Fragmenta entomologica, 49 (1): 37-55 (2017)

eISSN: 2284-4880 (online version)
pISSN: 0429-288X (print version)

Research article
Submitted: April 26th, 2017 - *Accepted*: June 5th, 2017 - *Published*: June 30th, 2017

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)

Vasily V. GREBENNIKOV

CFIA, K.W. Neatby Building, 960 Carling Ave., Ottawa, ON, K1A 0C6, Canada - vasily.grebennikov@inspection.gc.ca

Abstract
This paper reports discovery of a new genus *Lupangus* **gen. n.** with three new flightless weevils endemic to the forests of the Eastern Arc Mountains in Tanzania: *L. asterius* **sp. n.** (East Usambara; the type species), *L. jason* **sp. n.** (Uluguru) and *L. orpheus* **sp. n.** (Udzungwa). Maximum Likelihood phylogenetic analyses using parts of mitochondrial (COI), nuclear ribosomal (28S) genes, as well as the nuclear spacer region (ITS2) from 46 terminals grouped together the reciprocally monophyletic *Lupangus* (3 terminals) and *Typoderus* (3 terminals), with all three clades strongly supported. Phylogenetic analysis of 32 COI-5' sequences recovered *Lupangus* species as reciprocally monophyletic, with *L. orpheus* being the sister to the rest. Internal phylogeny within both *L. jason* and *L. orpheus* are geographically structured, while that of *L. asterius* is not. Temporal analysis of *Lupangus* evolution using COI-5' data assessed under slow and fast substitution rate schemes estimated separation of mitochondrial lineages leading to three *Lupangus* species at about 7–8 Ma and about 1.9–2.1 Ma, respectively. Temporal analyses consistently failed to suggest correlation between the timing of *Lupangus* evolution and the late Pleistocene climatic fluctuations, thus rejecting the hypothesis of faunal interchanges during the wettest periods of the last million years. Applicability of flightless weevils for dispersal-vicariance analysis is reviewed, and their mostly undocumented and taxonomically entangled diversity in the Tanzanian Eastern Arc Mountains is briefly highlighted.

Key words: molecular phylogeny, DNA barcoding, COI, ITS2, 28S.

urn:lsid:zoobank.org:pub:CB21FF4B-0EBF-4CBF-B54B-EFAE84FAF6FF

Introduction

Careful selection of geographical settings facilitates assessment of the spatial and temporal components of the organic evolution. Oceanic islands such as the Galápagos Islands or the Sunda Arc are in this respect the absolute favourites since the time of Charles Robert Darwin and Alfred Russel Wallace. Their nearest inland alternatives are the "sky islands", such as the Eastern Arc Mountains (EAM) of Eastern Africa. They consisting of 10 main forested blocks broadly scattered through Kenya (Taita Hills) and Tanzania (North Pare, South Pare, West Usambara, East Usambara, Uluguru, Nguru, Kaguru, Rubeho and Udzungwa; Fig. 1A). A few other adjacent forested areas, such as geologically young forested volcanoes (i.e. Mt. Kilimanjaro, Mt. Meru, Mt. Hanang, Fig. 1A) or lowland forests (i.e. Kimboza, Pugu Hills, Fig. 1A) do not belong to EAM, even though their Biota might have been variously connected with that of EAM in the geological past. The forests of EAM are remarkable for their archipelago-type habitats supporting disproportionately high biodiversity with many narrow endemics (Lovett & Wasser 1993). The predominant hypothesis behind this phenomenon is the high biotic and abiotic stability of EAM during the last 5–10 Ma, when most of the Afrotropical forest repeatedly shrank to a few small and widely separated core survival zones (Hamilton & Taylor 1991; Maley 1996). Indeed, the global glacial cycles of the Plio- and Pleistocene manifested themselves in Africa by repetitive droughts reducing the forest cover to a few small and widely separate refugia (deMenocal 2004). The EAM, however, owning to their height and relative proximity to the Indian Ocean, are believed to continuously precipitate atmospheric moisture sufficient to support rainforest and its associates animal life ever since the Miocene, when the African forest had it last maximum (Hamilton & Taylor 1991). This hypothesis is directly supported by the pollen, charcoal and carbon isotope evidence obtained from two deep soil probes taken in Udzungwa (Mumbi et al. 2008) and Uluguru (Finch et al. 2009) and reveling stable forest composition during the past 48,000 yr, that is through a period greatly exceeding the Last Glacial Maximum with its peak at about 25,000 ya.

The pivotal book edited by Lovett and Wasser (1993) placed EAM in the spotlight of modern phylogeographical

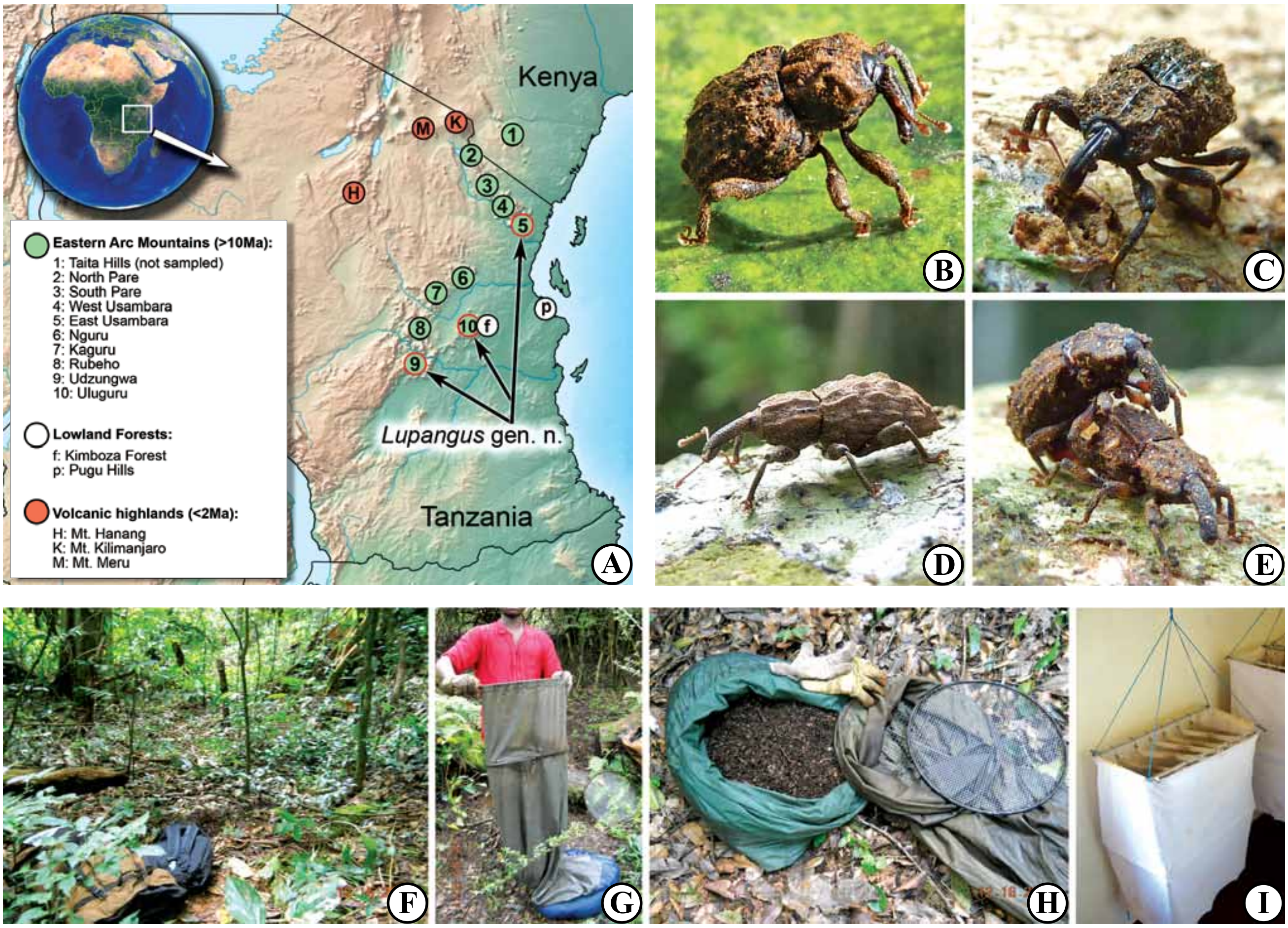

Kenya
Tanzania
Lupangus gen. n.
Eastern Arc Mountains (>10Ma):
1: Taita Hills (not sampled)
2: North Pare
3: South Pare
4: West Usambara
5: East Usambara
6: Nguru
7: Kaguru
8: Rubeho
9: Udzungwa
10: Uluguru
Lowland Forests:
f: Kimboza Forest
p: Pugu Hills
Volcanic highlands (<2Ma):
H: Mt. Hanang
K: Mt. Kilimanjaro
M: Mt. Meru
A
B
C
D
E
F
G
H
I

Fig. 1 – **A**, Map of sampled Tanzanian forests (generated with the online SimpleMappr tool by Shorthouse 2010); **B**,**C**, *Lupangus asterius*; **D**, *Typoderus furcatus* Marshall, 1957; **E**, *Typoderus* sp. sampled together with *L. asterius*; **F**, forest floor in East Usambara, habitat of *L. asterius*; **G**, sifter in operational position and with litter in the bag resting on the ground; **H**, typical sample with the final litter fraction and with collapsed sifter and finer mesh insert seen on the right; **I**, Winkler funnel with seven suspended bags in operational position.

studies. The EAM were thought to act as "species pumps" (Fjeldså & Lovett 1997; Murienne et al. 2013) accumulating and maintaining diverse and endemic forest-dependant clades of different age. Since then, a number of EAM organisms had their phylogeny assessed at the fine scale and involving the spatial and temporal aspect of evolution. The most suitable organisms for such analyses should meet criteria as: (1.) form a clade with the age of diversification not exceeding that of EAM forests; (2.) be common enough to permit predictable and efficient sampling; (3.) be biologically dependant on forests for survival; (4.) have relatively low dispersal capacity across intervening dry and hot savannah and (5.) have their diversity satisfactory known and Linnaean taxonomy stable. Different clades variously meeting these criteria have been recently employed to shed light on the phylogeographic past of EAM. In plants, widely cultivated African violets (*Saintpaulia* H. Wendl., Gesneriaceae) nearly endemic to EAM attracted much phylogeographical attention (Möller and Cronk 1997; Lindqvist & Albert 1999; Dimitrov et al. 2012). Low dispersing and moisture dependant Vertebrata were frequently targeted, such as frogs (Loader et al. 2014), caecilians (Loader et al. 2011), chameleons (Measey & Tolley 2011; Tolley et al. 2011; Ceccarelli et al. 2014), snakes (Menegon et al. 2014) and small mammals (rodents by Bryja et al. 2014; shrews by Stanley et al. 2015). Non-vertebrate animal life of EAM, although undoubtedly highly diverse and with a number of forest-dependant low-dispersal clades, is still too fragmentary known taxonomically to permit their reliable usage for phylogeographical purposes. Among the latter, attempts were focussed on katydids (Hemp et al. 2016), flat (Heiss & Grebennikov 2016) and assassin (Weirauch et al. 2017) bugs, ground beetles (Grebennikov et al. 2017) and weevils (Grebennikov 2015a). Some of these studies, however, relied on the non-recombinant mitochondrial COI gene, which when used alone (i.e. without nuclear markers) is prone to various shortcomings obscuring past evolutionary events (Funk & Omland 2003).

This paper highlights the discovery of a new weevil genus with three new species each narrowly endemic to a single EAM block, as well as finding its sister group and assessing it phylogeographically. The clade's representatives (Fig. 1B,C) were first detected in 2002 by sifting wet forest litter (Fig. 1F–I) near Amani village in the East Usambara Mountains, Tanzania (Fig. 1A) and for about a decade could not be assigned to any known genus. Based on current systematic practice, they belong to the presumably polyphyletic subfamily Molytinae comprising dozens of dubiously defined tribes and hundreds of genera, some of them recently discovered (Grebennikov 2014b) or inadequately known (Grebennikov 2016a). Externally, the East Usambara specimens resembled those of the Afrotropical genus *Typoderus* Marshall, 1953, with 11 poorly known species, some of which (Fig. 1D,E) were found in the same litter samples. Lack of adequate comparative material coupled with the absence of DNA data delayed phylogenetic assessment of the East Usambara specimens.

