localities in the Uluguru Mountains in 2010 and 2012 (Figs. 1 and 3b and c). Specimens were sampled by litter sifting as described in Grebennikov (2017), stored in 96% ethanol for DNA extraction, and subsequently glued on paper cardboard. Full specimen data in Darwin Core format, including GenBank accession numbers, and BOLD identifiers are available in Supplementary Material (specimens_data.xlsx). The morphology of 3 type specimens of *Grebennikovius basilewskyi* (Balthasar, 1960), including the holotype, was also examined.

The studied material is preserved in the following institutes and private collections:

> BDGC–Bruce D. Gill private collection, Ottawa, Canada
> JFJC–Jean-François Josso private collection, Muzillac, France
> MZH–Finnish Museum of Natural History LUOMUS, Helsinki, Finland
> PMOC–Philippe Moretto private collection, Toulon, France
> RMCA–Royal Museum for Central Africa, Tervuren, Belgium

When reporting labels of holotypes, double slashes (//) are used to separate different labels and single slash (/) for different lines on the same label. Comments to data and acronyms of the repository collections are given in square brackets ([]).

Morphological Study

To delimit morphospecies, we investigated external and male genital morphology in detail. Of the 64 specimens analyzed in this study, 4 were not considered in the morphological assessment due to the inability to locate their vouchers after DNA extraction. Criteria to identify putative species are those widely adopted in dung beetle taxonomy (see, e.g., Génier and Moretto 2017; Moctezuma and Halffter 2021). Morphology was examined under a Leica S9D stereomicroscope. Male genitalia were extracted, dissected, and cleaned in a 10% KOH solution and stored in a microvial with glycerol. Each vial was
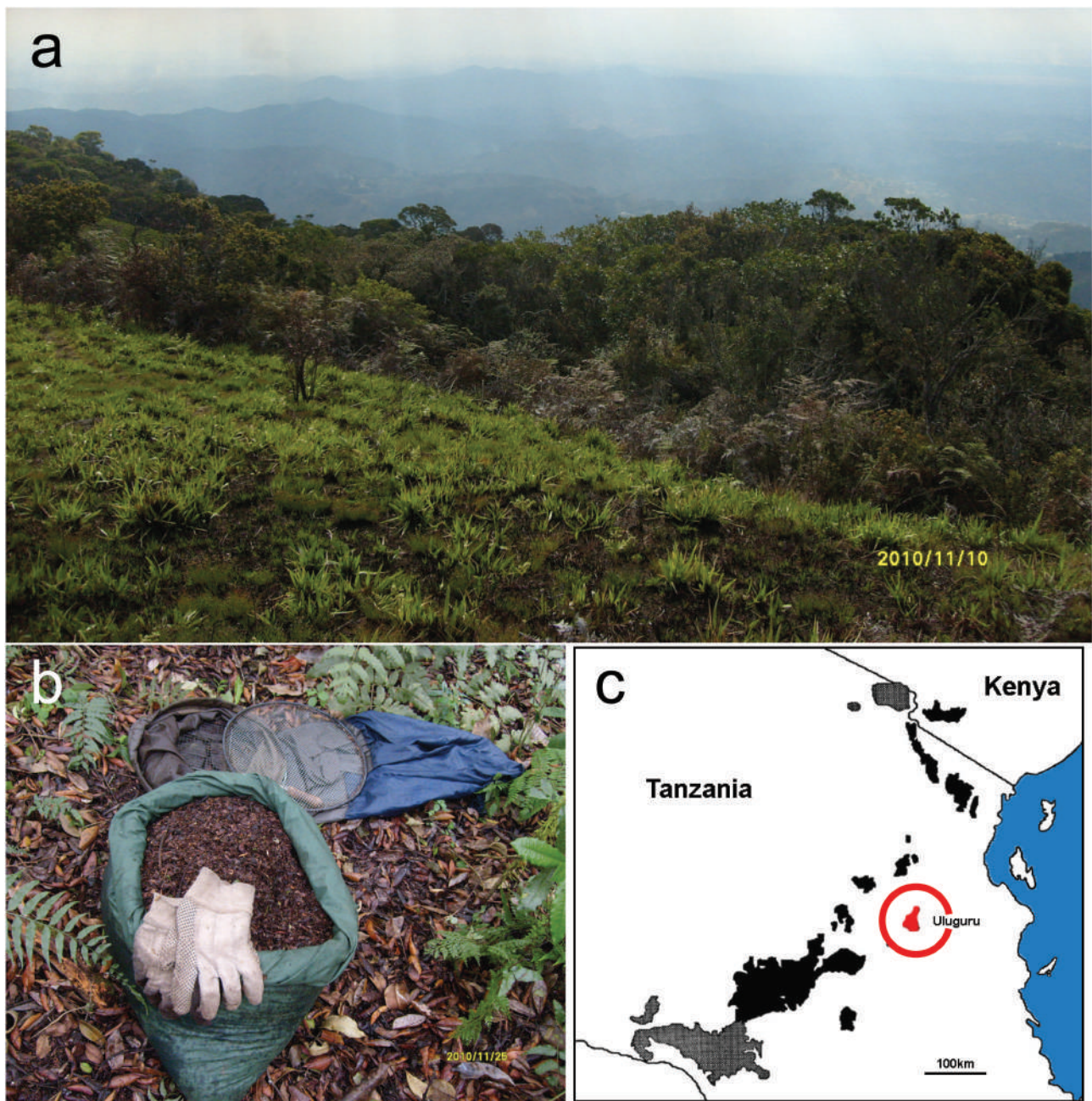
Fig. 1. Habitat of *Grebennikovius* species. a) View from the Lukwangule Plateau near where *G. armiger* n. sp. and *G. pafelo* n. sp. were collected. b) Tools used for litter sifting. c) Map of the Eastern Arc Mountains (in black) with the Uluguru Mountains highlighted in red. Modified from Burgess et al. (2007) (with permission).

pinned underneath the corresponding specimen. Pictures of external morphology were taken with a Canon EOS 5DS; pictures of genitalia were taken with a Canon EOS 250D connected to the stereomicroscope. Alcohol-based hand sanitizer was used to stabilize the position of the genitalia during photography.

Species Descriptions, Terminology, and Ontologies

In this article, species descriptions are written in natural language. Additionally, ontology-based semantic descriptions (Mikó et al. 2021) produced using Phenoscript language (Tarasov et al. 2023) will be available through an upcoming publication (Montanaro et al., in preparation). Phenoscript allows the user to write ontology-based descriptions, which are understandable by computers. In contrast to

traditional descriptions, this approach makes phenotypic data easily queryable and accessible for future analytical applications.

Terminology follows Tarasov and Génier (2015), Tarasov and Solodovnikov (2011), Génier (2019), and Génier and Moretto (2017). For cuticular structures, we adopted the terminology from the Insect Anatomy Ontology (AISM, available on Github) (Girón et al. 2023) and Coleoptera Anatomy Ontology (COLAO, available on Github) (Girón et al. 2023). The precise definitions of the terms are available through those references.

We use the term "meso-" and "metaventrite" instead of "meso-" and "metasternum" (see Lawrence and Slipinski 2013) and "tergite VIII" instead of "pygidium" (see also Cristovao and Vaz-de Mello 2021). Positional terms refer to the general insect body plan; for

example, we consider that protibial teeth are positioned on the dorsal (and not lateral or external) margin of protibiae. This is because the traditional "lateral margin" in Scarabaeinae refers, in fact, to what represents the dorsal margin of an insect leg. Similarly, we use positional terms referring to the whole insect body, not to specific body parts. For example, we avoid the use of "basal-apical" axis for regions of aedeagus, elytron, and tergite VIII. Instead, we use "proximal-distal" and, for tergite VIII, "anterior-posterior" axes.

Nomenclature

This article and the nomenclatural acts it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:0D3E2C9E-8886-41FB-AF78-702585AA3764.

DNA Dataset Construction

Molecular analyses were performed using the COI barcode region (Hebert et al. 2003; DeSalle and Goldstein 2019). DNA extraction and amplification were performed on 28 specimens following the protocol described in Grebennikov (2017) and resulted in 27 sequences 658–307 bps (mean length = 600 bps). Specimen records with sequences and other relevant information can be found on BOLD (Ratnasingham and Hebert 2007). Alignment was obtained using the online server of MAFFT v. 7 (Katoh and Standley 2013) and processed in MEGA v. 10.2.5 (Tamura et al. 2021). Three redundant sequences were removed and one for *Epactoides frontalis* (Montreuil, 2003) was retrieved from BOLD (identifier: GBCLS288-14) to be used as an outgroup. The final dataset (25 sequences, 654 bps long) is available in Supplementary Material.

Molecular Phylogenetic Inference

We used 2 datasets—unpartitioned and partitioned by codon position—to reconstruct phylogenetic relationships within *Grebennikovius*. Model selection was performed using JModelTest v. 2.1.10 (Darriba et al. 2012) based on the Akaike information criterion (AIC). Dated phylogenetic trees were obtained in BEAST v. 2.6.7 (Bouckaert et al. 2019) using the birth–death model with exponentially distributed birth rate prior (mean = 0.1).

We tested 2 different calibration approaches: (a) using a broad prior for the substitution rate equal to 0.018 substitutions per site per million years that was suggested by previous studies on insects (Papadopoulou et al. 2010; Andújar et al. 2012) and (b) using a secondary calibration point from Rossini et al. (2022) for the tree root. In the latter approach, the root age prior was set to *Normal* (mean = 32.28, sigma = 1.66); the standard deviation was found empirically to fit the 95% interval of the distribution. We tested both strict and a relaxed lognormal clock models for the secondary point calibration. The settings for all analyses are summarized in Table 1.

Markov chain Monte Carlo (MCMC) was run for 50M generations by sampling every 2,500th generation. Convergence was assessed in Tracer v. 1.7.2 (Rambaut et al. 2018). The trees obtained from the posterior distribution were processed in TreeAnnotator v. 1.10.4 (Drummond and Rambaut 2007) using a burnin fraction of 25%. The distribution of node ages was plotted in R (script available upon request).

The fit of strict and relaxed lognormal clock models was assessed using Bayes factor (Oaks et al. 2019). Marginal likelihoods were calculated using path sampling (Lartillot and Philippe 2006) in BEAST with alpha = 0.3, 50 steps, and 20M MCMC generations. Two runs for each BEAST analysis were performed to check convergence.

Lastly, MrBayes (Ronquist et al. 2012) was used to infer a posterior sample of nonultrametric trees used for species delimitation with bPTP (see below). Settings were similar to those in BEAST analyses (Table 1 and Supplementary Material).

Molecular Species Delimitation

Molecular species delimitation (MSD) was performed to infer species boundaries in addition to morphological evidence. Multiple studies have revealed the unreliability of single MSD methods due to inherent biases. Therefore, it was recommended to prioritize consistency by employing multiple approaches (Dellicour and Flot 2018; Ranasinghe et al. 2022). Herein, we used 3 different methods: (a) GMYC, the generalized mixed Yule coalescent process (Fujisawa and Barraclough 2013; Pons et al. 2006); (b) bPTP, the Bayesian Poisson tree process (Zhang et al. 2013); and (c) ABGD, the automatic barcode gap discovery (Puillandre et al. 2012).

GMYC was applied to the ultrametric trees obtained in BEAST using partitioned datasets; the analysis was performed in R following the instructions by Michonneau (2016) (see Supplementary Material). The trees from MrBayes were used for the bPTP analysis on the webserver (https://species.h-its.org/ptp). We used default settings with the burnin set to 0.25 and outgroup removed. The ABGD analysis was conducted on the webserver (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) using 50 steps and relative gap width = 1.

Mantel Test and Pairwise Distances

We performed a Mantel test to assess correlation between genetic and geographical distances (Diniz-Filho et al. 2013). Genetic distance was calculated as pairwise F_{ST} in Arlequin (Excoffier and Lischer 2010)

Table 1. Settings of phylogenetic analyses using BEAST and MrBayes. The calibration approaches are explained in Materials and Methods

Run	Software	Dataset	Subst. model codon 1	Subst. model codon 2	Subst. model codon 3	Calibration approach	Clock type
P1	BEAST	Partitioned	TRN	HKY	TRN+I	1	Strict
P2	BEAST	Partitioned	TRN	HKY	TRN+I	2	Strict
P3	BEAST	Partitioned	TRN	HKY	TRN+I	2	Relaxed lognormal
UP1	BEAST	Unpartitioned	TIM3+G+I	TIM3+G+I	TIM3+G+I	1	Strict
UP2	BEAST	Unpartitioned	TIM3+G+I	TIM3+G+I	TIM3+G+I	2	Strict
UP3	BEAST	Unpartitioned	TIM3+G+I	TIM3+G+I	TIM3+G+I	2	Relaxed lognormal
MB	MrBayes	Partitioned	GTR	HKY	GTR+G	—	Strict

using Tamura–Nei substitution model (Tamura and Nei 1993). A matrix of pairwise geographical distances was obtained in R using specimen coordinates. The test was performed in R using 9,999 permutations and Spearman's correction (see Supplementary Material). Pairwise distances (%) were calculated in MEGA using Tamura–Nei substitution model.