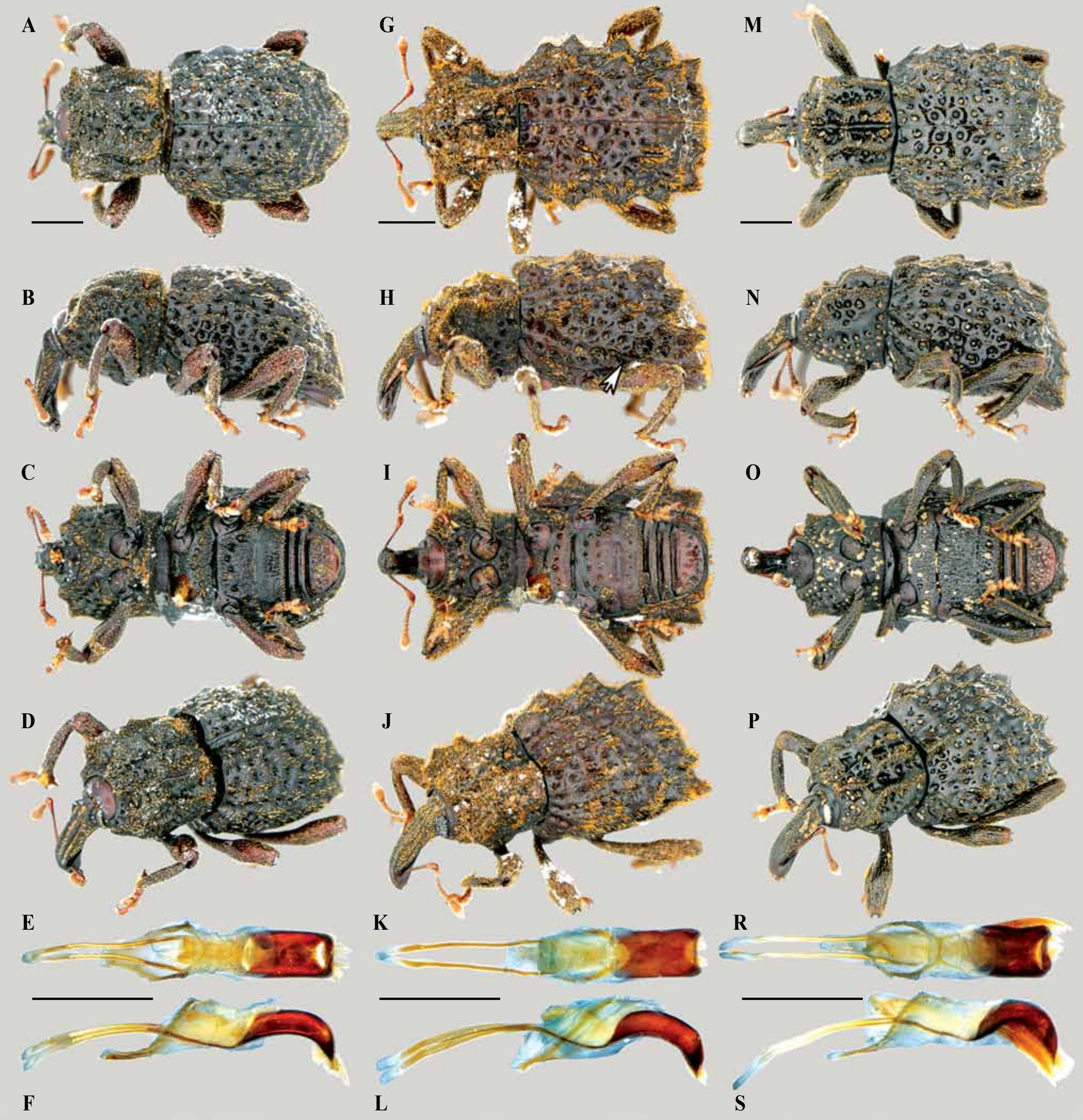


Fig. 2 – *Lupangus* spp., holotypes. **A**–**F**, *L. asterius*; **G**–**L**, *L. jason*; **M**–**S**, *L. orpheus*. Habitus: **A**,**G**,**M**, dorsal, **B**,**H**,**N**, left lateral, **C**,**I**,**O**, ventral, **D**,**J**,**P**, left fronto-lateral; aedeagus: **E**,**K**,**R**, dorsal and **F**,**L**,**S**, lateral. Scale bars: 1 mm.

During this time sampling in EAM (Fig. 1) and in some nearby forested areas was conducted. It revealed similarly shaped, although morphologically distinct beetles in Uluguru and Udzungwa, but nowhere else (Fig. 1A).

Discovery of these novel, morphologically similar, and phylogenetically puzzling weevils, seemingly restricted to three EAM blocks, evoked a number of evolutionary questions. First, do they form a clade, and if so, what might be its sister-group? Second, do specimens from each of the three localities form a reciprocally monophyletic group? Third, how did these flightless and presumably low-dispersing, habitat-dependent organisms come to populate three blocks of wet forest widely separated by seemingly highly unsuitable dry and hot savannah? More specifically, can the observed distribution be explained through normal ecological dispersal (Heads 2014) with subsequent subdivision into three allopatric lineages by means of climatically induced wet forest fragmentation (= vicariance) or, alternatively, may a hypothesis of long range dispersal be justifiably evoked? The latter, although infrequent, has been convincingly demonstrated for a number of animals crossing large saltwater barriers, such as at least some among 40 species of eyeless arthropods of the Galapagos Islands (Peck 1990), non-human primates of the New World (de Queiroz 2014), and minute blind and wingless *Orthotyphlus* Zaballos & Mateu, 1998 ground beetles colonizing New Caledonia (Andújar et al. 2016). Last but not least, how old are the evolutionary events leading to the present-day diversity and distribution of these novel beetles and do they coincide with the Pliocene-Pleistocene climatic fluctuations? This paper attempts to shed light on all these evolutionary questions by doing a series of DNA-based phylogenetic analyses and interpreting the results taxonomically, phylogenetically and phylogeographically.

Material and methods

Specimen sampling. A total of 130 individual litter samples were taken in 2010–14 in 14 discrete Tanzanian forests of different age and genesis, representing those on EAM (9), volcanic highlands (3) and lowland forests (2; Fig. 1A, Table 1). Fine fraction of the litter (Fig. 1H) was physically separated by using a large sifter (35 cm in diameter, Fig. 1G). The same litter was sifted twice: first through a larger mesh (square side: 10 mm) firmly fixed in the sifter, following by sifting through a wire insert (square side: 5 mm; Fig. 1H). Such two-step sifting was judged more efficient in processing a larger volume of litter without clogging the finer mesh, particularly in wet conditions. Taking a sifting sample lasted for about 2–4 hours and the wet mass of the final fraction (< 5 mm) was on average 7.7 kg (Fig. 1H, varying between 1.4 kg and 36.1 kg per sample with the total of 996 kg of fine litter fraction sampled, Table 1). Living organisms were then extracted (typically the following night) by placing approximately one handful of litter in a mesh bag suspended inside Winkler non-electric funnels and operated in a shelter (Fig. 1I). Funnels were suspended for 2–8 hours and then re-loaded with the same litter at least once with the aim to shake and excite organisms and thus to stimulate their active movement and, therefore, extraction. Six funnels each containing nine mesh bags were simultaneously in operation. A container at the bottom of each funnel containing extracted live organisms was emptied into a Whirl-Pak sealable plastic bag with 96% ethanol. Ethanol was drained and replaced at least three times in intervals of 1–3 days, to effectively remove water from specimens. All specimens from the same sifting sample received identical geographical labels (given verbatim in Table 1). During fieldwork, specimens were stored for up to two months at room temperature and then brought into a lab and placed in a freezer at –9°C. All herein reported specimens (including all outgroup taxa) are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes in Ottawa, Canada (CNC). Each specimen can be traced through a unique identifier label pinned under a beetle and bearing the code CNCCOLVG0000XXXX; the last four X's correspond to a unique number referred to on the topologies (Figs 3–5).

Tissue submission and DNA laboratory procedures. Within two months of their capture, specimens were sorted from the samples and processed for DNA barcoding using the standard animal COI-5' fragment (Hebert et al. 2003; Ratnasingham & Hebert 2007). One leg per specimen (normally a right femur cut open on both ends to expose muscle tissue, and additionally partly crushed with forceps) was placed in two drops of 95% ethanol in a sealed well on a standard 96-well microplate used for tissue submission. Three sets of primers were used (Table 2) to amplify one mitochondrial and two nuclear DNA markers (Table 3). All laboratory work related to DNA extraction, purification and sequencing was performed in a commercial laboratory "Canadian Center for DNA Barcode" (CCDB, http://www.ccdb.ca/) at the University of Guelph, Ontario, Canada, following the standard laboratory protocol (Ivanova et al. 2006, Ivanova et al. no date). Resulting sequences and additional relevant information such as gel images and trace files were uploaded to the "Barcode of Life Database" (= BOLD, http://www.boldsystems.org/).

Alignment and dataset concatenation. Each of three DNA markers (Table 3) was aligned using a different strategy. Alignment of the COI fragment was trivial and did not result in introduction of insertions or deletions (= indels). Alignments were checked for stop codons and frame shifts. Alignment of ITS2 and 28S was done using the online version of MAFFT 7 (Katoh et al. 2002; Katoh & Toh 2008a), with the Q-INS-i algorithm (Katoh & Toh 2008b) utilising the secondary structure information and resulted in introduction of 959 and 70 indels, respectively (Table

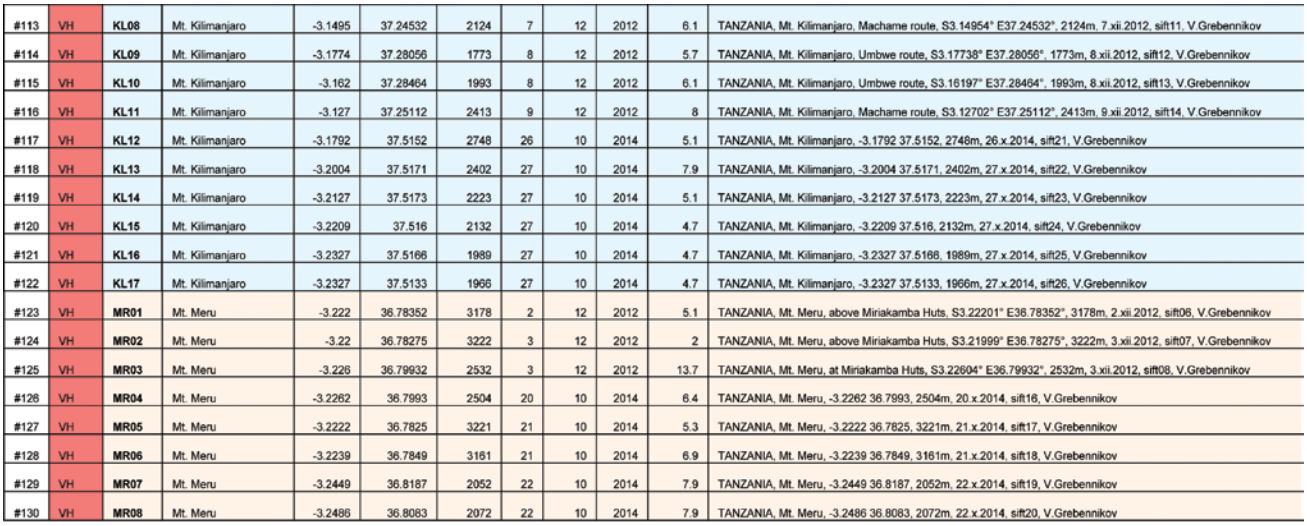
Table 1 – Sampled localities. EAM: Eastern Arc Mountains, LF: lowland forests, VH: volcanic highlands.

	Forest type	Sample code	Locality	Latitude (decimal)	Longitude (decimal)	Altitude (m)	Day	Month	Year	Weight (kg)	Label
#1	EAM	**EU01**	East Usambara Mts.	-5.1	38.64139	724	12	12	2011	5.7	TANZANIA, E Usambara Mts., Amani NR, 5°06'00"S 38°38'29"E, 12.xii.2011, 724m, sift.01, V.Grebennikov
#2	EAM	**EU02**	East Usambara Mts.	-5.1	38.64139	699	12	12	2011	6	TANZANIA, E Usambara Mts., Amani NR, 5°06'00"S 38°38'29"E, 12.xii.2011, 699m, sift.02, V.Grebennikov
#3	EAM	**EU03**	East Usambara Mts.	-5.0989	38.62167	1020	13	12	2011	4.6	TANZANIA, E Usambara Mts., Amani NR, 5°05'56"S 38°37'18"E, 13.xii.2011, 1020m, sift.03, V.Grebennikov
#4	EAM	**EU04**	East Usambara Mts.	-5.0989	38.62167	953	14	12	2011	8.1	TANZANIA, E Usambara Mts., Amani NR, 5°05'56"S 38°37'18"E, 14.xii.2011, 953m, sift.04, V.Grebennikov
#5	EAM	**EU05**	East Usambara Mts.	-5.1761	38.60028	1004	15	12	2011	5	TANZANIA, E Usambara Mts., Amani NR, 5°10'34"S 38°36'01"E, 15.xii.2011, 1004m, sift.05, V.Grebennikov
#6	EAM	**EU06**	East Usambara Mts.	-5.1656	38.60083	996	15	12	2011	4	TANZANIA, E Usambara Mts., Amani NR, 5°09'56"S 38°36'03"E, 15.xii.2011, 996m, sift.06, V.Grebennikov
#7	EAM	**EU07**	East Usambara Mts.	-5.0989	38.62167	970	16	12	2011	6.1	TANZANIA, E Usambara Mts., Amani NR, 5°05'56"S 38°37'18"E, 16.xii.2011, 970m, sift.07, V.Grebennikov
#8	EAM	**EU08**	East Usambara Mts.	-5.1011	38.65222	501	17	12	2011	6.1	TANZANIA, E Usambara Mts., Amani NR, 5°06'04"S 38°39'08"E, 17.xii.2011, 501m, sift.08, V.Grebennikov
#9	EAM	**KG01**	Kaguru Mts.	-6.3775	36.92361	1833	28	12	2011	18.1	TANZANIA, Kaguru Mts. at Masenge vil., 6°22'39"S 36°55'25"E, 28.xii.2011, 1833m, sift.16, V.Grebennikov
#10	EAM	**KG02**	Kaguru Mts.	-6.3756	36.93167	1875	28	12	2011	4.7	TANZANIA, Kaguru Mts. at Masenge vil., 6°22'32"S 36°55'54"E, 28.xii.2011, 1875m, sift.17, V.Grebennikov
#11	EAM	**KG03**	Kaguru Mts.	-6.3544	36.96222	1615	29	12	2011	10.6	TANZANIA, Kaguru Mts. at Masenge vil., 6°21'16"S 36°57'44"E, 29.xii.2011, 1615m, sift.18, V.Grebennikov
#12	EAM	**KG04**	Kaguru Mts.	-6.3608	36.95	1560	30	12	2011	5.7	TANZANIA, Kaguru Mts. at Masenge vil., 6°21'39"S 36°57'00"E, 30.xii.2011, 1560m, sift.19, V.Grebennikov
#13	EAM	**KG05**	Kaguru Mts.	-6.3631	36.94833	1608	30	12	2011	6.7	TANZANIA, Kaguru Mts. at Masenge vil., 6°21'47"S 36°56'54"E, 30.xii.2011, 1608m, sift.20, V.Grebennikov
#14	EAM	**NG01**	Nguru Mts.	-6.0694	37.55083	711	29	10	2010	5	TANZANIA, Nguru Mts. at Turiani, S06°04'10" E037°33'03", 29.x.2010, 711m, sifting01, V.Grebennikov
#15	EAM	**NG02**	Nguru Mts.	-6.0672	37.55722	677	30	10	2010	3	TANZANIA, Nguru Mts. at Turiani, S06°04'02" E037°33'26", 30.x.2010, 677m, sifting02, V.Grebennikov
#16	EAM	**NG03**	Nguru Mts.	-6.0747	37.53861	1277	31	10	2010	1.4	TANZANIA, Nguru Mts. at Turiani, S06°04'29" E037°32'19", 31.x.2010, 1277m, sifting03, V.Grebennikov
#17	EAM	**NG04**	Nguru Mts.	-6.0425	37.53889	1059	1	11	2010	2.4	TANZANIA, Nguru Mts. at Turiani, S06°02'33" E037°32'20", 1.xi.2010, 1059m, sifting04, V.Grebennikov
#18	EAM	**NG05**	Nguru Mts.	-6.1067	37.53	1236	3	11	2010	6.1	TANZANIA, Nguru Mts. at Turiani, S06°06'24" E037°31'48", 3.xi.2010, 1236m, sifting05, V.Grebennikov
#19	EAM	**NG06**	Nguru Mts.	-6.0747	37.53861	1227	4	11	2010	3	TANZANIA, Nguru Mts. at Turiani, S06°04'29" E037°32'19", 4.xi.2010, 1277m, sifting06, V.Grebennikov
#20	EAM	**NG07**	Nguru Mts.	-6.1067	37.53	1236	5	11	2010	5	TANZANIA, Nguru Mts. at Turiani, S06°06'24" E037°31'48", 5.xi.2010, 1236m, sifting07, V.Grebennikov
#21	EAM	**NG08**	Nguru Mts.	-6.1067	37.53	1254	3	1	2012	12.3	TANZANIA, Nguru Mts. at Mhonda, 6°06'24"S 37°31'48"E, 3.i.2012, 1254m, sift.21, V.Grebennikov
#22	EAM	**NG09**	Nguru Mts.	-6.1061	37.54389	967	4	1	2012	16.3	TANZANIA, Nguru Mts. at Mhonda, 6°06'22"S 37°32'38"E, 4.i.2012, 967m, sift.22, V.Grebennikov
#23	EAM	**NG10**	Nguru Mts.	-6.0739	37.55861	623	5	1	2012	5.9	TANZANIA, Nguru Mts. at Mhonda, 6°04'26"S 37°33'31"E, 5.i.2012, 623m, sift.23, V.Grebennikov
#24	EAM	**NP01**	North Pare Mts.	-3.7385	37.65134	2119	23	12	2012	6.1	TANZANIA, North Pare Mts., Kindoroko For., S3.73846° E37.65134°, 2119m, 23.xii.2012, sift24, V.Grebennikov
#25	EAM	**NP02**	North Pare Mts.	-3.7431	37.65022	1987	23	12	2012	7	TANZANIA, North Pare Mts., Kindoroko For., S3.74313° E37.65022°, 1987m, 23.xii.2012, sift25, V.Grebennikov
#26	EAM	**NP03**	North Pare Mts.	-3.5813	37.67454	1602	24	12	2012	9.6	TANZANIA, North Pare Mts., Minja For., S3.58126° E37.67454°, 1602m, 24.xii.2012, sift26, V.Grebennikov
#27	EAM	**NP04**	North Pare Mts.	-3.5804	37.67695	1613	24	12	2012	6.1	TANZANIA, North Pare Mts., Minja For., S3.58038° E37.67695°, 1613m, 24.xii.2012, sift27, V.Grebennikov
#28	EAM	**NP05**	North Pare Mts.	-3.7292	37.65329	1678	25	12	2012	6.1	TANZANIA, North Pare Mts., Kindoroko For., S3.72921° E37.65329°, 1678m, 25.xii.2012, sift28, V.Grebennikov
#29	EAM	**NP06**	North Pare Mts.	-3.7307	37.65358	1797	25	12	2012	9.1	TANZANIA, North Pare Mts., Kindoroko For., S3.73069° E37.65358°, 1797m, 25.xii.2012, sift29, V.Grebennikov
#30	EAM	**NP07**	North Pare Mts.	-3.6861	37.63745	1830	26	12	2012	8.1	TANZANIA, North Pare Mts., Kamwala For., S3.68606° E37.63745°, 1830m, 26.xii.2012, sift30, V.Grebennikov
#31	EAM	**NP08**	North Pare Mts.	-3.6857	37.63665	1844	26	12	2012	6.7	TANZANIA, North Pare Mts., Kamwala For., S3.68565° E37.63665°, 1844m, 26.xii.2012, sift31, V.Grebennikov
#32	EAM	**NP09**	North Pare Mts.	-3.7297	37.64447	1954	27	12	2012	5.9	TANZANIA, North Pare Mts., Kindoroko For., S3.72965° E37.64447°, 1954m, 27.xii.2012, sift32, V.Grebennikov
#33	EAM	**NP10**	North Pare Mts.	-3.7261	37.64778	1811	27	12	2012	7.4	TANZANIA, North Pare Mts., Kindoroko For., S3.72605° E37.64778°, 1811m, 27.xii.2012, sift33, V.Grebennikov
#34	EAM	**RB01**	Rubeho Mts.	-6.8306	36.56889	1993	20	12	2011	3.1	TANZANIA, Rubeho Mts. at Ipondelo vil., 6°49'50"S 36°34'08"E, 20.xii.2011, 1993m, sift.09, V.Grebennikov
#35	EAM	**RB02**	Rubeho Mts.	-6.8389	36.57556	1875	21	12	2011	10.1	TANZANIA, Rubeho Mts. at Ipondelo vil., 6°50'20"S 36°34'32"E, 21.xii.2011, 1875m, sift.10, V.Grebennikov
#36	EAM	**RB03**	Rubeho Mts.	-6.8389	36.57556	1895	21	12	2011	5.1	TANZANIA, Rubeho Mts. at Ipondelo vil., 6°50'20"S 36°34'32"E, 21.xii.2011, 1895m, sift.11, V.Grebennikov
#37	EAM	**RB04**	Rubeho Mts.	-6.8389	36.57556	1833	21	12	2011	4.9	TANZANIA, Rubeho Mts. at Ipondelo vil., 6°50'20"S 36°34'32"E, 21.xii.2011, 1833m, sift.12, V.Grebennikov
#38	EAM	**RB05**	Rubeho Mts.	-6.8306	36.56889	1997	23	12	2011	6.9	TANZANIA, Rubeho Mts. at Ipondelo vil., 6°49'50"S 36°34'08"E, 23.xii.2011, 1997m, sift.13, V.Grebennikov
#39	EAM	**RB06**	Rubeho Mts.	-6.8308	36.575	1982	23	12	2011	6.1	TANZANIA, Rubeho Mts. at Ipondelo vil., 6°49'51"S 36°34'30"E, 23.xii.2011, 1982m, sift.14, V.Grebennikov
#40	EAM	**RB07**	Rubeho Mts.	-6.8617	36.79417	763	24	12	2011	5	TANZANIA, Rubeho Mts, 6°51'42"S 36°47'39"E, 24.xii.2011, 763m, sift.15, V.Grebennikov
#41	EAM	**SP01**	South Pare Mts.	-4.2678	37.93151	2460	2	1	2013	5.6	TANZANIA, South Pare Mts., Chome For., S4.26776° E37.93151°, 2460m, 2.i.2013, sift34, V.Grebennikov
#42	EAM	**SP02**	South Pare Mts.	-4.268	37.93071	2412	2	1	2013	7.4	TANZANIA, South Pare Mts., Chome For., S4.26804° E37.93071°, 2412m, 2.i.2013, sift35, V.Grebennikov
#43	EAM	**SP03**	South Pare Mts.	-4.2687	37.92944	2318	3	1	2013	4.9	TANZANIA, South Pare Mts., Chome For., S4.26865° E37.92944°, 2318m, 3.i.2013, sift36, V.Grebennikov
#44	EAM	**SP04**	South Pare Mts.	-4.2695	37.92814	2239	3	1	2013	7.4	TANZANIA, South Pare Mts., Chome For., S4.26945° E37.92814°, 2239m, 3.i.2013, sift37, V.Grebennikov
#45	EAM	**SP05**	South Pare Mts.	-4.2706	37.92595	2159	3	1	2013	12.1	TANZANIA, South Pare Mts., Chome For., S4.27064° E37.92595°, 2159m, 3.i.2013, sift38, V.Grebennikov
#46	EAM	**SP06**	South Pare Mts.	-4.2788	37.90684	1876	4	1	2013	7.6	TANZANIA, South Pare Mts., small for., S4.27875° E37.90684°, 1876m, 4.i.2013, sift39, V.Grebennikov
#47	EAM	**SP07**	South Pare Mts.	-4.2715	37.92347	2072	4	1	2013	12.4	TANZANIA, South Pare Mts., Chome For., S4.27145° E37.92347°, 2072m, 4.i.2013, sift40, V.Grebennikov
#48	EAM	**SP08**	South Pare Mts.	-4.3062	37.97156	1648	6	1	2013	9.3	TANZANIA, South Pare Mts., Chome For., S4.30624° E37.97156°, 1648m, 6.i.2013, sift41, V.Grebennikov
#49	EAM	**SP09**	South Pare Mts.	-4.1292	37.88445	1629	7	1	2013	12.6	TANZANIA, South Pare Mts., Kwizu For., S4.12924° E37.88445°, 1629m, 7.i.2013, sift42, V.Grebennikov
#50	EAM	**UD01**	Udzungwa Mts.	-7.845	36.8761	454	6	10	2014	7.9	TANZANIA, Udzungwa Mts., -7.845 36.8761, 454m, 6.x.2014. sift01, V.Grebennikov
#51	EAM	**UD02**	Udzungwa Mts.	-7.8447	36.8577	1113	7	10	2014	6.6	TANZANIA, Udzungwa Mts., -7.8447 36.8577, 1113m, 7.x.2014, sift02, V.Grebennikov
#52	EAM	**UD03**	Udzungwa Mts.	-7.8419	36.8546	1083	7	10	2014	8.9	TANZANIA, Udzungwa Mts., -7.8419 36.8546, 1083m, 7.x.2014, sift03, V.Grebennikov
#53	EAM	**UD04**	Udzungwa Mts.	-7.7684	36.9	720	8	10	2014	7.9	TANZANIA, Udzungwa Mts., -7.7684 36.9, 720m, 8.x.2014, sift04, V.Grebennikov
#54	EAM	**UD05**	Udzungwa Mts.	-7.7552	36.8979	680	8	10	2014	7.6	TANZANIA, Udzungwa Mts., -7.7552 36.8979, 680m, 8.x.2014, sift05, V.Grebennikov
#55	EAM	**UD06**	Udzungwa Mts.	-7.8061	36.851	794	9	10	2014	7.6	TANZANIA, Udzungwa Mts., -7.8061 36.851, 794m, 9.x.2014, sift06, V.Grebennikov