Results

Morphological Species Delimitation

Based on external and genital morphology, *Grebennikovius* specimens are unambiguously classified into 4 morphospecies (Fig. 2), the type species *G. basilewskyi*, and 3 new species: *G. armiger* **n. sp.**, *G. pafelo* **n. sp.**, and *G. lupanganus* **n. sp**. They mainly differ in male secondary sexual traits, integument punctuation, head and body shape, and male genitalia (see the Taxonomy section). Particularly, cuticular protrusions on male legs provide good characters to distinguish species (Fig. 6). The most striking example is found between *G. armiger* and *G. pafelo*, which differ greatly in the shape of metafemora.

The 4 morphospecies are found in different localities. *Grebennikovius basilewskyi* occurs in the vicinity of the Bunduki village (central Uluguru); *Grebennikovius lupanganus* is found at the Lupanga Peak (North Uluguru); and *Grebennikovius armiger* and *G. pafelo* occur, respectively, in the western and eastern slopes delimiting the Lukwangule Plateau (South Uluguru) (Fig. 3b and c).
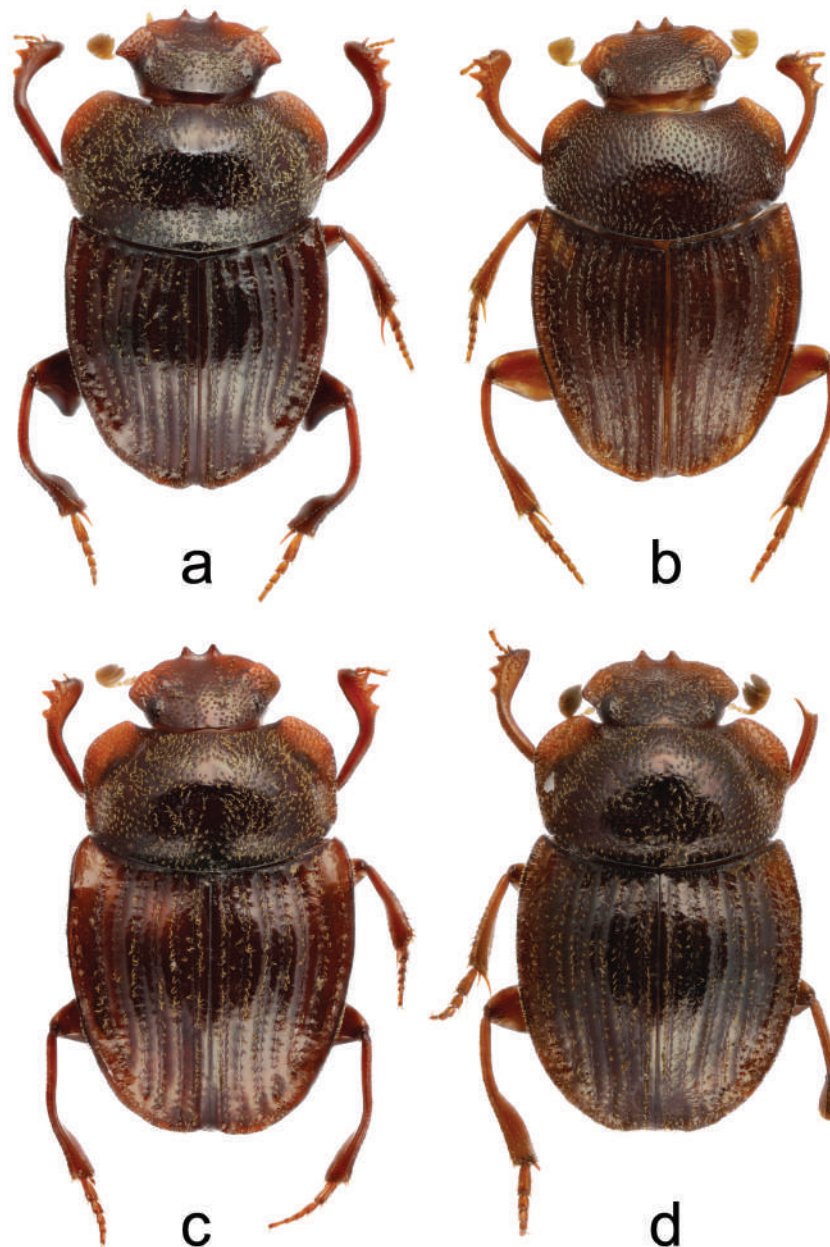
Fig. 2. Male habiti of *Grebennikovius* species. a) *G. armiger* n. sp., paratype; b) *G. basilewskyi* (Balthasar, 1960), recently collected specimen; c) *G. pafelo* n. sp., holotype; d) *G. lupanganus* n. sp., holotype.
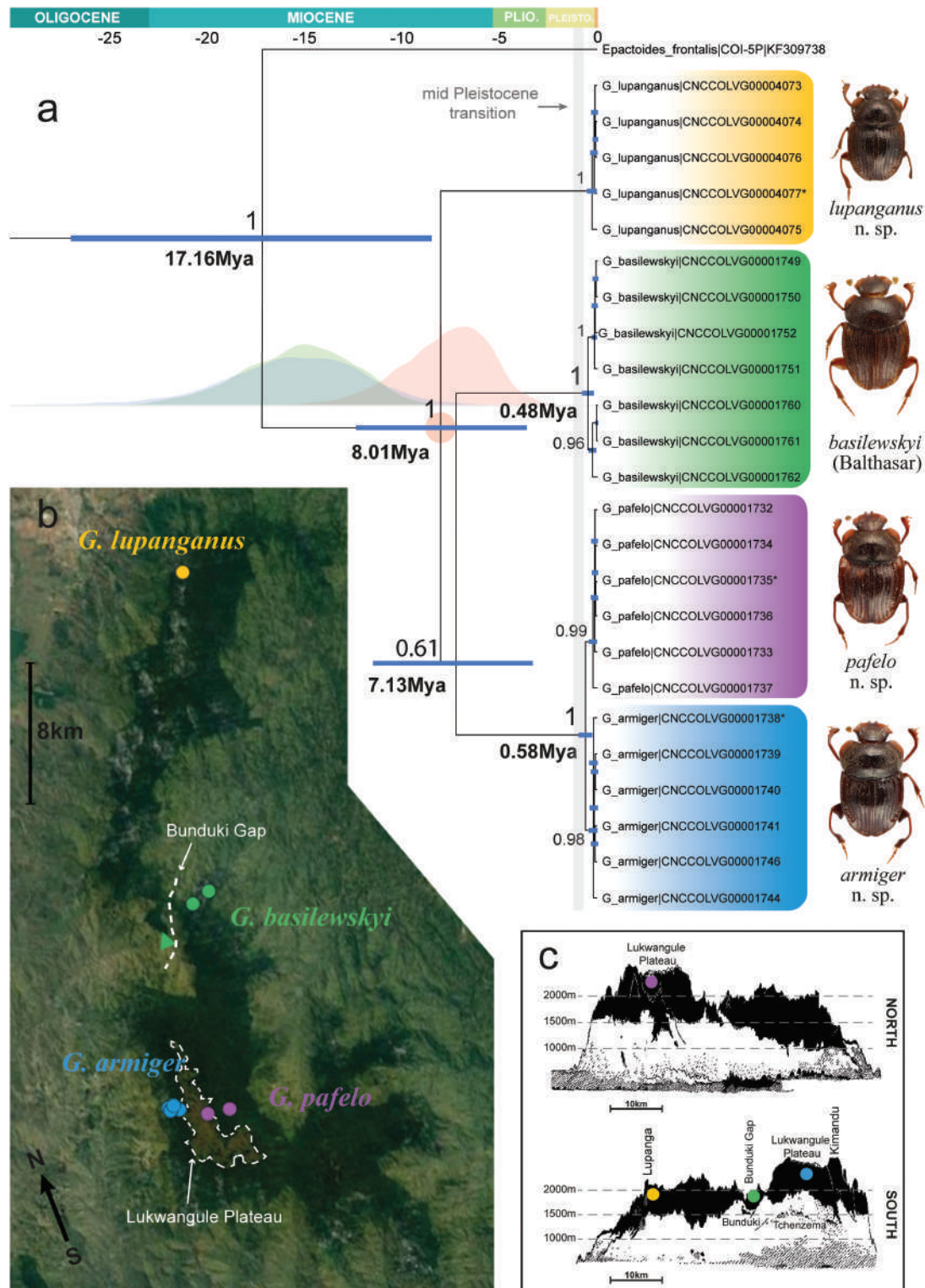
Fig. 3. a) Timetree and b, c) distribution of *Grebennikovius*. a) BEAST tree from the analyses with the fixed substitution rate (P1); numbers above and below nodes indicate posterior probabilities and inferred age means, respectively; error bars on nodes represent 95% HPD age intervals; sequences from holotype specimens are marked with an asterisk. Density plots next to the node marked with red circle (MRCA of *Grebennikovius*) show the distributions of node ages for different partitioned analyses (P1 in red, P2 in green, and P3 in blue). b) Distribution of *Grebennikovius* species on the Ulugurus (green triangle indicates the type locality of *G. basilewskyi*). c) Distribution of *Grebennikovius* species on elevation profiles of the Ulugurus.

Phylogenetic Reconstructions and Dating

Phylogenetic inference recovers 4 highly supported clades of specimens consistent with morphospecies. The trees obtained with different calibration approaches yielded largely similar topologies, which only differ in age estimates ([Fig. 3a](#) and [Supplementary Material](#)).

Marginal likelihoods (ML) were constantly higher in the trees from the partitioned dataset (Bayes factors = $ML_{partitioned}-ML_{unpartitioned} > 200$). Partitioning is usually favored as it accommodates varying evolutionary substitution patterns among sites (Kainer and Lanfear 2015). For this reason, we omit the unpartitioned analyses from further discussion. The partitioned model with fixed substitution rates (P1, Table 1) yielded a substantially better marginal likelihood score compared to other partitioned analyses (ML_{P1} = $-1,671.01 > ML_{P2} = -1,686.950.35 > ML_{P3} = -1,688.530.15$). Only trees P1 and P2 will be used in the Discussion section hereafter. The topology obtained with MrBayes is consistent with those obtained with BEAST.

In all analyses, *G. lupanganus* is the sister species of the remaining *Grebennikovius*, while *G. armiger* and *G. pafelo* appear as recently diverged sister species. Age estimates vary considerably between the 2 calibration approaches, with the fixed-rate approach (P1) yielding node ages that are almost half as old as those from the secondary calibration point approach (P2 and P3). The most recent common ancestor (MRCA) of *Grebennikovius* emerged around 8.01 My for P1, 14.90 My for P2, and 16.32 My for P3 (Fig. 3a and Supplementary Material). P2 and P3 have almost identical age distributions despite using different clock models (strict vs. relaxed lognormal).

MSD Results

Regardless of the calibration approach, GMYC recognized 5 species of *Grebennikovius* in addition to the outgroup (Fig. 4b). All of them match the morphospecies, except for *G. basilewskyi* that splits into 2 groups corresponding to specimens from 2 different localities.

bPTP recognizes *G. basilewskyi* and *G. lupanganus* but lumps *G. armiger* and *G. pafelo* (Fig. 4a). Lastly, ABGD, for barcode gaps between 0.31% and 2.02%, finds the same groups as bPTP. *Grebennikovius basilewskyi* splits into 3 groups with lower thresholds, while with higher values, all species become lumped (see Supplementary Material).

Correlation Between Genetic and Geographical Distances

The Mantel test recovered a strong correlation between F_{ST} and geographical distances ($r = 0.68$, P-value<0.01). F_{ST} values calculated between populations of different morphospecies range between 0.72 and 0.97, while the 2 sampled populations of *G. basilewskyi* have $F_{ST} = 0.665$. Average pairwise distances between different species range between 7.84% (*G. armiger*–*G. basilewskyi*) and 9.34% (*G. basilewskyi*–*G. lupanganus*), with the exception of *G. armiger*–*G. pafelo* where divergence is 1.44%.

Taxonomy

Grebennikovius Mlambo, Scholtz & Deschodt, 2019

Phacosoma Boucomont, 1914: Balthasar 1960: 57.
Madaphacosoma Paulian, 1975: Paulian 1975: 222.
Epactoides Olsoufieff, 1947: Wirta and Montreuil 2008: 652.
Grebennikovius Mlambo, Scholtz & Deschodt, 2019: Mlambo et al. 2019: 113.

Type species: *Phacosoma basilewskyi* Balthasar, 1960, by monotypy.