continued

	Forest type	Sample code	Locality	Latitude (decimal)	Longitude (decimal)	Altitude (m)	Day	Month	Year	Weight (kg)	Label
#56	EAM	**UD07**	Udzungwa Mts.	-7.8062	36.8441	912	9	10	2014	7.9	TANZANIA, Udzungwa Mts., -7.8062 36.8441, 912m, 9.x.2014, sift07, V.Grebennikov
#57	EAM	**UD08**	Udzungwa Mts.	-7.8125	36.8241	1423	10	10	2014	9.3	TANZANIA, Udzungwa Mts., -7.8125 36.8241, 1423m, 10.x.2014, sift08, V.Grebennikov
#58	EAM	**UD09**	Udzungwa Mts.	-7.8191	36.8268	1905	10	10	2014	3.1	TANZANIA, Udzungwa Mts., -7.8191 36.8268, 1905m, 10.x.2014, sift09, V.Grebennikov
#59	EAM	**UD10**	Udzungwa Mts.	-7.818	36.8263	1793	10	10	2014	7.9	TANZANIA, Udzungwa Mts., -7.818 36.8263, 1793m, 10.x.2014, sift10, V.Grebennikov
#60	EAM	**UD11**	Udzungwa Mts.	-7.8158	36.826	1693	11	10	2014	4.4	TANZANIA, Udzungwa Mts., -7.8158 36.826, 1693m, 11.x.2014, sift11, V.Grebennikov
#61	EAM	**UD12**	Udzungwa Mts.	-7.8146	36.8256	1585	11	10	2014	4.4	TANZANIA, Udzungwa Mts., -7.8146 36.8256, 1585m, 11.x.2014, sift12, V.Grebennikov
#62	EAM	**UD13**	Udzungwa Mts.	-7.8125	36.8241	1431	11	10	2014	6.3	TANZANIA, Udzungwa Mts., -7.8125 36.8241, 1431m, 11.x.2014, sift13, V.Grebennikov
#63	EAM	**UL01**	Uluguru Mts.	-7.1236	37.63333	2220	7	11	2010	2	TANZANIA, east slope southern Uluguru Mts., S07°07'25" E037°37'60", 7.xi.2010, 2220m, sifting08, V.Grebennikov
#64	EAM	**UL02**	Uluguru Mts.	-7.1067	37.62111	2429	7	11	2010	4	TANZANIA, Uluguru Mts., at Tchenzema vil. S07°07'19" E037°37'16", 7.xi.2010, 2429m, sifting09, V.Grebennikov
#65	EAM	**UL03**	Uluguru Mts.	-7.115	37.60944	2408	8	11	2010	11.3	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'54" E037°36'34", 8.xi.2010, 2408m, sifting10, V.Grebennikov
#66	EAM	**UL04**	Uluguru Mts.	-7.0939	37.61917	2654	10	11	2010	5	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°05'38" E037°37'09", 10.xi.2010, 2654m, sifting11, V.Grebennikov
#67	EAM	**UL05**	Uluguru Mts.	-7.1139	37.605	2318	11	11	2010	5	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'50" E037°36'18", 11.xi.2010, 2318m, sifting12, V.Grebennikov
#68	EAM	**UL06**	Uluguru Mts.	-7.1106	37.60389	2208	11	11	2010	8.1	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'38" E037°36'14", 11.xi.2010, 2208m, sifting13, V.Grebennikov
#69	EAM	**UL07**	Uluguru Mts.	-7.1122	37.60444	2258	13	11	2010	7.1	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'44" E037°36'16", 13.xi.2010, 2258m, sifting14, V.Grebennikov
#70	EAM	**UL08**	Uluguru Mts.	-7.1131	37.61417	2547	15	11	2010	4	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'47" E037°36'51", 15.xi.2010, 2547m, sifting15, V.Grebennikov
#71	EAM	**UL09**	Uluguru Mts.	-7.1106	37.60306	2135	16	11	2010	9.1	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'38" E037°36'11", 16.xi.2010, 2135m, sifting16, V.Grebennikov
#72	EAM	**UL10**	Uluguru Mts.	-7.1222	37.64361	2038	18	11	2010	4	TANZANIA, east slope southern Uluguru Mts., S07°07'20" E037°38'37", 18.xi.2010, 2038m, sifting17, V.Grebennikov
#73	EAM	**UL11**	Uluguru Mts.	-7.1222	37.64361	2058	18	11	2010	6.1	TANZANIA, east slope southern Uluguru Mts., S07°07'20" E037°38'37", 18.xi.2010, 2058m, sifting18, V.Grebennikov
#74	EAM	**UL12**	Uluguru Mts.	-7.1133	37.605	2301	19	11	2010	6.1	TANZANIA, Uluguru Mts. at Tchenzema vil., S07°06'48" E037°36'18", 19.xi.2010, 2301m, sifting19, V.Grebennikov
#75	EAM	**UL13**	Uluguru Mts.	-7.0214	37.65278	1602	22	11	2010	6	TANZANIA, Uluguru Mts. at Bunduki vil., S07°01'17" E037°39'10", 22.xi.2010, 1602m, sifting20, V.Grebennikov
#76	EAM	**UL14**	Uluguru Mts.	-7.0639	37.65278	1592	22	11	2010	2.3	TANZANIA, Uluguru Mts. at Bunduki vil., S07°01'17" E037°39'10", 22.xi.2010, 1592m, sifting21, V.Grebennikov
#77	EAM	**UL15**	Uluguru Mts.	-7.0639	37.61528	2263	23	11	2010	4	TANZANIA, Uluguru Mts. at Bunduki vil., S07°03'50" E037°36'55", 23.xi.2010, 2263m, sifting22, V.Grebennikov
#78	EAM	**UL16**	Uluguru Mts.	-7.0564	37.62333	2051	23	11	2010	5.3	TANZANIA, Uluguru Mts. at Bunduki vil., S07°03'23" E037°37'24", 23.xi.2010, 2051m, sifting23, V.Grebennikov
#79	EAM	**UL17**	Uluguru Mts.	-7.0042	37.63056	1848	24	11	2010	11.4	TANZANIA, Uluguru Mts. at Bunduki vil., S07°00'15" E037°37'50 ", 24.xi.2010, 1848m, sifting24, V.Grebennikov
#80	EAM	**UL18**	Uluguru Mts.	-7.0664	37.63806	1818	25	11	2010	3.7	TANZANIA, Uluguru Mts. at Bunduki vil., S07°03'59" E037°38'17", 25.xi.2010, 1818m, sifting25, V.Grebennikov
#81	EAM	**UL19**	Uluguru Mts.	-7.0664	37.63778	1846	25	11	2010	5.1	TANZANIA, Uluguru Mts. at Bunduki vil., S07°03'59" E037°38'16", 25.xi.2010, 1846m, sifting26, V.Grebennikov
#82	EAM	**UL20**	Uluguru Mts.	-7.0183	37.6625	1569	26	11	2010	5	TANZANIA, Uluguru Mts. at Bunduki vil., S07°01'06" E037°39'45", 26.xi.2010, 1569m, sifting27, V.Grebennikov
#83	EAM	**UL21**	Uluguru Mts.	-6.865	37.70778	1921	10	1	2012	7.7	TANZANIA, Uluguru Mts., Lupanga Peak, 6°51'54"S 37°42'28"E, 10.i.2012, 1921m, sift.27, V.Grebennikov
#84	EAM	**UL22**	Uluguru Mts.	-6.8647	37.70778	1919	10	1	2012	3.3	TANZANIA, Uluguru Mts., Lupanga Peak, 6°51'53"S 37°42'28"E, 10.i.2012, 1919m, sift.28, V.Grebennikov
#85	EAM	**WU01**	West Usambara Mts.	-4.8105	38.49858	1618	11	1	2013	31.7	TANZANIA, W. Usambara Mts., Mazumbai For., S4.81054° E38.49858°, 1618m, 11.i.2013, sift43, V.Grebennikov
#86	EAM	**WU02**	West Usambara Mts.	-4.8157	38.49492	1788	12	1	2013	14.4	TANZANIA, W. Usambara Mts., Mazumbai For., S4.81566° E38.49492°, 1788m, 12.i.2013, sift44, V.Grebennikov
#87	EAM	**WU03**	West Usambara Mts.	-4.8242	38.4959	1930	12	1	2013	11	TANZANIA, W. Usambara Mts., Mazumbai For., S4.82420° E38.49590°, 1930m, 12.i.2013, sift45, V.Grebennikov
#88	EAM	**WU04**	West Usambara Mts.	-4.8263	38.4969	1860	12	1	2013	13.9	TANZANIA, W. Usambara Mts., Mazumbai For., S4.82634° E38.49690°, 1860m, 12.i.2013, sift46, V.Grebennikov
#89	EAM	**WU05**	West Usambara Mts.	-4.7659	38.36022	1576	14	1	2013	36.1	TANZANIA, W. Usambara Mts., Mkusu For., S4.76585° E38.36022°, 1576m, 14.i.2013, sift47, V.Grebennikov
#90	EAM	**WU06**	West Usambara Mts.	-4.7313	38.25688	1863	16	1	2013	17.7	TANZANIA, W. Usambara Mts., Shume For., S4.73129° E38.25688°, 1863m, 16.i.2013, sift48, V.Grebennikov
#91	EAM	**WU07**	West Usambara Mts.	-4.7301	38.25277	1943	16	1	2013	3.7	TANZANIA, W. Usambara Mts., Shume For., S4.73014° E38.25277°, 1943m, 16.i.2013, sift49, V.Grebennikov
#92	LF	**KM01**	Kimboza For.	-7.0222	37.80361	288	8	1	2012	6.1	TANZANIA, Kimboza forest, 7°01'20"S 37°48'13"E, 8.i.2012, 288m, sift.24, V.Grebennikov
#93	LF	**KM02**	Kimboza For.	-7.0217	37.80778	263	8	1	2012	16.1	TANZANIA, Kimboza forest, 7°01'18"S 37°48'28"E, 8.i.2012, 263m, sift.25, V.Grebennikov
#94	LF	**KM03**	Kimboza For.	-7.0242	37.80806	217	9	1	2012	8.4	TANZANIA, Kimboza forest, 7°01'27"S 37°48'29"E, 9.i.2012, 217m, sift.26, V.Grebennikov
#95	LF	**KM04**	Kimboza For.	-7.0218	37.8035	295	16	10	2014	35.7	TANZANIA, Kimboza For., -7.0218 37.8035, 295m, 16.x.2014, sift15, V.Grebennikov
#96	LF	**PG01**	Pugu Hills	-6.9101	39.09678	166	19	1	2013	18.4	TANZANIA, Pugu Hills, at reservoir, S6.91012° E39.09678°, 166m, 19.i.2013, sift50, V.Grebennikov
#97	VH	**HN01**	Mt. Hanang	-4.4162	35.40281	2652	13	12	2012	6.1	TANZANIA, Mt. Hanang, NE slope, S4.41621° E35.40281°, 2652m, 13.xii.2012, sift15, V.Grebennikov
#98	VH	**HN02**	Mt. Hanang	-4.4136	35.40384	2553	13	12	2012	6.1	TANZANIA, Mt. Hanang, NE slope, S4.41355° E35.40384°, 2553m, 13.xii.2012, sift16, V.Grebennikov
#99	VH	**HN03**	Mt. Hanang	-4.4133	35.40477	2541	13	12	2012	6.1	TANZANIA, Mt. Hanang, NE slope, S4.41330° E35.40477°, 2541m, 13.xii.2012, sift17, V.Grebennikov
#100	VH	**HN04**	Mt. Hanang	-4.4198	35.40159	2792	14	12	2012	5.1	TANZANIA, Mt. Hanang, NE slope, S4.41977° E35.40159°, 2792m, 14.xii.2012, sift18, V.Grebennikov
#101	VH	**HN05**	Mt. Hanang	-4.4086	35.40864	2263	14	12	2012	7.9	TANZANIA, Mt. Hanang, NE slope, S4.40857° E35.40864°, 2263m, 14.xii.2012, sift19, V.Grebennikov
#102	VH	**HN06**	Mt. Hanang	-4.4308	35.41593	2283	15	12	2012	8.6	TANZANIA, Mt. Hanang, NE slope, S4.43077° E35.41593°, 2283m, 15.xii.2012, sift20, V.Grebennikov
#103	VH	**HN07**	Mt. Hanang	-4.4318	35.41357	2366	15	12	2012	9.3	TANZANIA, Mt. Hanang, NE slope, S4.43175° E35.41357°, 2366m, 15.xii.2012, sift21, V.Grebennikov
#104	VH	**HN08**	Mt. Hanang	-4.4306	35.41616	2275	16	12	2012	8.4	TANZANIA, Mt. Hanang, NE slope, S4.43058° E35.41616°, 2275m, 16.xii.2012, sift22, V.Grebennikov
#105	VH	**HN09**	Mt. Hanang	-4.4611	35.39741	2383	17	12	2012	10	TANZANIA, Mt. Hanang, S slope, S4.46111° E35.39741°, 2383m, 17.xii.2012, sift23, V.Grebennikov
#106	VH	**KL01**	Mt. Kilimanjaro	-3.1842	37.51344	2668	25	11	2012	6.1	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.18424° E37.51344°, 2668m, 25.xi.2012, sift01, V.Grebennikov
#107	VH	**KL02**	Mt. Kilimanjaro	-3.1774	37.51137	2841	27	11	2012	6.1	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.17737° E37.51137°, 2841m, 27.xi.2012, sift02, V.Grebennikov
#108	VH	**KL03**	Mt. Kilimanjaro	-3.1776	37.51692	2755	27	11	2012	6.9	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.17761° E37.51692°, 2755m, 27.xi.2012, sift03, V.Grebennikov
#109	VH	**KL04**	Mt. Kilimanjaro	-3.193	37.51515	2590	27	11	2012	4	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.19304° E37.51515°, 2590m, 27.xi.2012, sift04, V.Grebennikov
#110	VH	**KL05**	Mt. Kilimanjaro	-3.2147	37.51822	2198	28	11	2012	5.7	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.21470° E37.51822°, 2198m, 28.xi.2012, sift05, V.Grebennikov
#111	VH	**KL06**	Mt. Kilimanjaro	-3.2014	37.51903	2370	6	12	2012	6.1	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.20141° E37.51903°, 2370m, 6.xii.2012, sift09, V.Grebennikov
#112	VH	**KL07**	Mt. Kilimanjaro	-3.2191	37.51472	2156	6	12	2012	6.1	TANZANIA, Mt. Kilimanjaro, Marangu route, S3.21909° E37.51472°, 2156m, 6.xii.2012, sift10, V.Grebennikov

continued



	Forest type	Sample code	Locality	Latitude (decimal)	Longitude (decimal)	Altitude (m)	Day	Month	Year	Weight (kg)	Label
#113	VH	**KL08**	Mt. Kilimanjaro	-3.1495	37.24532	2124	7	12	2012	6.1	TANZANIA, Mt. Kilimanjaro, Machame route, S3.14954° E37.24532°, 2124m, 7.xii.2012, sift11, V.Grebennikov
#114	VH	**KL09**	Mt. Kilimanjaro	-3.1774	37.28056	1773	8	12	2012	5.7	TANZANIA, Mt. Kilimanjaro, Umbwe route, S3.17738° E37.28056°, 1773m, 8.xii.2012, sift12, V.Grebennikov
#115	VH	**KL10**	Mt. Kilimanjaro	-3.162	37.28464	1993	8	12	2012	6.1	TANZANIA, Mt. Kilimanjaro, Umbwe route, S3.16197° E37.28464°, 1993m, 8.xii.2012, sift13, V.Grebennikov
#116	VH	**KL11**	Mt. Kilimanjaro	-3.127	37.25112	2413	9	12	2012	8	TANZANIA, Mt. Kilimanjaro, Machame route, S3.12702° E37.25112°, 2413m, 9.xii.2012, sift14, V.Grebennikov
#117	VH	**KL12**	Mt. Kilimanjaro	-3.1792	37.5152	2748	26	10	2014	5.1	TANZANIA, Mt. Kilimanjaro, -3.1792 37.5152, 2748m, 26.x.2014, sift21, V.Grebennikov
#118	VH	**KL13**	Mt. Kilimanjaro	-3.2004	37.5171	2402	27	10	2014	7.9	TANZANIA, Mt. Kilimanjaro, -3.2004 37.5171, 2402m, 27.x.2014, sift22, V.Grebennikov
#119	VH	**KL14**	Mt. Kilimanjaro	-3.2127	37.5173	2223	27	10	2014	5.1	TANZANIA, Mt. Kilimanjaro, -3.2127 37.5173, 2223m, 27.x.2014, sift23, V.Grebennikov
#120	VH	**KL15**	Mt. Kilimanjaro	-3.2209	37.516	2132	27	10	2014	4.7	TANZANIA, Mt. Kilimanjaro, -3.2209 37.516, 2132m, 27.x.2014, sift24, V.Grebennikov
#121	VH	**KL16**	Mt. Kilimanjaro	-3.2327	37.5166	1989	27	10	2014	4.7	TANZANIA, Mt. Kilimanjaro, -3.2327 37.5166, 1989m, 27.x.2014, sift25, V.Grebennikov
#122	VH	**KL17**	Mt. Kilimanjaro	-3.2327	37.5133	1966	27	10	2014	4.7	TANZANIA, Mt. Kilimanjaro, -3.2327 37.5133, 1966m, 27.x.2014, sift26, V.Grebennikov
#123	VH	**MR01**	Mt. Meru	-3.222	36.78352	3178	2	12	2012	5.1	TANZANIA, Mt. Meru, above Miriakamba Huts, S3.22201° E36.78352°, 3178m, 2.xii.2012, sift06, V.Grebennikov
#124	VH	**MR02**	Mt. Meru	-3.22	36.78275	3222	3	12	2012	2	TANZANIA, Mt. Meru, above Miriakamba Huts, S3.21999° E36.78275°, 3222m, 3.xii.2012, sift07, V.Grebennikov
#125	VH	**MR03**	Mt. Meru	-3.226	36.79932	2532	3	12	2012	13.7	TANZANIA, Mt. Meru, at Miriakamba Huts, S3.22604° E36.79932°, 2532m, 3.xii.2012, sift08, V.Grebennikov
#126	VH	**MR04**	Mt. Meru	-3.2262	36.7993	2504	20	10	2014	6.4	TANZANIA, Mt. Meru, -3.2262 36.7993, 2504m, 20.x.2014, sift16, V.Grebennikov
#127	VH	**MR05**	Mt. Meru	-3.2222	36.7825	3221	21	10	2014	5.3	TANZANIA, Mt. Meru, -3.2222 36.7825, 3221m, 21.x.2014, sift17, V.Grebennikov
#128	VH	**MR06**	Mt. Meru	-3.2239	36.7849	3161	21	10	2014	6.9	TANZANIA, Mt. Meru, -3.2239 36.7849, 3161m, 21.x.2014, sift18, V.Grebennikov
#129	VH	**MR07**	Mt. Meru	-3.2449	36.8187	2052	22	10	2014	7.9	TANZANIA, Mt. Meru, -3.2449 36.8187, 2052m, 22.x.2014, sift19, V.Grebennikov
#130	VH	**MR08**	Mt. Meru	-3.2486	36.8083	2072	22	10	2014	7.9	TANZANIA, Mt. Meru, -3.2486 36.8083, 2072m, 22.x.2014, sift20, V.Grebennikov

Table 2 – List of primers used.

Fragment	Cocktail	Name	Sense	Sequence (5′ to 3′)	References
COI-5’	C_LepFolF	LepF1	F	ATTCAACCAATCATAAAGATATTGG	Hebert et al. 2003a,b
COI-5’	C_LepFolF	LCO1490	F	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
COI-5’	C_LepFolR	LepR1	R	TAAACTTCTGGATGTCCAAAAAATCA	Hebert et al. 2003a,b
COI-5’	C_LepFolR	HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
ITS2	n/a	CAS5p8sFc	F	TGAACATCGACATTTYGAACGCACAT	Ji et al. 2003
ITS2	n/a	CAS28sB1d	R	TTCTTTTCCTCCSCTTAYTRATATGCTTAA	Ji et al. 2003
28S	n/a	D2B	F	GTCGGGTTGCTTGAGAGTGC	Saux et al. 2004
28S	n/a	D3Ar	R	TCCGTGTTTCAAGACGGGTC	Saux et al. 2004

3). No parts of the alignment were excluded from the analysis. Three aligned single-fragment datasets were concatenated using Mesquite 3.11 (Maddison & Maddison 2011). The concatenated matrix contained 42% of gaps (mainly from indels in ITS2).