Diagnosis. Genus of the tribe Epactoidini as defined by Rossini et al. (2022). It shares the main morphological characters of *Epactoides*, including the equally sclerotized ventral membranes of parameres, the axial and subaxial endophallites fused, and the absence of frontolateral peripheral endophallite (see Rossini et al. 2022, Fig. 2). Similar to some species of *Epactoides*, *Grebennikovius*
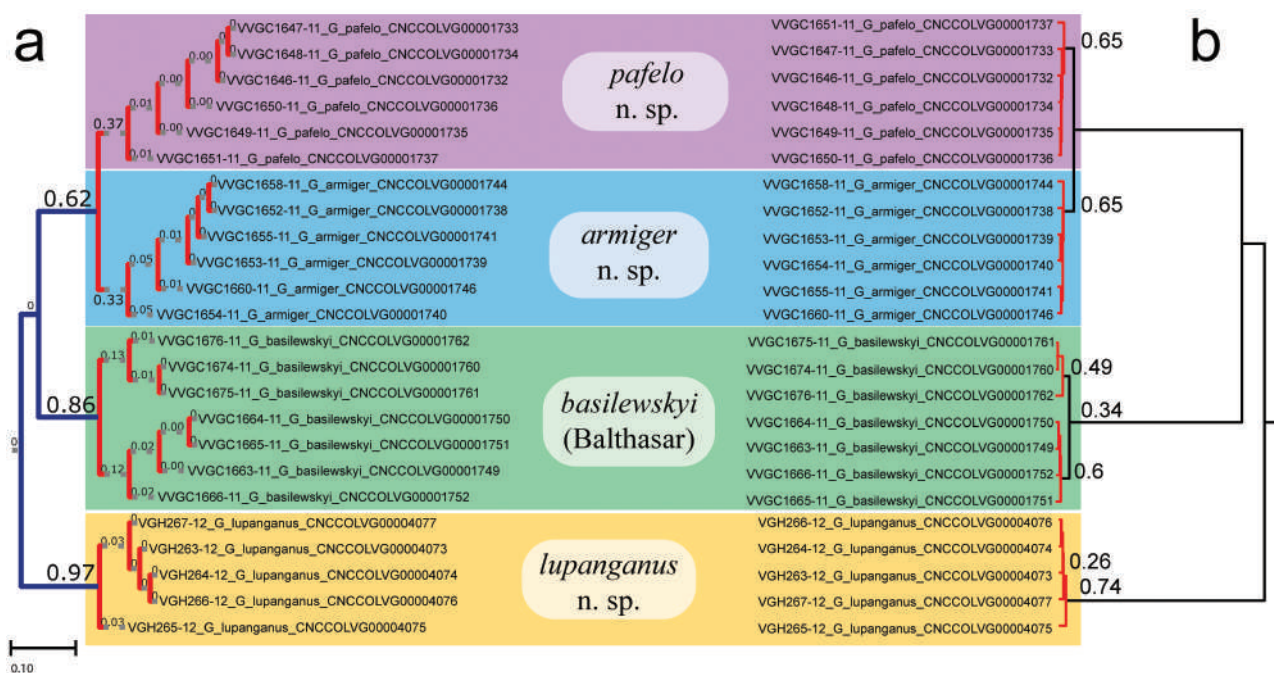
Fig. 4. Results of molecular species delimitation for *Grebennikovius*. a) bPTP recovered 3 species, lumping *G. pafelo* and *G. armiger*; values on nodes are the support for each clade being a distinct species. b) GMYC recovered 5 species and split *G. basilewskyi* into 2 groups; support values higher than 0.25 are plotted on corresponding nodes.

has a ridge on the anterior edge of mesofemora, a broad procoxal cavity in males, and a tooth on the ventral carina of procoxae—the latter being particularly developed in *Grebennikovius*. *Grebennikovius* species are overall more flattened dorsoventrally, which makes the lateral edge of the carina on the eighth interstria particularly evident. Lastly, *Grebennikovius* is endemic of the Uluguru Mountains in Tanzania, while *Epactoides* is found exclusively in Madagascar and in La Réunion island.

Redescription. Body. Oval-shaped, flattened, surface brown to reddish brown, darker ventrally, antennae yellowish. **Length**: 3.0–4.9 mm. **Head.** Strongly tapering anteriorly; clypeus with 2 sharp, upturned triangular teeth in the middle; lateral margin slightly notched in correspondence of genoclypeal sulcus; covered with simple punctures, punctures smaller anteriorly; posteromedial edge of head with small smooth area; antennae with 9 segments. **Pronotum.** Convex on disc, flattened anterolaterally; maximum width on anterior half; lateral edges obtusely angled on anterior half; posterior angles rounded; base broadly rounded; covered with setigerous ocellate punctures; lateral region with smooth, glossy area medially. **Elytra and hind wings.** With 9 striae; punctures of striae large, diameter almost 3 times strial width; interstria 8 carinate, sides below interstria slanted inward, carina almost reaching suture of elytra distally; scutellum concealed in dorsal view. Wings strongly reduced. **Ventral body surface.** Hypomeral depression deep. Procoxal cavities broad, approximately as wide as 3/4 or more of half pronotal width in males, 1/2 of half pronotal width in females. Mesometaventral sulcus rounded medially, straight laterally; meso- and metaventrite covered with simple punctures; metaventrite evenly convex. **Tergite VIII.** Entirely bordered, covered with ocellate setigerous punctures. **Legs.** Protibiae with 3 teeth; middle and hind tibiae expanded distally. Dorsal margin of metafemur carinate. Pro- and meta-legs sexually dimorphic. *Males*: ventral carina of male procoxae toothed medially (Fig. 5c); profemora and metafemora with protrusions; protibiae strongly curved, their apex expanded and bearing a row of short, thick setae, apex expanded below tarsal insertion; metatibiae sinuated, expanded distally. *Females*: legs without protrusions; protibiae shorter and less strongly curved; metatibiae straight, evenly widened distally. **Male genitalia.** Parameres symmetrical, notched proximoventrally, narrowing distally. Ventral membranes of parameres equally sclerotised. Lamella copulatrix elongated. Axial and subaxial endophallites fused together, frontolateral peripheral endophallite absent.

Distribution and ecology. *Grebennikovius* species inhabit the montane rainforests of the Uluguru Mountains in Tanzania. Nothing is known about their life history, reproductive, and feeding behavior. All known specimens were collected from forest litter.

Remarks. Morphologically, *Grebennikovius* species closely resemble some *Epactoides* (*e.g.*, *E. major* (Paulian, 1991) and *E. mangabeensis* Wirta & Montreuil, 2008), making it difficult to justify the separation of the 2 genera on a morphological basis (Rossini et al. 2022). Mlambo et al. (2019) invoked brachyptery and related elytral modifications to support the creation of the genus. However, brachyptery is a very common phenomenon in Coleoptera (Wagner and Liebherr 1992), and it is suggested to have evolved 2 times in mountain-inhabiting *Epactoides* (Wirta and Montreuil 2008). The main argument for *Grebennikovius*' status as a separate genus, in addition to its sister-group relationship with *Epactoides*, is its unique geographical occurrence. The average COI barcode distance between the 2 genera (see Supplementary Material) is 15.77%, which overlaps with both interspecific and intergeneric distances found in other animals (Ward 2009; Park et al. 2011; Magoga et al. 2018).

Grebennikovius armiger n. sp.

Figs. 2a, 5e, h, 6a, b,i,m–o

(urn:lsid:zoobank.org:act:883D3610-BE9D-4818-AE90-7D729A205190)

Type locality: Uluguru Mountains, near Tchenzema village, West of Lukwangule Plateau (Tanzania).

Material examined. Holotype ♂: Tanzania, Uluguru Mts., at Tchenzema vil., 7°06'54"S 37°36'34"E, 2,408 m, sifting, 8.xi.2010, V. Grebennikov *legit* [MZH]. **Paratypes** (8♂♂, 15♀♀): *idem* holotype [1♂♂ & 1♀, MZH; 1♂ & 1♀, BDGC]; Tanzania, Uluguru Mts., at Tchenzema vil., 7°06'44"S 37°36'16"E, 2,258 m, sifting, 13.xi.2010, V. Grebennikov *legit* [3♂♂ & 1♀, MZH; 1♂ & 1♀, JFJC]; Tanzania, Uluguru Mts., at Tchenzema vil., 7°06'50"S 37°36'18"E, 2,318 m, sifting, 11.xi.2010, V. Grebennikov *legit* [1♀, MZH]; Tanzania, Uluguru Mts., at Tchenzema vil., 7°06'48"S 37°36'18"E, 2,301 m, sifting, 19.xi.2010, V. Grebennikov *legit* [2♂♂ & 10♀♀, MZH].

Holotype labeling: "TANZANIA, Uluguru Mts. / at Tchenzema vil., / S07°06'54" E037°36'34", / 8.xi.2010, 2,408 m, / sifting10, V.Grebennikov [printed in black on white cardboard] // CNC-COLVG / 00001738 [printed in black on white cardboard] // *Grebennikovius* / *armiger* n. sp. ♂/ HOLOTYPE [in bold] / Montanaro, Grebennikov, / Rossini & Tarasov, 2023 [printed in black with Arial 4 on red cardboard; ♂symbol handwritten] // http://id.Luomus.fi/ / GAC.37252 / Univ. of Helsinki / LUOMUS, 2023 [printed in black on white cardboard]".

Diagnosis. *Grebennikovius armiger* can be readily identified by the acutely angled and sharp genae (Fig. 5e), which are obtusely angled and rounded in all other species (Fig. 5f). Additionally, males can be identified on the basis of the broad triangular tooth on metafemora (Fig. 6a) (the other species have a small proximal tubercle), the pronotum wider than elytra (Fig. 2a) (narrower than elytra in the other species), the very slender parameres (Fig. 6m and o), and the lamella copulatrix not expanded distally and with a small additional sclerite proximally (Fig. 6n).

Description. Male (holotype). Length: 4.3 mm. **Head.** Genae acutely angled, sharp; surface covered with ocellate setigerous punctures separated by approximately 1 diameter on frons, becoming smaller and sparser anteriorly; posterior edge of vertex with small smooth space medially. **Pronotum.** Wider than elytra, very convex on disc, strongly flattened anterolaterally, steeply declivous posteriorly; posterolateral edges oblique; longitudinally depressed medially from base to almost anterior edge; depression smooth, rest of surface covered with ocellate setigerous punctures separated by 1-2 diameters on disc; with row of large, oval and ocellate punctures posteriorly. **Elytra and hind wings.** Elytral surface depressed along proximal edge of interstriae 5–6; carina of interstria 8 expanded distally. Hind wings extremely reduced. **Ventral body surface.** Punctures of metaventrite separated by 1–2 diameters, becoming smaller posteriorly. **Tergite VIII.** Depressed along anterior margin, with single tubercle on each side. **Legs.** Ventral margin of profemur expanded and tapering at middle. Expansion of protibial apex below tarsal insertion rounded. Ventral margin of metafemur with medial, broad subtriangular tooth. Metatibia strongly bent at middle, abruptly widened in distal third, dorsal edge sinuated on distal half. **Genitalia.** Parameres very
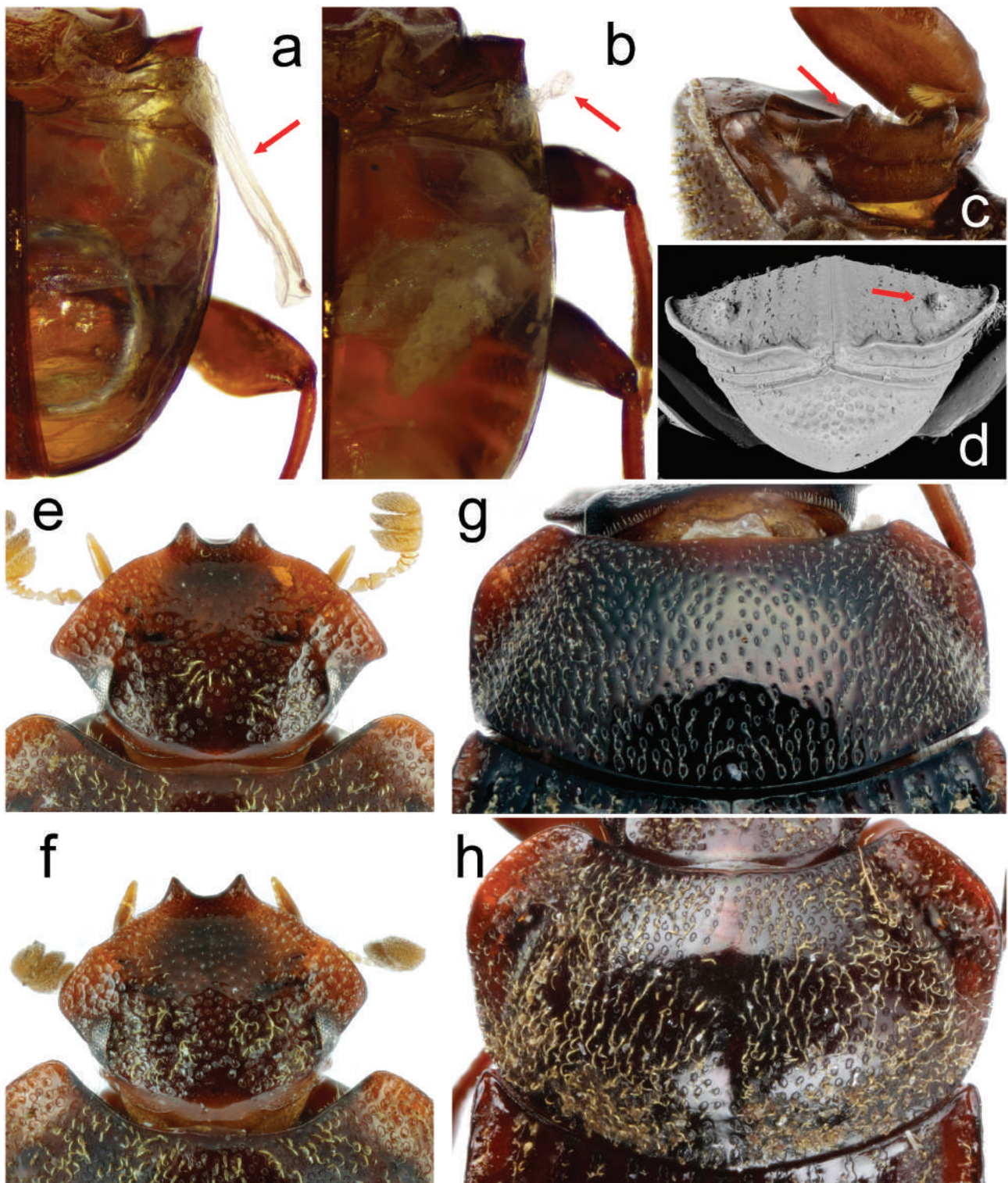
Fig. 5. a–h) Morphological details of *Grebennikovius* species. a) *Grebennikovius basilewskyi* right hind wing (red arrow); b) *G. pafelo* left hind wing (red arrow); c) procoxa of *G. lupanganus*, red arrow pointing at procoxal tooth; d) *G. basilewskyi*, posterior view of male, red arrow pointing at the tubercle of fifth elytral interstria, picture by Max Söderholm (MZH); e) *G. armiger*, dorsal view of male head; f) *G. pafelo*, dorsal view of female head; g) *G. basilewskyi*, dorsal view of male pronotum; h) *G. armiger*, dorsal view of male pronotum.

elongated. Endophallus bearing elongated, almost straight lamella copulatrix associated with small additional sclerification.

Female. Pronotum narrower than elytra. Legs without protrusions, as described in the genus description.

Variation. Body length ranges from 4.1 to 4.9 mm. Variation is limited to body size and development of male secondary sexual characters (i.e., leg protrusions), which are more developed in larger individuals.
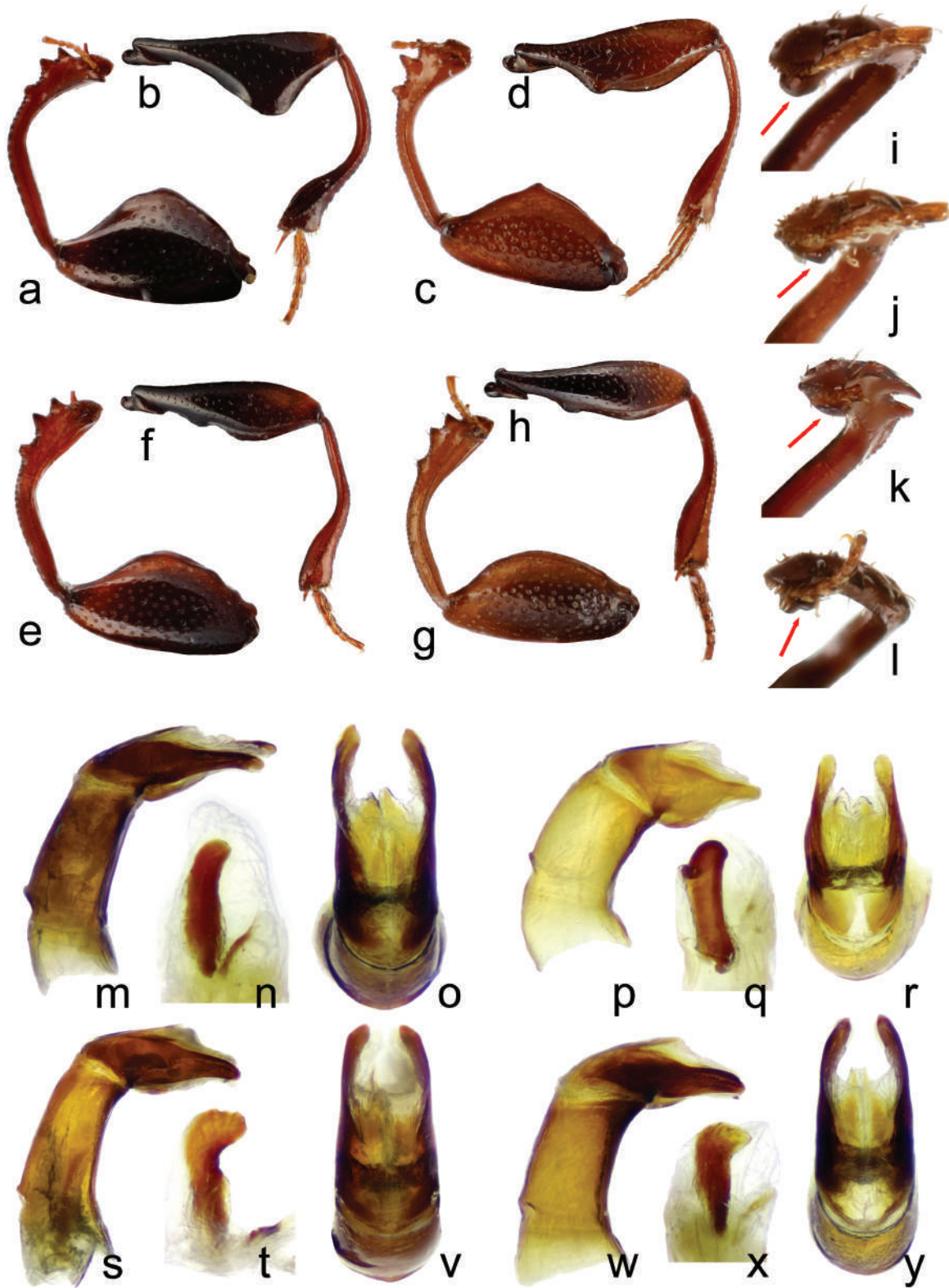
Fig. 6. Morphological details of male *Grebennikovius* spp. For each species, in alphabetical order, are depicted: fore leg (ventral view), hind leg (ventral view), protibial apex (oblique view), aedeagus (lateral view), lamella copulatrix, and parameres (dorsal view). a, b, i, m–o) *G. armiger*; c, d, j, p–r)*G. basilewskyi*; e, f, k, s–v) *G. pafelo*; g, h, l, w–y) *G. lupanganus* .

Distribution. This species was collected in forest litter on the Western slope of the Lukwangule Plateau, near Tchenzema village in southern Ulugurus (Figs. 1 and 3b and c).

Etymology. Latin noun meaning "arms-bearer," referring to the conspicuously modified legs in males and the pointed genae in both sexes. Noun in apposition.

Grebennikovius basilewskyi (Balthasar, 1960)

Figs. 2a, 5a,d,g, 6c–d,j, 6p–r
Phacosoma basilewskyi: Balthasar 1960: 57.
Madaphacosoma basilewskyi: Paulian 1975: 232; Mlambo et al. 2014: 116.
Grebennikovius basilewskyi: Mlambo et al. 2019: 113.

Type locality: Uluguru Mountains, vicinity of Bunduki, Mungula gorge (Tanzania).

Material examined. Holotype ♂: Tanzania, Uluguru Mountains, Bunduki, Mungula gorge, 1,500 m, transition forest, 1–6.v.1957 [RMCA]. **Paratypes (1♂, 1♀)**: Tanzania, Uluguru Mountains, top of Kidunda, 1,800–1,950 m, mountain forest, 3.v.1957 [RMCA]. **Other material (20♂♂, 6♀♀)**: Tanzania, Uluguru Mts. at Bunduki village, S07°01'06" E037°39'45", 1569m, 26.xi.2010, sifting, V. Grebennikov *legit* [15♂♂ & 5♀♀, MZH; 1♂ & 1♀, BDGC]; Tanzania, Uluguru Mts. at Bunduki village, S07°01'17" E037°39'10", 1,592 m, 22.xi.2010, sifting, V. Grebennikov *legit* [3♂♂, MZH; 1♂, PMOC].

Holotype labeling. HOLOTYPUS [printed red label] // Forêt / transition, / dans l'humus [printed light blue label] // COLL. MUS. CONGO / Tanganyika Terr.: Bundu- / ki, Uluguru Mts., 1500 m. / gorge Mungula 1/6-V-1957 [printed white label] // Mission Zoolog. I.R.S.A.C. / en Afrique orientale / (P. Basilewsky et / N. Leleup) [printed white label] // Phacosoma / basilewskyi / ♂n. sp. Balth. / Holotypus [handwritten white label, last line printed] // RMCA ENT / 000015068 [printed white label, with a QR code].

Diagnosis. This species can be readily identified by the tubercle on the fifth interstria (Fig. 5d), absent in the other species. Other unique features are the evenly punctured pronotal disc, longitudinally depressed only on the posterior half (Fig. 5g) (with a longitudinal smooth depression in the other species, Fig. 5h), the sharp tooth on the ventral edge of male profemur (Fig. 6c) (profemoral ventral edge rounded or at most obtusely angular in the other species), the medial bulge in the posterior part of female tergite VIII (absent in the other species), the short parameres (Fig. 6p and r) and the lamella copulatrix with a lateral distal protrusion and without additional proximal sclerites (Fig. 6q).

Redescription. Male (holotype). Length: 3.8 mm. **Head**. Genae obtusely angled; surface covered with ocellate setigerous punctures separated by 1 diameter; posterior edge of vertex with small smooth space medially. **Pronotum**. Less wide than elytra, evenly convex on disc; external edges oblique posteriorly; with longitudinal median groove posteriorly; evenly covered with ocellate punctures, separated by 1-2 diameters on disc, punctuation denser laterally, decreasing in size from base to anterior pronotal edge. **Elytra and hind wings**. Interstriae slightly convex; interstria 7 swollen proximally; interstriae 6 and 7 with yellowish spots proximally; interstria 5 tuberculated distally; sutural interstria raised in distal half; carina of interstria 8 expanded distally. Wings approximately as long as half of elytral length (Fig. 5a). **Ventral body surface**. Punctures of mesoventrite separated by 1–2 puncture diameters, distinctly smaller medially. **Tergite VIII**. Depressed anteriorly, strongly transversely swollen medially. **Legs**. Ventral margin of profemur with sharp tooth medially. Expansion of protibial apex below tarsal insertion pointed. Ventral margin of metafemur with bulging on proximal 1/3, femur enlarged distally to the bulging. Metatibia widening distally, dorsal edge straight on distal half. **Genitalia**. Phallobase strongly curved proximally; parameres relatively short, enlarged proximoventrally, distal end narrow. Lamella copulatrix elongated, rounded distally, with small and sharp projection near the distal apex.

Female. Tergite VIII depressed along anterior margin, with a median bulge present slightly before the posterior margin. Legs without protrusions, as described in the genus description.

Variation. Body length ranges from 3.0 to 4.0 mm. Secondary sexual characters of legs are attenuated in smaller males. The proximal yellowish spots on interstriae 6 and 7 can be more or less visible.

Distribution. The species was collected in forest litter close to Bunduki village in central Ulugurus (Fig. 3b and c). The holotype and the paratypes were collected in the "gorge Mungula" and on the "top of Kidunda," respectively. The localities could not be georeferenced, but according to Basilewsky and Leleup (1960), both of them are in the vicinity of Bunduki.

Etymology. The species was dedicated by Balthasar (1960) to Pierre Basilewsky (1913–1993), Russian (in exile) and Belgian entomologist who directed an expedition in East Africa in 1957, during which the species was collected (source: Wikispecies 2022).

Grebennikovius lupanganus n. sp.

Figs. 2d, 5c, 6g–h,l,w–y
(urn:lsid:zoobank.org:act:1D32A7F2-0376-436B-976F-54C2EEEC430C)

Type locality: Uluguru Mountains, Lupanga Peak (Tanzania).