Analytical strategy, matrix design and phylogenetic analyses. Three analyses were designed and implemented: **Analysis 1** (**A1**, **phylogenetic**) was designed with multiple goals to (a.) test monophyly of the herein hypothesised new clade of novel beetles from East Usambara, Uluguru and Udzungwa; (b.) if found to be monophyletic, then assess its relationships with the genus *Typoderus*, itself a taxonomic unit of questionable monophyly and (c.) if both form a clade, place it in the practically non-existing phylogenetic framework of Molytinae weevils. For these purposes, a matrix was created containing 46 terminals sequenced for three markers (COI, ITS2 and 28S; Table 4) and containing three terminals representing novel beetles from East Usambara, Uluguru and Udzungwa, three terminals representing three named *Typoderus* species, 30 other various Molytinae, nine non-Molytinae Curculionidae, and a member of the closely related family Dryophthoridae to root obtained topologies. Phylogenetic analysis was conducted using the CIPRES Science Gateway (Miller et al. 2010) using the maximum likelihood (ML) method. ML trees were obtained using RAxML 7.2.7 (Stamatakis 2006), with default parameters unless otherwise stated. The concatenated matrix was partitioned into three frag-

Table 3 – DNA fragments used in analyses.

Fragment	#	min	max	aligned	positions
COI	46	369	658	658	1 to 658
ITS2	42	223	618	1577	659 to 2235
28S	46	341	600	700	2236 to 2905

Table 4 – GenBank accessions of sequences used in the concatenated analyses.

Voucher	Subfamily	Species	Country	CO1	ITS2	28S
CNCCOLVG00000431	Molytinae	*Anchonidium unguiculare*	Morocco	HM417678	none	KY110382
CNCCOLVG00000434	Dryophthorinae	*Sphenophorus parumpunctatus*	Morocco	HM417724	KY110320	KY110384
CNCCOLVG00000487	Molytinae	*Thalasselephas maximus*	Russia	HM417677	KY110313	KY110375
CNCCOLVG00000703	Molytinae	*Pissodes punctatus*	China	HQ987002	KY110304	KY110366
CNCCOLVG00000704	Molytinae	*Ectatorhinus adamsii*	China	HQ987003	KY110315	KY110377
CNCCOLVG00001678	Cossninae	*Himatium*	Tanzania	JN265954	KY110323	KY110388
CNCCOLVG00001791	Cossninae	*Trichopentarthrum uluguricus*	Tanzania	JN265975	KY110327	KY110392
CNCCOLVG00002163	Molytinae	*Lupangus jason*	Tanzania	KY110619	KY110339	KY110404
CNCCOLVG00002277	Molytinae	*Trachodisca*	China	KY110613	KY110321	KY110385
CNCCOLVG00002708	Molytinae	*Niphadomimus electra*	China	KJ427734	KY110306	KY110368
CNCCOLVG00002731	Molytinae	*Niphadomimus maia*	China	KJ427744	KY110324	KY110389
CNCCOLVG00002955	Molytinae	*Lobosoma rausense*	Russia	KJ427738	KY110316	KY110378
CNCCOLVG00002970	Cossoninae	*Carphonotus testaceus*	Canada	KY110606	KY110309	KY110371
CNCCOLVG00003019	Molytinae	*Typoderus furcatus*	Tanzania	KJ445682	KY250483	KY250478
CNCCOLVG00003060	Molytinae	*Lupangus asterius*	Tanzania	KY034280	KY250485	KY250480
CNCCOLVG00003280	Molytinae	*Prothrombosternus tarsalis*	Tanzania	KU748541	KY110337	KY110402
CNCCOLVG00003638	Molytinae	*Typoderus subfurcatus*	Tanzania	KY034353	KY250486	KY250481
CNCCOLVG00003648	Cossoninae	*Caenopentarthrum quadricolle*	Tanzania	KY110607	KY110310	KY110372
CNCCOLVG00004355	Molytinae	*Otibazo polyphemus*	Vietnam	KJ841732	KY110328	KY110393
CNCCOLVG00004537	Molytinae	*Morimotodes ismene*	China	KJ871649	KY110338	KY110403
CNCCOLVG00004845	Molytinae	*Paocryptorrhinus hustachei*	Tanzania	KJ841728	KY110333	KY110398
CNCCOLVG00004846	Molytinae	*Thrombosternus cucullatus*	Tanzania	KJ445714	KY110335	KY110400
CNCCOLVG00004991	Molytinae	*Niphadonothus gentilis*	Tanzania	KX360489	KY110336	KY110401
CNCCOLVG00005001	Molytinae	*Aparopionella*	Tanzania	KX360455	KY110318	KY110381
CNCCOLVG00005848	Molytinae	*Adexius scrobipennis*	Poland	KJ445686	KY110305	KY110367
CNCCOLVG00006337	Molytinae	*Plinthus confusus*	Georgia	KY110612	KY110319	KY110383
CNCCOLVG00006485	Molytinae	*Plinthus amplicollis*	Georgia	KY110617	KY110331	KY110396
CNCCOLVG00006552	Molytinae	*Aparopion costatum*	Georgia	KJ445700	none	KY110387
CNCCOLVG00006608	Molytinae	*Leiosoma reitteri*	Georgia	KJ445698	KY110322	KY110386
CNCCOLVG00006683	Molytinae	*Euthycus*	Taiwan	KJ445702	KY110325	KY110390
CNCCOLVG00006858	Molytinae	*Darumazo distinctus*	Taiwan	KY110611	KY110317	KY110380
CNCCOLVG00006872	Molytinae	*Euthycus*	Taiwan	KJ445687	KY110308	KY110370
CNCCOLVG00007166	Molytinae	*Typoderus antennarius*	Tanzania	KY250487	KY250484	KY250479
CNCCOLVG00007318	Molytinae	*Catapionus fossulatus*	Russia	KU748528	KY110302	KY110364
CNCCOLVG00007388	Cryptorhynchinae	*Shirahoshizo juglandis*	Russia	KY110608	KY110311	KY110373
CNCCOLVG00007530	Cryptorhynchinae	*Cryptorhynchus lapathi*	Russia	KY110605	KY110303	KY110365
CNCCOLVG00007531	Molytinae	*Niphades verrucosus*	Russia	KY110610	KY110314	KY110376
CNCCOLVG00007714	Molytinae	*Lupangus orpheus*	Tanzania	KY034258	none	KY110363
CNCCOLVG00008474	Molytinae	*Lepyrus palustris*	Poland	KX360483	KY110332	KY110397
CNCCOLVG00008480	Molytinae	*Leiosoma deflexum*	Poland	KY110614	KY110326	KY110391
CNCCOLVG00008484	Entiminae	*Trachodes hispidus*	Poland	KX360436	KY110307	KY110369
CNCCOLVG00008873	Molytinae	*Peribleptus*	Nepal	KX360450	none	KY110379
CNCCOLVG00008909	Entiminae	*Graptus triguttatus*	Czech Republic	KY110616	KY110330	KY110395
CNCCOLVG00008915	Molytinae	*Peribleptus*	Vietnam	KY110615	KY110329	KY110394
CNCCOLVG00008936	Molytinae	*Acicnemis albofasciatus*	Russia	KY110609	KY110312	KY110374
CNCCOLVG00009056	Entiminae	*Nastus*	Kazakhstan	KY110618	KY110334	KY110399

ments and an independent GTR+G model was applied to each data partition. This evolutionary model is the most complex for nucleotide transitions, since it gives a different rate for each of them and accounts for rate heterogeneity (G). It is also the only model implemented in RAxML. The best scoring ML tree was selected among 100 searches on the original alignment with different randomized parsimony starting trees. Support values were obtained with 1000 bootstrap (Felsenstein 1985a) replicates as strong (> 75%), moderate (40%–75%) and low (< 40%). GenBank accession numbers for all 46 specimens are given in Table 4, while their locality data, specimen images, electropherograms and sequences can be found online in a public BOLD dataset dx.doi.org/10.5883/DS-LUPANG2.

Analysis 2 (**A2**, **phylogeographic**) was designed based on the results of **A1** (corroborated sister-group relationships between the herein reported new clade and monophyletic *Typoderus*) and was aimed to assess the interrelationships within the new clade, including testing the hypothesis of reciprocal monophyly of its three geographical groups (from East Usambara, Uluguru and Udzungwa, respectively). For this purpose a matrix was created containing 31 ingroup terminals sequenced for the mitochondrial marker COI-5' and representing populations from East Usambara (12), Uluguru (7) and Udzungwa (12), plus a single representative of its sister group (*Typoderus*) to root obtained topologies. Phylogenetic analysis was conducted using the ML method implemented in MEGA7 (Kumar et al. 2016) with a GTR+G model determined in MEGA7 as having the best fit. Support values were obtained with 1000 bootstrap replicates. GenBank accession numbers for all 32 specimens are on the topology (Fig. 4), while locality data, specimen images, electropherograms and sequences can be found online in a public BOLD dataset dx.doi.org/10.5883/DS-LUPANG1.

Analysis 3 (**A3**, **temporal**) was designed based on the results of **A2** (corroborated reciprocal monophyly of the three geographical groups) and was aimed to estimate relative and absolute time of the main evolutionary events leading to the present day diversity and distribution of the new clade. For this purpose the **A2** matrix was reduced in size to only 11 terminals best representing the two most basal levels of branching inside each geographical clade, as detected in **A2**. Bayesian phylogenetic analyses in BEAST 1.8 (Drummond et al. 2012) was used to simultaneously estimate an ultrametric phylogenetic tree and ages of diversification. Lacking fossils and unambiguous biogeographical events to calibrate the phylogeny, a uniform a priori substitution rate was implemented. Two calibration schemes were used, each utilizing a different rate. The first calibration scheme was based on the rate of 0.018 nucleotide substitutions per site per million years per lineage (subs/s/Myr/l), in agreement with results obtained for COI-5' in other beetles (Papadopoulou et al. 2010; Andújar et al. 2012), other insects (Brower 1994) and other arthropods (Bauzà-Ribot et al. 2012). The second scheme was based on the unusually high rate of 0.0793 subs/s/Myr/l estimated for the biologically most similar *Trigonopterus* Fauvel, 1862 weevils inhabiting forest litter of the Oriental region (analysis 2 in Tänzler et al. 2016), in agreement with the hypothesis that molecular evolution in flightless beetles, especially groups inhabiting stable habitats, might be highly accelerated (Mitterboeck & Adamowicz 2013). Monophyly of the East Usambara + Uluguru clade was enforced, following the topology obtained in **A2**. The GTR+G evolutionary model was used and the MCMC chains ran for 10 million generations. Consensus trees were estimated with TreeAnnotator (Drummond et al. 2012) discarding the 25% initial trees as a burn-in fraction, after checking ESS of likelihood, evolutionary rates and root age values, and ensuring that the tree likelihood values had reached a plateau. Posterior probabilities were considered as a measure of node support.

Taxonomic procedures. The ingroup organisms dealt with in this paper are new to science and it is therefore necessary to perform their formal taxonomic description. Since higher taxonomic categories do not exist objectively and need to be decided by the first reviser based on the best available evidence (Ward 2011), the following logic was implemented. The herein reported new clade, since morphologically easily diagnosable from its sister group genus *Typoderus*, should be ranked as a new genus. Its evolutionary lineages from East Usambara, Uluguru and Udzungwa, since reciprocally monophyletic and morphologically distinct, should be ranked as either one broadly defined, or three narrowly defined new species. Both approaches are logically equally valid by fully meeting all three primary taxon-naming criteria (Vences et al. 2013): monophyly of the taxon in an inferred species tree, clade stability and phenotypic diagnosability. The choice between one versus three species scheme should, therefore, be based on practical need to have names when referring to these organisms (Ward 2011). It should be explicitly stressed that logically sound species naming, like any assertion about a biological object, requires explicit knowledge of branching phylogeny (Felsenstein 1985b) and cannot be argued in oversimplified terms of "intraspecific genetic distance". Since specimens representing three geographically determined lineages are easily distinguished morphologically, each lineage was given a rank of a Linnaean species. All three new species are mutually allopatric and it is, therefore, impossible to assess how effectively they can preserve their genetic and morphological distinctness in case of possible introgression with other herein described congeners. The situation of putative allopatric "species" repeatedly diversifying and then succumbing to introgression was recently termed "Sisyphean evolution" and illustrated on the iconic Darwin's finches (McKay & Zink 2015). This Sisyphean scenario is likely widespread in nature and might, perhaps, be applicable to the new species described below. It is, therefore, important to fully embrace the understanding that any taxonomic arrangement represents the practical situation of today (= *status quo*) and should, therefore, be revised if and when new conflicting evidence came to light.