Material examined. Holotype ♂: Tanzania, Uluguru Mts., Lupanga Peak, 6°51'54"S 37°42'28"E, 1,921 m, sifting, 10.i.2012, V. Grebennikov *legit* [MZH]. **Paratype (♀)**: *idem* holotype [MZH].

Holotype labeling: "TANZANIA, Uluguru / Mts., Lupanga Peak / 6°51'54"S 37°42'28"E. / 10.i.2012, 1,921 m, / sift.27, V.Grebennikov [printed in black on white cardboard] // CNC-COLVG / 00004077 [printed in black on white cardboard] // *Grebennikovius* / *lupanganus* n. sp. ♂/ HOLOTYPE [in bold] / Montanaro, Grebennikov, / Rossini & Tarasov, 2023 [printed in black with Arial 4 on red cardboard; ♂symbol handwritten] // http://id.Luomus.fi/ / GAC.37250 / Univ. of Helsinki / LUOMUS, 2023 [printed in black on white cardboard]".

Diagnosis. This species is distinguishable from others by the combination of the following characteristics: fifth elytral interstria without tubercles, genae with obtuse angles and pronotum with oblique posterolateral margins. In males, the notched expansion below the protibial tarsal insertion (Fig. 6l) sets it apart from any other species.

Description. Male (holotype). Length: 4.0 mm. **Head**. Genae obtusely angled; covered with ocellate setigerous punctures separated by approximately 1 diameter; smooth area in the middle of vertex almost null. **Pronotum**. Less wide than elytra, convex on disc, strongly flattened anterolaterally; posterolateral edges oblique; longitudinally depressed in the middle from posterior region to anterior edge; depression smooth, rest of the surface covered with ocellate setigerous punctures separated by approximately 1–2 diameters on disc. **Elytra and hind wings**. Elytral surface depressed proximally. Hind wings extremely reduced. **Ventral body surface**. Punctures of metaventrite separated by approximately 1 own diameter, becoming smaller posteriorly. **Tergite VIII**. Convex, flattened posteriorly. **Legs**. Ventral margin of profemur slightly expanded medially. Expansion of protibial apex below tarsal insertion notched. Ventral margin of metafemur with a small bulging on proximal third. Metatibia widened in distal half. **Genitalia**. Parameres elongated; lateral edge of parameres slightly obtusely angled before apex in dorsal view. LC elongated, apex enlarged and bent, its tip rounded, associated with a small additional sclerification.

Female. Similar to male, except for the secondary sexual characters mentioned in the genus description.

Variation. Body length ranges from 4.0 to 4.7 mm. Due to the limited number of examined specimens, it is challenging to make any other statement regarding variation.

Distribution. The species was collected in forest litter on Lupanga Peak in northern Ulugurus (Fig. 3b and c).

Etymology. Latin adjective referring to the type locality, the Lupanga Peak.

Remarks. Three additional specimens (CNCCOLVG00004073, CNCCOLVG00004074, CNCCOLVG00004075) were collected at Lupanga Peak. They were utilized in the present molecular analyses and possibly referenced in the study by Mlambo et al. (2014). However, their morphology was not examined in this study as the specimens could not be located anymore after DNA extraction.

Grebennikovius pafelo n. sp.

Figs. 2c, 5b and f, 6e and f, k, s–v

(urn:lsid:zoobank.org:act:6AA504F4-91BB-4EF9-AF8F-31EDA06AD5F9)

Type locality: Uluguru Mountains, near Tchenzema village, East of Lukwangule Plateau (Tanzania).

Material examined. Holotype ♂: Tanzania, Uluguru Mts., at Tchenzema vil., 7°07'19"S 37°37'16"E, 2,429 m, sifting, 7.xi.2010, V. Grebennikov *legit* [MZH]. **Paratypes** (4♀♀): *idem* holotype [2♀♀, MZH]; Tanzania, east slope of southern Uluguru Mts., 7°07'25"S 37°37'59"E, 2,220 m, sifting, 7.xi.2010, V. Grebennikov *legit* [2♀♀, MZH].

Holotype labeling: "TANZANIA, Uluguru Mts., / at Tchenzema vil. / S07°07'19" E037°37'16", / 7.xi.2010, 2,429 m, / sifting09. V.Grebennikov [printed in black on white cardboard] // CNCCOLVG / 00001735 [printed in black on white cardboard] // Madaphacosoma / basilewskyi (Balth) / det. B.Gill 2011 [white cardboard, first 2 lines and date handwritten, the rest printed in black] // Grebennikovius / basilewskyi (Balth.) / Det. B.D.Gill 2020 [printed in black on white cardboard] // *Grebennikovius* / *Grebennikovius* / *pafelo* n. sp. ♂/ HOLOTYPE [in bold] / Montanaro, Grebennikov, / Rossini & Tarasov, 2023 [printed in black with Arial 4 on red cardboard; ♂symbol handwritten] // http://id.Luomus.fi / GAC.37245 / Univ. of Helsinki / LUOMUS, 2023 [printed in black on white cardboard]".

Diagnosis. This species can be distinguished from *G. armiger* and *G. lupanganus* by its posterolateral pronotal margins, which are parallel rather than oblique. It can be distinguished from *G. basilewskyi* for the absence of tubercles on the fifth interstria. Additionally, in males, protibiae of *G. pafelo* differ from those of the morphologically similar *G. lupanganus* due to the expansion below the protibial tarsal insertion (Fig. 6k), which is evenly rounded instead of being notched.

Description. **Male (holotype)**. **Length**: 4.9 mm. **Head**. Genae obtusely angled; covered with ocellate setigerous punctures separated by approximately 1 diameter; posterior edge of vertex with small smooth space medially. **Pronotum**. Less wide than elytra, very convex on disc, strongly flattened anterolaterally, steeply declivous posteriorly; posterolateral edges parallel; longitudinally depressed medially from posterior region to almost anterior edge, depression smooth; rest of surface covered with ocellate setigerous punctures separated by 1–2 diameters on disc; posterior edge with row of large, ovalar ocellate punctures. **Elytra and hind wings**. Elytral surface depressed along proximal edge of interstriae 4–6; elytral surface strongly depressed along medial side of carina of interstria 8. Hind wings extremely reduced (Fig. 5b). **Ventral body surface**. Punctures of metaventrite separated by approximately 1 diameter, becoming smaller posteriorly. **Tergite VIII**. Evenly convex. **Legs**. Ventral margin of profemur expanded medially. Expansion of protibial apex below tarsal insertion rounded. Ventral margin of metafemur with bulging on proximal third, femur notched and then enlarged distally to the bulging. Metatibia bent medially, widened in distal third, dorsal edge slightly sinuous on distal third. **Genitalia**. Parameres elongated; lateral edge of parameres evenly rounded in dorsal view. Lamella copulatrix elongated, apex enlarged and bent, its tip angular, associated with a small additional sclerification.

Female. Tergite VIII transversely swollen on anterior third. Legs without protrusions, as described in the genus description.

Variation. Body length ranges from 4.0 to 4.9 mm. No other significant variation was observed.

Distribution. The species was collected into forest litter on the East side of Lukwangule Plateau, near Tchenzema village in southern Ulugurus (Figs. 1 and 3b–c).

Etymology. Combination of the first syllable from the first names of 3 friends of GM: Paolo Majorano (wild naturalist), Federica Losacco (colleague at MZH), and Lorenza Cutrone (passionate biotechnologist). GM wants to heartfeltly thank his reckless companions with whom he spent part of his life in Helsinki—and beyond. Noun in apposition.

Remarks. An additional specimen was collected (CNCCOLVG00001732) and used for molecular analyses, but could not be located after DNA extraction.

Identification key to *Grebennikovius* species

1. Elytral interstria 5 tuberculated distally (Fig. 5d). Pronotum longitudinally depressed only on the posterior region, evenly covered with punctures ((Fig. 5g). Ventral margin of male profemora with a sharp tooth (Fig. 6c). Female tergite VIII with a ridge-like bulging close to the posterior margin, slightly swollen at anterior angles, the rest of the surface flat. Male genitalia as in Fig. 6p–r . . . . *G. basilewskyi* (Balthasar, 1960)

– Elytral interstria 5 not tuberculated. Pronotum longitudinally depressed and smooth medially (Fig. 5h). Ventral margin of male profemora without sharp protrusions. Female tergite VIII without bulging posteriorly 2

2. Genae acutely angled (Fig. 5e). Male pronotum wider than elytra. Male metafemurs with a broad triangular tooth on the ventral margin (Fig. 6a). Female tergite VIII transversely swollen, with a longitudinal depression medially. Male genitalia as in Fig. 6m–o . . . . *G. armiger* n. sp.

– Genae obtusely angled (Fig. 5f). Male pronotum narrower than elytra. Male metafemurs without broad triangular tooth, at most with a small bulging proximally. Female tergite VIII transversely swollen on anterior half, only slightly depressed longitudinally 3

3. Posterolateral margins of pronotum parallel. Expansion of male protibial apex below tarsal insertion rounded (Fig. 6k). Phallobase slender (Fig. 6s); parameres more abruptly curved inwards distally in dorsal view (Fig. 6v) . . . . *G. pafelo* n. sp.

– Posterolateral margins of pronotum oblique. Expansion of male protibial apex below tarsal insertion notched (Fig. 6l). Phallobase thicker (Fig. 6w); parameres evenly and slightly curved inwards distally in dorsal view (Fig. 6y) . . . . *G. lupanganus* n. sp.

Discussion

Microallopatric Speciation in *Grebennikovius*

Integration of data from different sources of evidence is very important to draw solid conclusions about species boundaries and to avoid unwarranted taxonomic acts (Luo et al. 2018; Hillis et al. 2021; Ranasinghe et al. 2022). Overall, our results show that phenotypic and molecular data are consistent in recovering 4 distinct species of *Grebennikovius*.

The morphological examination confidently identified 4 clusters of specimens, which correspond to 4 clades in the molecular phylogenetic trees. However, there are substantial differences in age estimations depending on the calibration approach used, resulting in wide credible intervals (CIs) (Fig. 3a). It is challenging to determine which calibration method is the most reliable, and the inclusion of additional genetic loci would be necessary to enhance estimation accuracy.

MSD methods yielded varying but overall consistent results. In the case of *G. basilewskyi*, GMYC split the 2 sampled geographically separated populations. Although biases of GMYC towards oversplitting were documented (Talavera et al. 2013; Dellicour and Flot 2018), a significant divergence between individuals of the 2 populations seem to really occur, as indicated by a high F_{ST} value (0.66). However, no significant morphological differences were observed between the 2 groups. This suggests that the populations of *G. basilewskyi* are, indeed, well structured based on COI barcodes, but are likely conspecific. On the other hand, bPTP and ABGD group together *G. armiger* and *G. pafelo*. This grouping can be likely attributed to their recent divergence, as indicated by a low barcode distance—1.44% compared to 7.84–9.34% in other species pairs. In beetles, the barcode distance is usually significantly larger than 2% (Astrin et al. 2012; Magoga et al. 2018; Huang et al. 2020; Zhang and Bu 2022; Schütte et al. 2023). However, it is important to highlight that, despite their minimal genetic divergence, *G. armiger* and *G. pafelo* exhibit significant morphological differences, leaving little room for doubt that they represent 2 distinct species. Notably, these differences are not restricted to rapidly evolving sexual traits—typically, male genitalia (Hosken and Stockley 2004) and male cuticular protrusions (Emlen et al. 2005)—but occur also in nonsexually dimorphic traits such as the shape of genae.

Each of the 4 species of *Grebennikovius* seems to be exclusive of a different part of the Uluguru Mountains (Fig. 3b), with geographical proximity reflecting genetic relatedness. Based on available data, it appears that these species are allopatric. However, since sampling is geographically limited, the possibility of parapatry or even sympatry cannot be excluded. For example, because of their geographic proximity, the sister species *G. armiger* and *G. pafelo*, separated by the Lukwangule Plateau, might turn out to be nonallopatric.

Considering that no *Grebennikovius* species is known outside of the Uluguru Mountains, it is plausible to assume that speciation occurred locally within these mountains. The divergence of 4 species in such a confined forest area is remarkably surprising. To our knowledge, such an exceptional case of microallopatric speciation (*i.e.*, allopatry on a very small geographical scale; Fitzpatrick et al. (2008)) was never documented before for scarabaeine dung beetles. It is also an uncommon phenomenon in the fauna of the EAMs as a whole since endemic EAM clades are typically represented by one species per mountain block (Burgess et al. 2007; Lovett and Wasser 2008; Grebennikov 2017).

Alternative Interpretations

Despite being consistent with the 4-species interpretation advocated herein, our results may be explained differently. Specifically, an alternative scenario could invoke the Sisyphean evolution (McKay and Zink 2015) or the ring species phenomenon (Kuchta and Wake 2016). The latter is particularly applicable to the *Grebennikovius* populations encircling the unsuitable Lukwangule Plateau. Remarkably, a similarly structured sampling and analysis of another forest-dwelling Uluguru clade with low-dispersal ability, the weevil *Typoderus admetus* Grebennikov, 2019 (Grebennikov 2019), failed to detect a correlation between morphology, DNA, and geography. As a result, this led to a single-species interpretation. May the herein documented morphological and genetic divergence of *Grebennikovius* interpreted as 4 distinct species simply be a consequence of geographically limited sampling? If so, do we follow in our ignorance of the 3 blind men who misinterpreted the elephant (a parable for a single polymorphic species) as a snake, a tree trunk, and a wall (multiple discrete species)? Only time will tell if this is the case within *Grebennikovius*.

Uluguru Forest Fragmentation, Ecological Specialization, and Flightlessness as Putative Drivers of Speciation

The exceptional microallopatric speciation observed in *Grebennikovius* can be attributed to an interplay of several factors. A major role was probably played by past forest fragmentations, a phenomenon that widely influenced the diversity of specialized taxa within Afrotropical forests (Davis et al. 2001; Couvreur et al. 2021; Daniel et al. 2021). Among dung beetles, a similar pattern of isolation in separate rainforest islands contributed to the radiation of the flightless genus *Temnoplectron* in Australia (Bell et al. 2004, 2007). In the EAMs, there is substantial phylogeographic evidence indicating that various taxa diverged allopatrically on different mountain blocks following forest breakups. Many of these diversification events occurred relatively recently during the Pliocene–Pleistocene climatic cycles (Dimitrov et al. 2012; Ceccarelli et al. 2014; Bryja et al. 2014; Hemp et al. 2015; Grebennikov 2017). However, strong within-block population differentiation was seldom hypothesized (Menegon et al. 2004, 2009; Müller et al. 2005) and evidence for it is very scarce (Measey and Tolley 2011).

It is reasonable to propose that forest isolation played a crucial role in the microallopatric diversification of *Grebennikovius*. However, due to uncertainties in age estimates and limited knowledge about the fine-scale past climatic and vegetational characteristics of the Uluguru Mountains, only speculative hypotheses can be put forward. Nonetheless, these hypotheses can still provide a plausible scenario.

In the first place, according to our reconstructions, the split between *Grebennikovius* and the Malagasy *Epactoides* occurred between 17 Mya (tree P1, CI = 26.9–8.6 Mya) and 32 Mya (tree P2, CI = 35.3–28.8 Mya), the latter being closer to the age estimated by Rossini et al. (2022). Credible intervals of different estimations partially overlap, suggesting that the real age of the divergence is likely comprised between those dates. Notwithstanding this uncertainty, the split between the 2 genera appears relatively old. Surprisingly, genetic differentiation does not seem to be paralleled by significant morphological changes. Morphology is in fact rather conserved between the 2 genera, the main difference being represented by brachyptery in *Grebennikovius* (see also Taxonomy section). This character was gained secondarily by the genus, similarly to what happened to some species of *Epactoides* (Wirta and Montreuil 2008) and *Ochicanthon* (Latha et al. 2011). Flightlessness probably represents an adaptation to living in isolated mountain forest habitats, as already reported for several dung beetle taxa (Kohlmann et al. 2019).

The estimated splits between *G. lupanganus* and the other species occurred at 14.9-8.0 Mya (CI$_{P2}$= 20.1 – 9.8 Mya; CI$_{P1}$= 12.5 – 3.9

Mya), while the split between *G. basilewskyi* and *G. pafelo* occurred at 13.2–7.1 Mya (CI$_{P2}$=18.9–8.3 Mya; CI$_{P1}$=11.4–3.4 Mya). These time periods roughly align with the repeated climatic oscillations that took place between the late Miocene and mid-Pleistocene (11–1.5 Mya) (Couvreur et al. 2021). The Ulugurus are divided into 2 sub-blocks, Uluguru North and Uluguru South, separated by the lower altitude Bunduki Gap (Fjeldså and Hansen 1995). Forest connection between them is currently faint and was historically exposed to interruptions (Nyenza et al. 2013). *Grebennikovius lupanganus* is found in the northern sub-block (Lupanga Peak), while the other species are located in the southern one. Thus, it is possible that the reciprocal isolation of Uluguru North and South, which was reinforced during past forest shrinkage, contributed to the early divergence of *G. lupanganus*.

Another interesting case is the divergence time between *G. armiger* and its sister *G. pafelo* (1.06–0.58 Mya; CI$_{P2}$=1.86–0.37 Mya; CI$_{P1}$ = 0.94–0.29 Mya), as well as between the 2 populations of *G. basilewskyi* (0.88–0.48 Mya; CI$_{P2}$ = 1.57–0.28 Mya; CI$_{P1}$ = 0.77–0.24 Mya). Ages are similar, with broadly overlapping credible intervals. These dates overlap with the mid-Pleistocene transition (1.2–0.7 Mya), a period characterized by changes in the pace of climatic fluctuations and the onset of glacial cycles with alternating warm and dry periods (0.9–0.6 Mya) (Dupont et al. 2001). Particularly, fluctuations in African rainforests were stronger shortly after 1.05 Mya. During this time, reduced forest connectivity at the small scale of Uluguru South may have been sufficient to drive significant population divergence, as observed in the case of *Kinyongia* chameleons in the Taita Hills (Measey and Tolley 2011).

Moreover, there exists a current barrier between *G. armiger* and *G. pafelo*, namely the Lukwangule Plateau (Figs. 1a and 3c). This grassland zone acts as a separation between the western and eastern forest slopes in southern Ulugurus, and is unsuitable for *Grebennikovius*. Even if the plateau is only 2–4 km wide and the forest remains continuous to the north and south of it, this area likely was (Finch et al. 2009) and continues to be an effective barrier to gene flow. Additionally, the 2 opposing slopes delimiting the Lukwangule Plateau present slightly different climatic conditions, with the western side being drier and the eastern side being moister (Fjeldså and Hansen 1995). Consequently, the separation between *G. armiger* and its sister *G. pafelo* could be the result of ecological niche differentiation due to adaptations to distinct microclimates.

An additional intriguing observation is that in all *Grebennikovius* species, COI sequences coalesce relatively recently, mostly within a time frame of less than 1 My. This suggests the possibility that *Grebennikovius* populations experienced significant bottlenecks during the mid and late Pleistocene, likely due to forest contraction during drier periods. This scenario requires further exploration in a comprehensive phylogeographic framework.

Lastly, the limited mobility of *Grebennikovius* species is probably an additional factor reinforcing the speciation process. These species are flightless and primarily inhabit forest litter, which significantly restricts their dispersal ability. Reduced mobility may have promoted population segregation and divergence in different microhabitats within the Ulugurus, a phenomenon commonly observed in insects (Fattorini 2007; Voje et al. 2009; Bray and Bocak 2016; Pérez-Delgado et al. 2022).

Conclusions

In this study, we examined the species diversity of *Grebennikovius* in the Uluguru Mountains. We used both morphological and molecular data to separate species. We recovered 4 distinct species, of which 3 are new to science, occurring in different parts of the block. This finding is unique, as the presence of several species with microallopatric distribution in such a small forest area has never been documented before in scarabaeine dung beetles. Moreover, microallopatry is uncommon for the EAMs in general. According to our evolutionary scenario, the stem lineage of *Grebennikovius* diverged from its closest relative *Epactoides* at a considerable time ago about 32–17 Mya. Since *Grebennikovius* species are highly specialized for rainforests, which provide a relatively stable environment, their morphology did not undergo substantial change over time, despite the considerable divergence period. Furthermore, environmental stability favored the loss of wings, resulting in further reduced dispersal ability. The combination of low mobility, preferences for specific microhabitats, and climatic fluctuations impacting rainforest connectivity during the Miocene and the mid-Pleistocene has likely promoted the unique case of microallopatric speciation observed within this genus. These findings provide further evidence of the Pleistocene as a significant period for species diversification in African rainforest taxa. Once again, the EAMs demonstrate their role as a natural laboratory for studying extreme and complex evolutionary phenomena.

Furthermore, we cannot rule out that additional species of *Grebennikovius* might be found in the Ulugurus, particularly in unexplored areas such as the Kimandu Hill peak (Fig. 3c). This study is based on samples collected using leaf litter sifting, a challenging yet effective method for capturing a wide range of litter-dwelling arthropods, including *Grebennikovius*. By employing this technique in focused sampling efforts, other rare EAM species may be found. Additionally, dung beetle researchers could potentially benefit from using this method in other tropical areas. Other techniques specifically tailored for dung beetles, such as dung-, mushroom-, and carrion-baited pitfall traps, could also serve as efficient means for collecting *Grebennikovius* and providing insights into their feeding preferences.

Lastly, our findings underscore the significance of preserving microenvironments. In regions like the EAMs, endemic organisms may be confined to single peaks or specific areas within forests. Consequently, the destruction of even a small patch of forest can result in the extinction of entire species. This highlights the critical importance of conservation efforts in safeguarding these delicate and unique ecosystems.

Specimen Collection Statement

The authors attest that all legal and regulatory requirements, including export and import collection permits, have been followed for the collection of specimens from source populations at any international, national, regional, or other geographic level for all relevant field specimens collected as part of this study.

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Supplementary Material

Supplementary material is available at *Insect Systematics and Diversity* online.

Author Contributions

Giulio Montanaro (Conceptualization [Equal], Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Methodology [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), Vasily V. Grebennikov (Conceptualization [Equal], Methodology [Equal], Resources [Equal], Writing—review & editing [Equal]), Michele Rossini (Conceptualization [Equal], Methodology [Equal], Supervision [Equal], Writing—review & editing [Equal]), Alessandro Grappputo (Methodology [Equal], Supervision [Equal], Writing—review & editing [Equal]), Enrico Ruzzier (Supervision [Equal], Writing—review & editing [Equal]), Sergei Tarasov (Conceptualization [Equal], Funding acquisition [Equal], Methodology [Equal], Supervision [Equal], Writing—review & editing [Equal])

References

Andújar C, Serrano J, Gómez-Zurita J. Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evol Biol.* 2012:12(1):1–17. https://doi.org/10.1186/1471-2148-12-40

Astrin JJ, Stüben PE, Misof B, Wägele JW, Gimnich F, Raupach MJ, Ahrens, D. Exploring diversity in cryptorhynchine weevils (Coleoptera) using distance-, character- and tree-based species delineation. *Mol Phylogenet Evol.* 2012:63(1):1–14. https://doi.org/10.1016/j.ympev.2011.11.018

Balthasar, V. Mission zoologique de l'irsac en afrique orientale (P. basilewsky et N. Leleup, 1957). résultats scientifiques. deuxième partie. 41. Coleoptera scarabaeidae: Scarabaeini, coprini, onitini et oniticellini. (101. beitrag zur kenntnis der scarabaeiden). *Annales du Musée Royal du Congo Belge, Série in-8°, Sciences Zoologiques.* Vol. 88. Tervuren (Belgium): Musée Royal du Congo Belge; 1960. p. 55–66.

Basilewsky P, Leleup N. I. Enumération et description des stations prospectées 11-26. In Unknown, editor. Mission zoologique de l'I.R.S.A.C. en Afrique Orientale (P. Basilewsky & N. Leleup, 1957). Résultats Scientifiques. Première Partie. Vol 81 of *Annales du Musée Royal du Congo Belge, Série in-8°, Sciences Zoologiques.* Tervuren (Belgium): Musée Royal du Congo Belge; 1960. p. 1–473.