To expedite the formal descriptive taxonomic process, suggestions by Riedel et al. (2013) are followed. Each new species is illustrated by the standardized images of the holotype and its genitalia, and DNA barcoding data are provided. Species-level diagnostic descriptions are given by means of Table 5, which consistently lists all easily observed morphological differences. No absolute measurements are reported (which are variously and inconsistently measured in weevils, i.e. with or without rostrum and/or the head capsule visible from outside), which should be instead calculated from scale bars provided for the Holotype images (and from those provided for all herein reported specimens and accessible online on public BOLD dataset dx.doi.org/10.5883/DS-LUPANG1). The subfamily Molytinae is taxonomically defined following mainly Alonso-Zarazaga & Lyal (1999), and without the recent addition of Cryptorhynchinae (Oberprieler et al. 2007; Lyal 2014; for reasons see Riedel et al. 2016).

Lupangus **gen. n.**

Type species: *Lupangus asterius* **sp. n.**, by present designation.

Diagnosis. Adult specimens of *Lupangus* can be immediately recognized among Molytinae weevils (including those of the genus *Typoderus*, its sister clade) by the combination of at least two easily observed characters: markedly vertical eyes about 4–5 x as high as wide (Fig. 4; not more than 2.5 x in *Typoderus*) and a deep transverse groove extending dorsally between the dorsal edge of eyes (Fig. 4; absent in *Typoderus*).

Description. Adult body robust and heavily sclerotized, dark coloured and medium-sized (about 5–7 mm between anterior edge of pronotum to elytral apices); head, body and legs with numerous thick and short yellowish to orange setae; body with numerous large punctures; pronotum and elytra between striae 2–3, 4–5 and 6–7 with longitudinal ridges bearing separate rounded elevations or sharp peaks. Head with eyes markedly vertical (about 4–5 x as high as wide); cornea of ommatidia markedly globular; rostrum delimited posteriorly by deep and narrow dorsal transverse groove extending between dorsal eye corners; rostrum with variously developed longitudinal grooves; antennae with scapus, funicle with 7 antennomeres and club with three antennomeres. Prothorax without postocular lobes or prosternal channel; procoxae moderately separated by prosternal process and closed posteriorly; mesocoxae moderately and metacoxae markedly separated. Hind wings absent. Elytra interlocked with meso- and metathorax and among themselves; each elytron with ten rows of punctures. Aedeagus short, bent and cylindrical, with ventrally-directed hair in apical part.

Species composition and distribution. The genus *Lupangus* consists of three new allopatric species restricted to the Eastern Arc Mountains in Tanzania. Elevation: 501–1921 m.

Table 5 – Discrete morphological characters and matrix for diagnostics of *Lupangus* weevils.

1. Pronotum, shape (dorsal view): nearly square, maximal anterior width not more than 1.1 x as wide as posterior (0); trapezoid, maximal anterior width 1.3 x as wide as posterior (1)
2. Pronotum, central longitudinal ridge, length compared to pronotal length at midline: absent or if present, then < 20% (0); present, about 30–50% (1); present, 70–100% (2)
3. Pronotum, deep central triangular depression on posterior edge: absent (0); present (1)
4. Pronotum, outer longitudinal ridge, dorsal view: small, not forming lateral pronotal contour (0); large, forming lateral pronotal contour (1)
5. Elytra, elevations between striae 2–3, 4–5 and 6–7, size and shape: slightly elevated and rounded (0); moderately elevated and obtuse (1); markedly elevated and sharp (1)
6. Elytra, basal half, elevations between striae 2 and 3, formed by: longitudinal ridge once interrupted (0); separate rounded peaks (1)
7. Elytra, number of separate rounded elevations between striae 2 and 3: four (0); six (1)
8. Elytra, number of separate rounded elevations between striae 4 and 5: three (0); four (1)
9. Elytra, short longitudinal groove obliterating striae 9 and 10 in their middle and ending in a pit: absent (0); present (Fig. 2H) (1)
10. Aedeagus, hair distribution: apical part only (0); apical part and ventral surface (1)

Species	Locality	1	2	3	4	5	6	7	8	9	10
L. asterius	East Usambara	0	1	1	0	0	0	n/a	0	0	0
L. jason	Uluguru	1	0	0	1	2	1	0	0	1	0
L. orpheus	Udzungwa	0	2	0	0	1	1	1	1	0	1

Biology. All known specimens of *Lupangus* were detected by sifting floor litter in wet primary Afromontane forests. Host plants, immature stages, parasites or any other biological aspects remain unknown.

Etymology. Toponymic, after Lupanga, one of the principal peaks of Uluguru; gender masculine.

Lupangus asterius sp. n.

(Figs 1B,C, 2A–F, 3–5)

Diagnostic description. Holotype, male (Fig. 2A–F). GenBank accession of DNA barcode: Fig. 4; combination of species-level morphological characters: Table 5.

Distribution. This species is known only from East Usambara, Tanzania. Elevation: 501–1020 m.

Etymology. The species epithet is a Latinized Greek mythical name of Asterius, an Argonaut from Thessalia; noun in apposition.

Material examined. Holotype, male (CNC), specimen #3060, **Tanzania**: “TANZANIA, E Usambara Mts., Amani NR, 5°10'34"S 38°36'01"E, 15.xii.2011, 1004m, sift.05, V.Grebennikov”. Paratypes (CNC): 11, as in Fig. 4.

Lupangus jason sp. n.

(Figs 2G–L, 3–5)

Diagnostic description. Holotype, male (Fig. 2G–L). GenBank accession of DNA barcode: Fig. 4; combination of species-level morphological characters: Table 5.

Distribution. This species is known only from Uluguru, Tanzania. Elevation: 1569–1921 m.

Etymology. The species epithet is a Latinized Greek mythical name of Jason, the leading Argonaut, husband of Medeia; noun in apposition.

Material examined. Holotype, male (CNC), specimen #3636, **Tanzania**: “TANZANIA, Uluguru Mts., Lupanga Peak, 6°51'54"S 37°42'28"E, 10.i.2012, 1921m, sift.27, V.Grebennikov”. Paratypes (CNC): 6, as in Fig. 4.

Lupangus orpheus sp. n.

(Figs 2M–S, 3–5)

Diagnostic description. Holotype, male (Fig. 2M–S). GenBank accession of DNA barcode: Fig. 4; combination of species-level morphological characters: Table 5.

Material examined. Holotype, male (CNC), specimen #7714, **Tanzania**: “TANZANIA, Udzungwa Mts., –7.8419 36.8546, 1083m, 7.x.2014, sift03, V.Grebennikov”. Paratypes (CNC): 11, as in Fig. 4.

Distribution. This species is known only from Udzungwa, Tanzania. Elevation: 1083–1693 m.

Etymology. The species epithet is a Latinized Greek mythical name of Orpheus, an Argonaut, a magically talented musician; noun in apposition.

Results of three DNA analyses

Analysis **A1** resulted in a topology (Fig. 3) with monophyletic *Lupangus* a sister to monophyletic *Typoderus*; all three clades are strongly supported. The rest of the topology is poorly resolved, with only a few clades showing strong support. The majority of these clades are formed by a few presumably most closely related terminals, such as genera each represented by more than one terminal or by pairs of genera such as *Trachodes* Germar, 1824 and *Acicnemis* Fairmaire, 1849, or by both Cryptorhynchinae genera. Cossoninae genera do not form a clade, two of them (*Trichopentathrum* Osella, 1976 and *Caenopentarthrum* Voss, 1965) are strongly linked to the molytine genus *Otibazo* Morimoto, 1961. *Lepyrus* Germar, 1817 is strongly supported as the sister of *Plinthus* Germar, 1817, and both of them to *Adexius* Schoenherr, 1834. Entiminae and the rest of Curculionidae are reciprocally monophyletic, except that the Molytinae *Prothrombosternus* Voss, 1965 is nested within Entiminae.

Analysis **A2** resulted in a topology (Fig. 4) with three clades of *Lupangus* corresponding to three newly described allopatric species, and with *L. orpheus* from Udzungwa being the sister to the rest. While specimens of *L. asterius* (from East Usambara) exhibit no phylogeographic structure (note that the specimens of sample EU08 define the basal-most dichotomy), both other species have such a structure. That of *L. orpheus* (from Udzungwa) is moderately pronounced with specimens from sample UD03 forming a shallow sister clade to the rest of the species sampled from the opposite side of Mt. Mwanihana some 400–600 m higher and about 5 km away. Phylogeographic structure of *L. jason* is the strongest, with the single specimen #3636 (Holotype) being the deeply divergent sister to the rest.

Analysis **A3** resulted in two topologically identical trees (Fig. 5) with different timescales. The slow evolutionary scheme (0.018 subs/s/Myr/l) suggested separation of mitochondrial lineages representing all three allopatric *Lupangus* species taking place at about 7–8 Ma, while the fast scheme (0.0793 subs/s/Myr/l) placed these events at about 1.9–2.1 Ma. The basalmost splits inside each of three species are dated between about 3 Ma (in *L. jason*