Bell K, Yeates D, Moritz C, Monteith G. Molecular phylogeny and biogeography of the dung beetle genus *Temnoplectron* Westwood (Scarabaeidae: Scarabaeinae) from Australia's wet tropics. *Mol Phylogenet Evol.* 2004:31(2):741–753. https://doi.org/10.1016/j.ympev.2003.09.010

Bell KL, Moritz C, Moussalli A, Yeates DK. Comparative phylogeography and speciation of dung beetles from the Australian wet tropics rainforest. *Mol Ecol.* 2007:16(23):4984–4998. https://doi.org/10.1111/j.1365-294X.2007.03533.x

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, et al. Beast 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput Biol.* 2019:15(4):e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bray TC, Bocak L. Slowly dispersing neotenic beetles can speciate on a penny coin and generate space-limited diversity in the tropical mountains. *Sci Rep.* 2016:6(1):33579. https://doi.org/10.1038/srep33579

Bryja J, Mikula O, Patzenhauerová H, Oguge NO, Šumbera R, Verheyen E. The role of dispersal and vicariance in the Pleistocene history of an East African mountain rodent, *Praomys delectorum*. *J Biogeogr.* 2014:41(1):196–208. https://doi.org/10.1111/jbi.12195

Burgess N, Butynski TM, Cordeiro NJ, Doggart N, Fjeldså J, Howell K, Kilahama FB, Loader S, Lovett JC, Mbilinyi B, et al. The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biol Conserv.* 2007:134(2):209–231. https://doi.org/10.1016/j.biocon.2006.08.015

Burgess N, Doggart N, Lovett J C. (2002). The Uluguru mountains of eastern Tanzania: the effect of forest loss on biodiversity. *Oryx.* 2007:36(2):140–152. https://doi.org/10.1017/S0030605302000212

Ceccarelli, F. S., Menegon, M., Tolley, K. A., Tilbury, C. R., Gower, D. J., Laserna, M. H., Kasahun, R., Rodriguez-Prieto, A., Hagmann, R., and Loader, S. P. (2014). Evolutionary relationships, species delimitation and biogeography of Eastern Afromontane horned chameleons (Chamaeleonidae: Trioceros). *Mol Phylogenet Evol.* 2014:80:125–136. https://doi.org/10.1016/j.ympev.2014.07.023

Cordeiro NJ, Seddon N, Capper DR, Ekstrom JM, Howell KM, Isherwood IS, Msuya CA, Mushi JT, Perkin AW, Pople RG, et al. Notes on the ecology and status of some forest mammals in four Eastern Arc Mountains, Tanzania. *J East Afr Nat Hist.* 2005:94(1):175–189. https://doi.org/10.2982/0012-8317(2005)94[175:NOTEAS]2.0.CO;2

Couvreur TL, Dauby G, Blach-Overgaard A, Deblauwe V, Dessein S, Droissart V, Hardy OJ, Harris DJ, Janssens SB, Ley AC, et al. Tectonics, climate and the diversification of the tropical African terrestrial flora and fauna. *Biol Rev.* 2021:96(1):16–51. https://doi.org/10.1111/brv.12644

Cristovao JP, Vaz-de Mello FZ. The terminalia of the superfamily Scarabaeoidea (Coleoptera): specific glossary, dissecting methodology, techniques and previously unrecorded sexual dimorphism in some difficult groups. *Zool J Linn Soc.* 2021:191(4):1001–1043. http://dx.doi.org/10.1093/zoolinnean/zlaa079

Daniel GM, Sole CL, Scholtz CH, Davis AL. Historical diversification and biogeography of the endemic southern African dung beetle genus, *Epirinus* (Scarabaeidae: Scarabaeinae). *Biol J Linn Soc.* 2021:133(3):751–765. https://doi.org/10.1093/biolinnean/blab051

Daniel GM, Strümpher WP, Josso J-F. Dung beetle fauna from Mount Mabu, Mozambique. Part 1: a new species of *Onthophagus* Latreille, 1802, and a checklist of species belonging to group 3 (d'orbigny 1913) (Coleoptera: Scarabaeidae: Scarabaeinae). *Zootaxa.* 2023:5258(4):429–442. https://doi.org/10.11646/zootaxa.5258.4.4

Darriba D, Taboada GL, Doallo R, Posada D. jmodeltest 2: more models, new heuristics and parallel computing. *Nat Methods.* 2012:9(8):772–772. https://doi.org/10.1038%2Fnmeth.2109

Davis AL, Scholtz CH, Harrison JDG. Cladistic, phenetic and biogeographical analysis of the flightless dung beetle genus, Gyronotus van Lansberge (Scarabaeidae: Scarabaeinae), in threatened eastern afrotropical forests. *J Nat Hist.* 2001:35(11):1607–1625. https://doi.org/10.1080/002229301317092351

Davis ALV, Frolov AV, Scholtz CH. *The African dung beetle genera.* Pretoria (South Africa): Protea Book House; 2008. p. 272. http://dx.doi.org/10.1649/0010-065X-64.4.394

De Queiroz K. Species concepts and species delimitation. *Syst Biol.* 2007:56(6):879–886. https://doi.org/10.1080/10635150701701083

Dellicour S, Flot J-F. The hitchhiker's guide to single-locus species delimitation. *Mol Ecol Res.* 2018:18(6):1234–1246. https://doi.org/10.1111/1755-0998.12908

deMenocal PB. African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth Planet Sci Lett.* 2004:220(1–2):3–24. https://doi.org/10.1016/S0012-821X(04)00003-2

DeSalle R, Goldstein P. Review and interpretation of trends in DNA barcoding. *Front Ecol Evol.* 2019:7:302. https://doi.org/10.3389/fevo.2019.00302

Dimitrov D, Nogues-Bravo D, Scharff N. Why do tropical mountains support exceptionally high biodiversity? The Eastern Arc Mountains and the drivers of *Saintpaulia* diversity. *PloS One*. 2012:7(11):e48908. https://doi.org/10.1371/journal.pone.0048908

Diniz-Filho JAF, Soares TN, Lima JS, Dobrovolski R, Landeiro VL, Telles MPdC, Rangel TF, Bini LM. Mantel test in population genetics. *Genet Mol Biol*. 2013:36:475–485. https://doi.org/10.1590/S1415-47572013000400002

Doggart N, Perkin A, Kiure J, Fjeldså J, Poynton J, Burgess N. Changing places: how the results of new field work in the Rubeho Mountains influence conservation priorities in the Eastern Arc Mountains of Tanzania. *Afr J Ecol*. 2006:44(2):134–144. https://doi.org/10.1111/j.1365-2028.2006.00572.x

Drummond AJ, Rambaut A. Beast: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol*. 2007:7(1):1–8. https://doi.org/10.1111/j.1365-294X.2007.03533.x

Dupont LM, Donner B, Schneider R, Wefer G. Mid-Pleistocene environmental change in tropical Africa began as early as 1.05 ma. *Geology*. 2001:29(3):195–198. https://doi.org/10.1130/0091-7613(2001)029%3C0195:MPECIT%3E2.0.CO;2

Emlen DJ, Marangelo J, Ball B, Cunningham CW. Diversity in the weapons of sexual selection: horn evolution in the beetle genus Onthophagus (Coleoptera: Scarabaeidae). *Evolution*. 2005:59(5):1060–1084. https://doi.org/10.1111/j.0014-3820.2005.tb01044.x

Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res*. 2010:10(3):564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fattorini S. Historical relationships of African mountains based on cladistic analysis of distributions and endemism of flightless insects. *Afr Entomol*. 2007:15(2):340–355. https://doi.org/10.4001/1021-3589-15.2.340

Finch J, Leng MJ, Marchant R. Late quaternary vegetation dynamics in a biodiversity hotspot, the Uluguru mountains of Tanzania. *Quat Res*. 2009:72(1):111–122. https://doi.org/10.1016/j.yqres.2009.02.005

Fitzpatrick B, Fordyce J, Gavrilets S. What, if anything, is sympatric speciation? *J Evol Biol*. 2008:21(6):1452–1459. https://doi.org/10.1111/j.1420-9101.2008.01611.x

Fjeldså J, Lovett JC. Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiver Conserv*. 1997:6(3):325–346. https://doi.org/10.1023/A:1018356506390

Fjeldså JSJ, Hansen L. Section 1. Executive summary. In: Svendsen J, Hansen L, editors. *Report on The Uluguru Biodiversity Survey 1993. Part A*. Bedfordshire (UK): The Royal Society for the Protection of Birds, International Department, The Lodge; 1995. p. 4–22. http://dx.doi.org/10.13140/2.1.3243.8728

Fujisawa T, Barraclough TG. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: a revised method and evaluation on simulated data sets. *Syst Biol*. 2013:62(5):707–724. https://doi.org/10.1093/sysbio/syt033

Génier F. Endophallites: a proposed neologism for naming the sclerotized elements of the insect endophallus (Arthropoda: Insecta). *Anna Soc Entomol Fr*. 2019:55(6):482–484. https://doi.org/10.1080/00379271.2019.1685907

Génier F, Moretto P. Digitonthophagus Balthasar, 1959: taxonomy, systematics, and morphological phylogeny of the genus revealing an African species complex (Coleoptera: Scarabaeidae: Scarabaeinae). *Zootaxa*. 2017:4248(1):1–110. https://doi.org/10.11646/zootaxa.4248.1.1

Girón JC, Tarasov S, González Montaña LA, Matentzoglu N, Smith AD, Koch M, Boudinot BE, Bouchard P, Burks R, Vogt L, et al. Formalizing invertebrate morphological data: a descriptive model for cuticle-based skeleto-muscular systems, an ontology for insect anatomy, and their potential applications in biodiversity research and informatics. *Syst Biol*. 2023:72(5):1084–1100. https://doi.org/10.1093/sysbio/syad025

Girón JC, Lumen R, Montanaro G, Tarasov S, Smith A. The Coleoptera Anatomy Ontology (COLAO) (version v2023-04-15). GitHub Repository; 2023.

Grebennikov VV. Wingless paocryptorrhinus (Coleoptera: Curculionidae) rediscovered in tanzania: synonymy, four new species and a mtDNA phylogeography. *Bonn Zool Bull*. 2015:64:1–15. https://zoologicalbulletin.de/BzB_Volumes/Volume_64_1/001_015_BZB64_1_Grebennikov_VV.pdf

Grebennikov VV. Phylogeography and sister group of lupangus, a new genus for three new flightless allopatric forest litter weevils endemic to the Eastern Arc Mountains, Tanzania (Coleoptera: Curculionidae, Molytinae). *Fragm Entomol*. 2017:49(1):37–55. https://doi.org/10.13133/2284-4880/229

Grebennikov VV. Independent morphological and genetic diversification in close geographic and phylogenetic proximity: Typoderus weevils (Coleoptera) in the Uluguru mountains, Tanzania. *Arthropod Syst Phylogeny*. 2019:77(2):251–265. https://doi.org/10.4081/fe.2017.229

Grebennikov VV. Phylogenetic placement of a new paleoendemic pill scarab from the Udzungwa Mountains, Tanzania, triggers biogeographic interpretations (Coleoptera: Hybosoridae: Ceratocanthinae). *Fragm Entomol*. 2021:53(2):283–298. https://doi.org/10.13133/2284-4880/547

Grebennikov VV, Heiss E. Survey and DNA barcoding of flat bugs (Hemiptera: Aradidae) in the Tanzanian forest archipelago reveal a phylogeographically structured fauna largely unknown at the species level. *Eur J Entomol*. 2018:512–523. https://doi.org/10.14411/eje.2018.051

Hebert PD, Cywinska A, Ball SL, DeWaard JR. Biological identifications through DNA barcodes. *Proc R Soc Lond B Biol Sci*. 2003:270(1512):313–321. https://doi.org/10.1098/rspb.2002.2218

Hemp C, Kehl S, Schultz O, Waegele JW, Hemp A. Climatic fluctuations and orogenesis as motors for speciation in East Africa: case study on *Parepistaurus* Karsch, 1896 (Orthoptera). *Syst Entomol*.:40(1):17–34. https://doi.org/10.1111/syen.12092

Hillis DM, Chambers EA, Devitt TJ. Contemporary methods and evidence for species delimitation. *Ichthyol Herpetol*. 2021:109(3):895–903. https://doi.org/10.1643/h2021082

Hosken DJ, Stockley P. Sexual selection and genital evolution. *Trends Ecol Evol*. 2004:19(2):87–93. https://doi.org/10.1016/j.tree.2003.11.012

Huang W, Xie X, Huo L, Liang X, Wang X, Chen X. An integrative DNA barcoding framework of ladybird beetles (Coleoptera: Coccinellidae). *Sci Rep*. 2020:10(1):10063. https://doi.org/10.1038/s41598-020-66874-1

Josso JF. Description d'une espèce ailée de janssensantus paulian, 1976, (Coleoptera, Scarabaeidae, Deltochilini). *Cathar. Rev*. 2022:(23):43–45.

Kainer D, Lanfear R. The effects of partitioning on phylogenetic inference. *Mol Biol Evol*. 2015:32(6):1611–1627. https://doi.org/10.1093/molbev/msv026

Katoh K, Standley DM. Mafft multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 2013:30(4):772–780. https://doi.org/10.1093/molbev/mst010

Kilwanila SI, Lyimo CM, Makundi RH, Rija AA. Isolating greater cane rat populations (*Thryonomys swinderianus*) from Eastern Arc Mountains, Tanzania: linking diversity to morphometric and molecular characteristics. *Diversity*. 2023:15(5):626. https://doi.org/10.3390/d15050626

Kitula M, Kajembe G, Zahabu E, Luoga E, Katani J, Ngowi S, Tron E. Influence of forest land tenure regimes on forest condition in Uluguru Mountains, Tanzania. *Tanzania J For Nat Conserv*. 2012:82(1):68–91.

Kohlmann B, Solís Á, Alvarado GE. Description of *Onthophagus humboldti* and *Uroxys bonplandi*, two new scarab beetles (Coleoptera, Scarabaeidae, Scarabaeinae) from Costa Rica, with notes on tropical mountain brachyptery and endemicity. *ZooKeys*. 2019:881:23. https://doi.org/10.3897/zookeys.881.38026

Kuchta SR, Wake DB. Wherefore and whither the ring species? *Copeia*. 2016:104(1):189–201. https://doi.org/10.1643/OT-14-176

Lartillot N, Philippe H. Computing Bayes factors using thermodynamic integration. *Syst Biol*. 2006:55(2):195–207. https://doi.org/10.1080/10635150500433722

Latha M, Cuccodoro G, Sabu TK, Vinod K. Taxonomy of the dung beetle genus *Ochicanthon vazdemello* (Coleoptera: Scarabaeidae: Scarabaeinae) of the Indian subcontinent, with notes on distribution patterns and flightlessness. *Zootaxa*. 2011:2745(1):1–29. https://doi.org/10.11646/zootaxa.2745.1.1

Lawrence J, Slipinski A. Australian beetles volume 1: morphology, classification and keys. Vol. 1. CSIRO Publishing; 2013. https://doi.org/10.1071/9780643097292

Lovett J, Marchant R, Taplin J, Küper W. The oldest rainforests in Africa: stability or resilience for survival and diversity? 198–229. In Purvis A, Gittleman JL, Brooks T, editors. Evolutionary Biology 8, Phylogeny and Conservation. Cambridge University Press, New York (USA); 2005. p. 1–448. https://doi.org/10.1017/CBO9780511614927.009

Lovett JC, Wasser SK. *Biogeography and ecology of the rain forests of eastern Africa*. Cambridge (UK): Cambridge University Press; 2008.

Luo A, Ling C, Ho SY, Zhu C-D. Comparison of methods for molecular species delimitation across a range of speciation scenarios. *Syst Biol*. 2018:67(5):830–846. https://doi.org/10.1093/sysbio/syy011

Magoga G, Sahin DC, Fontaneto D, Montagna M. Barcoding of Chrysomelidae of Euro-Mediterranean area: efficiency and problematic species. *Sci Rep*. 2018:8(1):13398. https://doi.org/10.1038/s41598-018-31545-9

McKay BD, Zink RM. Sisyphean evolution in Darwin's finches. *Biol Rev*. 2015:90(3):689–698. https://doi.org/10.1111/brv.12127

Measey GJ, Tolley KA. Sequential fragmentation of Pleistocene forests in an East Africa biodiversity hotspot: chameleons as a model to track forest history. *PLoS One*. 2011:6(10):e26606. https://doi.org/10.1371/journal.pone.0026606

Menegon M, Lyakurwa JV, Loader SP, Tolley KA. Cryptic diversity in pygmy chameleons (Chamaeleonidae: Rhampholeon) of the Eastern Arc Mountains of Tanzania, with description of six new species. *Acta Herpetol*. 2022:17(2):85–113. https://doi.org/10.36253/a_h-12978

Menegon M, Salvidio S, Loader S. Five new species of nectophrynoides noble 1926 (Amphibia Anura Bufonidae) from the Eastern Arc Mountains, Tanzania. *Trop Zool*. 2004:17(1):97–121. https://doi.org/10.1080/03946975.2004.10531201

Menegon M, Tolley KA, Jones T, Rovero, F, Marshall AR, Tilbury CR. A new species of chameleon (Sanria: Chamaeleonidae: Kinyongia) from the Magombera Forest and the Udzungwa Mountains National Park, Tanzania. *Afr J Herpetol*. 2009:58(2):59–70. https://doi.org/10.1080/21564574.2009.9650026

Michonneau, F. (2016). Using gmyc for species delineation. https://doi.org/10.5281/zenodo.838260.

Mikó I, Masner L, Ulmer JM, Raymond M, Hobbie J, Tarasov S, Margaría CB, Seltmann KC, Talamas EJ. A semantically enriched taxonomic revision of *Gryonoides* Dodd, 1920 (Hymenoptera, Scelionidae), with a review of the hosts of Teleasinae. *J Hymenopt Res*. 2021:87:523–573. https://doi.org/10.3897/jhr.87.72931.

Mlambo S, Scholtz CH, Deschodt CM, et al. *Grebennikovius*, a new genus to accommodate *Epactoides basilewskyi* (Balthasar, 1960) (Coleoptera: Scarabaeidae: Deltochilini). *Zootaxa*. 2019:4544(1):113–118. https://doi.org/10.11646/zootaxa.4544.1.6

Mlambo S, Sole CL, Scholtz CH. Affinities of the canthonini dung beetles of the Eastern Arc Mountains. *Organ Divers Evolut*. 2014:14(1):115–120. https://doi.org/10.1007/s13127-013-0158-y

Moctezuma V, Halffter G. Taxonomic revision of the *Phanaeus endymion* species group (Coleoptera: Scarabaeidae), with the descriptions of five new species. *Eur J Taxon*. 2021:747:1–71. https://orcid.org/0000-0002-4532-0302

Monaghan MT, Inward DJ, Hunt T, Vogler AP. A molecular phylogenetic analysis of the scarabaeinae (dung beetles). *Mol Phylogenet Evol*. 2007:45(2):674–692. https://doi.org/10.1016/j.ympev.2007.06.009

Müller H, Measey GJ, Loader SP, Malonza PK. A new species of *Boulengerula tornieri* (Amphibia: Gymnophiona: Caeciliidae) from an isolated mountain block of the Taita Hills, Kenya. *Zootaxa*. 2005:1004(1):37–50. https://doi.org/10.11646/zootaxa.1004.1.4

Mumbi CT, Marchant R, Hooghiemstra H, Wooller M. Late quaternary vegetation reconstruction from the Eastern Arc Mountains, Tanzania. *Quat Res*. 2008:69(2):326–341. https://doi.org/10.1016/j.yqres.2007.10.012

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. *Nature*. 2000:403(6772):853–858. https://doi.org/10.1038/35002501

Nyenza OM, Nzunda EF, Katani JZ. Socio-ecological resilience of people evicted for establishment of Uluguru Nature Reserve in Morogoro region, Tanzania. *For Trees Livelih*. 2013:22(3):190–203. https://doi.org/10.1080/14728028.2013.810405

Oaks JR, Cobb KA, Minin VM, Leaché, AD. Marginal likelihoods in phylogenetics: a review of methods and applications. *Syst Biol*. 2019:68(5):681–697. https://doi.org/10.1093/sysbio/syz003

Papadopoulou A, Anastasiou I, Vogler AP. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Mol Biol Evol*. 2010:27(7):1659–1672. https://doi.org/10.1093/molbev/msq051

Park DS, Foottit R, Maw E, Hebert PD. Barcoding bugs: DNA-based identification of the true bugs (Insecta: Hemiptera: Heteroptera). *PLoS One*. 2011:6(4):e18749. https://doi.org/10.1371/journal.pone.0018749

Paulian, R. Sur quelques canthonina (coléoptères scarabaéides) montagnards de madagascar. *Ann Soc Entomol Fr*. 1975:11:221–252.

Pérez-Delgado AJ, Arribas P, Hernando C, López H, Arjona Y, Suárez-Ramos D, Emerson BC, Andújar C. Hidden island endemic species and their implications for cryptic speciation within soil arthropods. *J Biogeogr*. 2022:49(7):1367–1380. https://doi.org/10.1111/jbi.14388

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst Biol*. 2006:55(4):595–609. https://doi.org/10.1080/10635150600852011

Puillandre N, Lambert A, Brouillet S, Achaz G. ABGD, automatic barcode gap discovery for primary species delimitation. *Mol Ecol*. 2012:21(8):1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Syst Biol*. 2018:67(5):901–904. https://doi.org/10.1093/sysbio/syy032

Ranasinghe UGSL, Eberle J, Thormann J, Bohacz C, Benjamin SP, Ahrens D. Multiple species delimitation approaches with COI barcodes poorly fit each other and morphospecies—an integrative taxonomy case of Sri Lankan *Sericini chafers* (Coleoptera: Scarabaeidae). *Ecol Evol*. 2022:12(5):e8942. https://doi.org/10.1002/ece3.8942

Ratnasingham S, Hebert PD. Bold: the barcode of life data system (http://www.barcodinglife. org). *Mol Ecol Notes*. 2007:7(3):355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. Mrbayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 2012:61(3):539–542. https://doi.org/10.1093/sysbio/sys029

Rossini M, Grebennikov V, Merrien T, Miraldo A, Viljanen H, Tarasov S. Paleogene forest fragmentation and out-of-Africa dispersal explain radiation of the paleotropical dung beetle tribe Epactoidini trib. nov.(Coleoptera: Scarabaeinae). *Syst Entomol*. 2022:47(4):655–667. https://doi.org/10.1111/syen.12564

Rovero F, Menegon M, FjeldsAa J, Collett L, Doggart N, Leonard C, Norton G, Owen N, Perkin A, Spitale D, et al. (2014). Targeted vertebrate surveys enhance the faunal importance and improve explanatory models within the Eastern Arc Mountains of Kenya and Tanzania. *Divers Distrib*. 2014:20(12):1438–1449. https://doi.org/10.1111/ddi.12246

Scholtz C, Howden H. A revision of the African cathonina (Coleoptera: Scarabaeidae: Scarabaeinae). *J Entomol Soc South Afr*. 1987:50(1):75–119.

Schütte A, Stüben P, Astrin J. Molecular weevil identification project: a thoroughly curated barcode release of 1300 Western Palearctic weevil species (Coleoptera, Curculionoidea). *Biodivers Data J*. 2023:11:e96438. https://doi.org/10.3897/BDJ.11.e96438

Skarbek, C. A review of endemic species in the Eastern Arc Afromontane region: importance, inferences, and conservation. *Macalester Rev Biogeogr*. 2008:1(1):3. https://digitalcommons.macalester.edu/biogeography/vol1/iss1/3

Talavera G, DincăV, Vila R. Factors affecting species delimitations with the GMYC model: insights from a butterfly survey. *Methods Ecol Evol*. 2013:4(12):1101–1110. https://doi.org/10.1111/2041-210X.12107

Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*. 1993:10(3):512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura K, Stecher G, Kumar S. Mega11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol*. 2021:38(7):3022–3027. https://doi.org/10.1093/molbev/msab120

Tarasov S, Dimitrov D. Multigene phylogenetic analysis redefines dung beetles relationships and classification (Coleoptera: Scarabaeidae: Scarabaeinae). *BMC Evol Biol.* 2016:16(1):1–19. https://doi.org/10.1186/s12862-016-0822-x

Tarasov S, Génier F. Innovative Bayesian and parsimony phylogeny of dung beetles (Coleoptera, Scarabaeidae, Scarabaeinae) enhanced by ontology-based partitioning of morphological characters. *PLoS One.* 2015:10(3):e0116671. https://doi.org/10.1371/journal.pone.0116671

Tarasov S, Montanaro G, Losacco F, Porto D. Towards fair principles in biodiversity research: enabling computable taxonomic descriptions and ecological data with phenoscript. *Biodiver Inf Sci Stand.* 2023:7:e111862. https://doi.org/10.3897/biss.7.111862

Tarasov SI, Solodovnikov AY. Phylogenetic analyses reveal reliable morphological markers to classify mega-diversity in onthophagini dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). *Cladistics.* 2011:27(5):490–528. https://doi.org/10.1111/j.1096-0031.2011.00351.x

Voje KL, Hemp C, Flagstad Ø, SÆTRE GP, Stenseth NC. Climatic change as an engine for speciation in flightless orthoptera species inhabiting African mountains. *Mol Ecol.* 2009:18(1):93–108. https://doi.org/10.1111/j.1365-294X.2008.04002.x

Wagner DL, Liebherr JK. Flightlessness in insects. *Trends in Ecol Evol.* 1992:7(7):216–220. https://doi.org/10.1016/0169-5347(92)90047-F

Ward RD. DNA barcode divergence among species and genera of birds and fishes. *Mol Ecol Res.* 2009:9(4):1077–1085. https://doi.org/10.1111/j.1755-0998.2009.02541.x

Wikispecies. Pierre basilewsky. Wikispecies; 2022 [accessed 19 September 2023]. https://species.wikimedia.org/wiki/Pierre_Basilewsky

Wirta H, Montreuil O. Evolution of the *Canthonini longitarsi* (Scarabaeidae) in Madagascar. *Zool Scrip.* 2008:37(6):651–663. https://doi.org/10.1111/j.1463-6409.2008.00352.x

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JW. Integrative taxonomy, or iterative taxonomy? *Syst Entomol.* 2011:36(2):209–217. https://doi.org/10.1111/j.1365-3113.2010.00558.x

Zhang H, Bu W. Exploring large-scale patterns of genetic variation in the *COI* gene among insecta: implications for DNA barcoding and threshold-based species delimitation studies. *Insects.* 2022:13(5):425. https://doi.org/10.3390/insects13050425

Zhang J, Kapli P, Pavlidis P, Stamatakis A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics.* 2013:29(22):2869–2876. https://doi.org/10.1093/bioinformatics/btt499