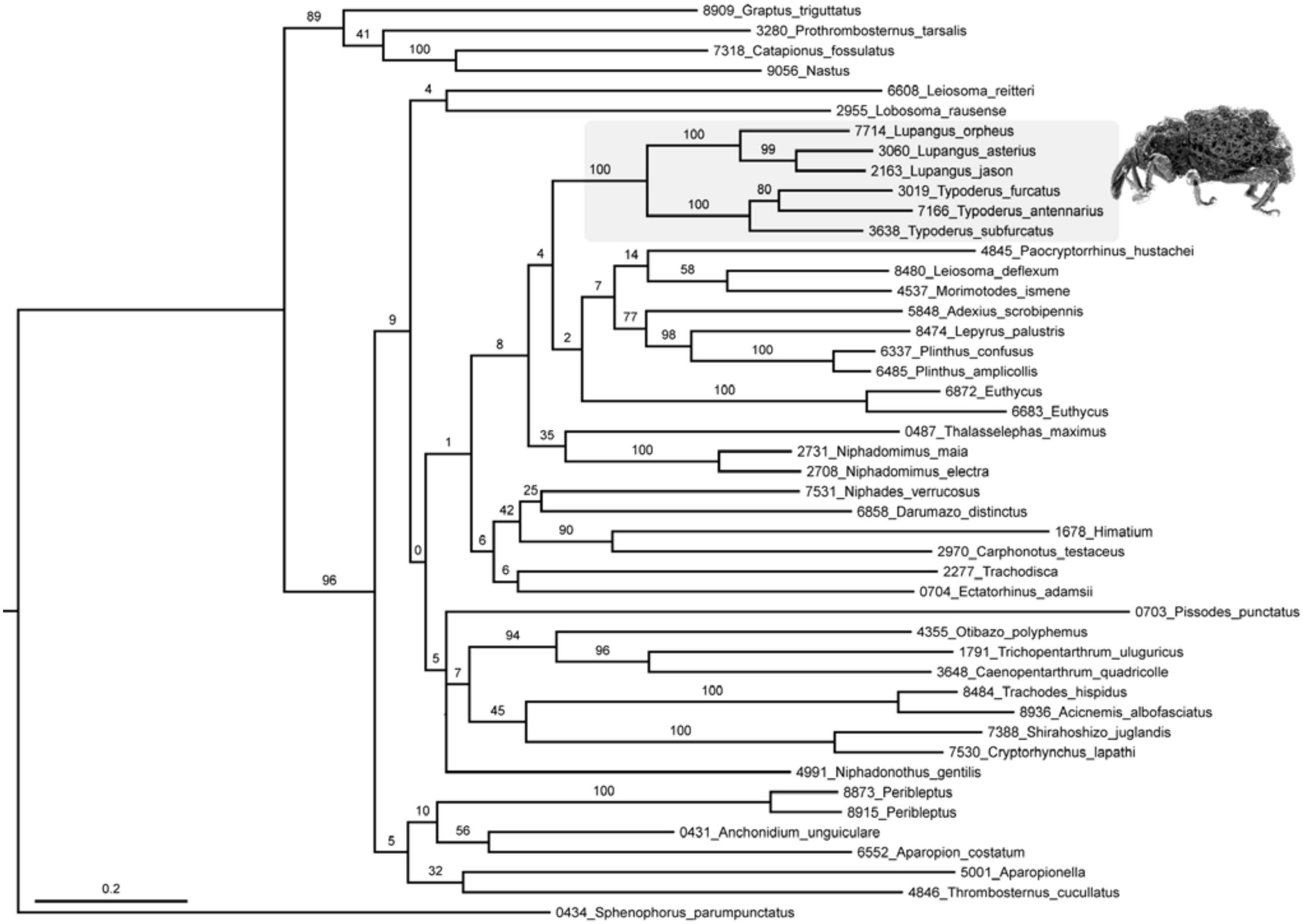

89
41
100
4
100
99
100
100
80
4
14
58
7
77
98
100
2
100
8
9
35
100
1
25
42
90
6
6
0
96
94
96
5
7
100
45
100
100
10
56
5
32
0.2
8909_Graptus_triguttatus
3280_Prothrombosternus_tarsalis
7318_Catapionus_fossulatus
9056_Nastus
6608_Leiosoma_reitteri
2955_Lobosoma_rausense
7714_Lupangus_orpheus
3060_Lupangus_asterius
2163_Lupangus_jason
3019_Typoderus_furcatus
7166_Typoderus_antennarius
3638_Typoderus_subfurcatus
4845_Paocryptorrhinus_hustachei
8480_Leiosoma_deflexum
4537_Morimotodes_ismene
5848_Adexius_scrobipennis
8474_Lepyrus_palustris
6337_Plinthus_confusus
6485_Plinthus_amplicollis
6872_Euthycus
6683_Euthycus
0487_Thalasselephas_maximus
2731_Niphadomimus_maia
2708_Niphadomimus_electra
7531_Niphades_verrucosus
6858_Darumazo_distinctus
1678_Himatium
2970_Carphonotus_testaceus
2277_Trachodisca
0704_Ectatorhinus_adamsii
0703_Pissodes_punctatus
4355_Otibazo_polyphemus
1791_Trichopentarthrum_uluguricus
3648_Caenopentarthrum_quadricolle
8484_Trachodes_hispidus
8936_Acicnemis_albofasciatus
7388_Shirahoshizo_juglandis
7530_Cryptorhynchus_lapathi
4991_Niphadonothus_gentilis
8873_Peribleptus
8915_Peribleptus
0431_Anchonidium_unguiculare
6552_Aparopion_costatum
5001_Aparopionella
4846_Thrombosternus_cucullatus
0434_Sphenophorus_parumpunctatus

Fig. 3 – Phylogeny of Molytinae, as obtained with RAxML with the combined COI, ITS2 and 28S dataset of 2905 aligned positions and partitioned by genes (Analysis **A1**). Numbers on nodes are bootstrap support values. Four digit voucher numbers precede terminal names.

under slow scheme) and about 0.25 Ma (in *L. orpheus* under fast scheme).

Discussion

Clade of *Lupangus* and *Typoderus* in unresolved Molytinae. The main result of analysis **A1** is the recovery of a strongly supported clade formed by reciprocally monophyletic *Lupangus* and *Typoderus*. This is the second pair of reciprocally monophyletic Afrotropical Molytinae genera convincingly shown to form a clade using phylogenetic analysis of DNA data; the other pair is *Amorphocerus* Schoenherr, 1826 and *Porthetes* Schoenherr, 1838 constituting the tribe Amorphocerini and known to develop exclusively on cycads of the genus *Encephalartos* (Zamiaceae) (Downie et al. 2008). The rest of Afrotropical Molytinae, consisting of many dozens, if not hundreds of genera (Alonso-Zarazaga & Lyal 1999), remains in the painful state of not only phylogenetic, but basic taxonomic neglect and obscurity (Grebennikov 2015a, 2016a). The herein analysed genus *Typoderus* (Fig. 1D, E) might be a good example of neglected genera, with all of its 11 nominal species known only from the original description published in the short period between Marshall (1953) and Voss (1965). Specimens of this genus were, however, exceedingly common and diverse in the majority of the 130 litter samples (Table 1), suggesting that the real species diversity (and its phylogeographic potential) is much higher. Both morphological characters of *Lupangus* stressed in the diagnosis (Fig. 4) seem autapomorphic to this genus, leaving *Typoderus* without known morphological support. From the presently released data one might suspect that *Lupangus* is an unusually shaped *Typoderus* sister to the three species represented in the analysis, but subordinate in *Typoderus* if the remaining species of this genus are considered. This, however, is unlikely, since analyses of a significant amount of unpublished data (about 400 *Typoderus* specimens sequenced for COI-5' and about 200 of them sequenced also for ITS2 and 28S, data not shown) consistently resulted in a monophyletic *Typoderus* excluding *Lupangus*.

Molytinae weevils lack a comprehensive molecular phylogeny compared with those proposed for some other comparably large weevils subfamilies such as Platypodi-

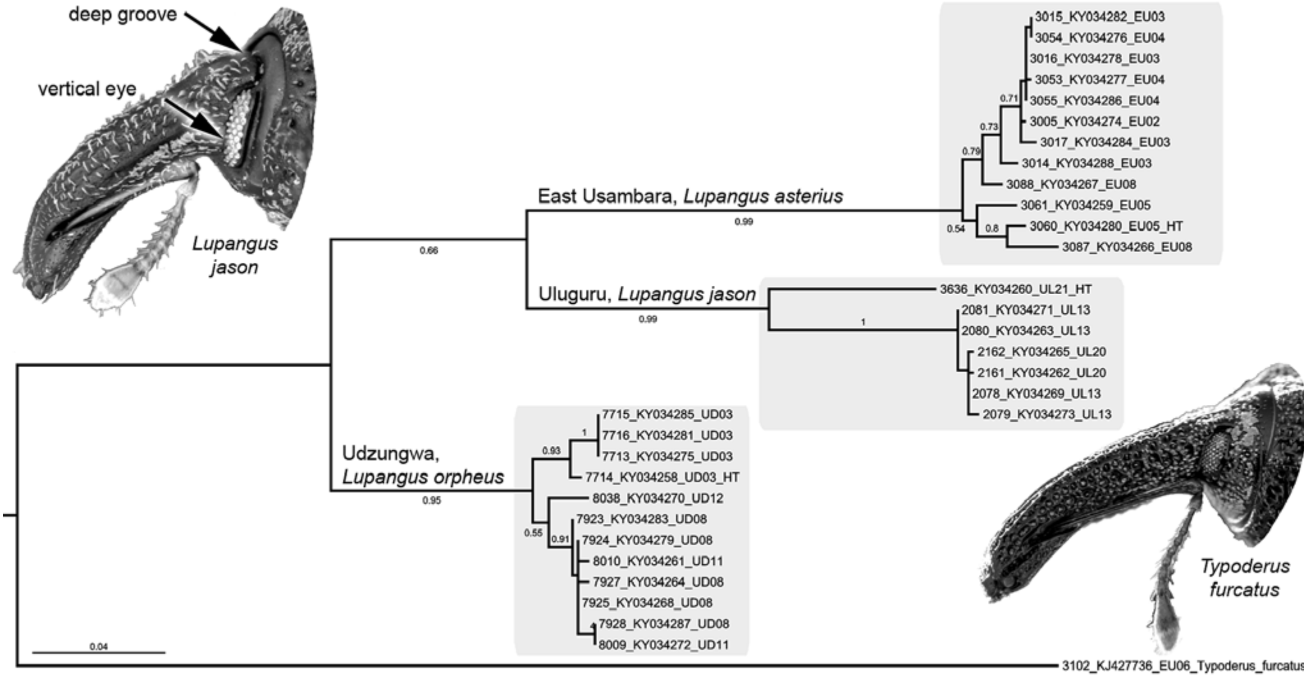

deep groove
vertical eye
Lupangus jason
3015_KY034282_EU03
3054_KY034276_EU04
3016_KY034278_EU03
3053_KY034277_EU04
0.71
3055_KY034286_EU04
0.73
3005_KY034274_EU02
3017_KY034284_EU03
0.79
3014_KY034288_EU03
3088_KY034267_EU08
East Usambara, Lupangus asterius
3061_KY034259_EU05
0.99
0.54
0.8
3060_KY034280_EU05_HT
3087_KY034266_EU08
0.66
3636_KY034260_UL21_HT
Uluguru, Lupangus jason
2081_KY034271_UL13
0.99
1
2080_KY034263_UL13
2162_KY034265_UL20
2161_KY034262_UL20
2078_KY034269_UL13
2079_KY034273_UL13
7715_KY034285_UD03
1
7716_KY034281_UD03
0.93
7713_KY034275_UD03
Udzungwa, Lupangus orpheus
7714_KY034258_UD03_HT
0.95
8038_KY034270_UD12
7923_KY034283_UD08
0.55
0.91
7924_KY034279_UD08
8010_KY034261_UD11
7927_KY034264_UD08
Typoderus furcatus
7925_KY034268_UD08
7928_KY034287_UD08
0.04
8009_KY034272_UD11
3102_KJ427736_EU06_Typoderus_furcatus

Fig. 4 – Phylogeny of *Lupangus*, as obtained with MEGA7 with COI sequences only (Analysis **A2**). Numbers on nodes are bootstrap support values. Four digit voucher numbers in terminal names precede GenBank accessions, followed by sample codes (Table 1); HT denotes the holotypes.

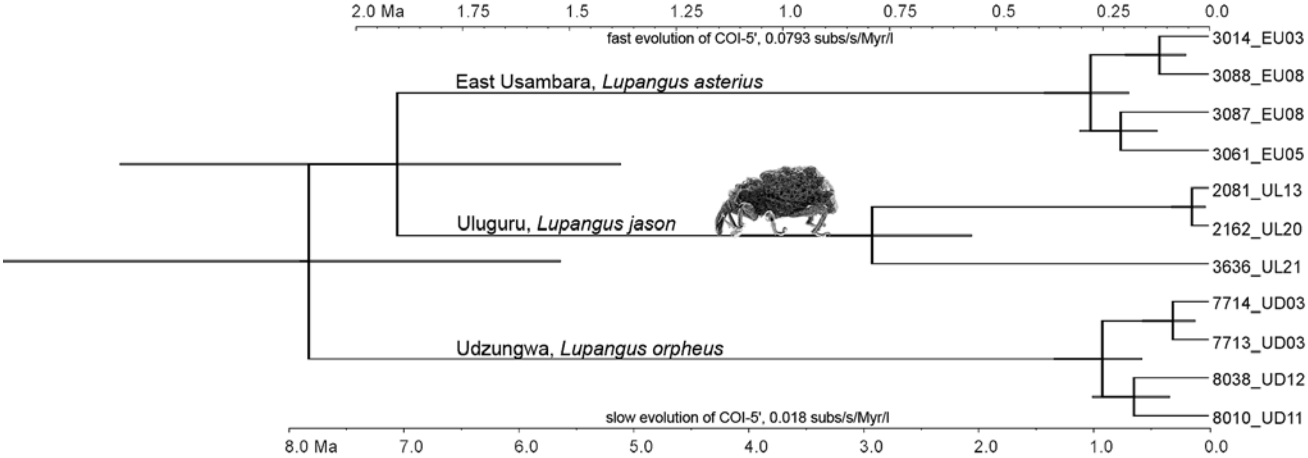

2.0 Ma
1.75
1.5
1.25
1.0
0.75
0.5
0.25
0.0
fast evolution of COI-5', 0.0793 subs/s/Myr/l
3014_EU03
East Usambara, Lupangus asterius
3088_EU08
3087_EU08
3061_EU05
2081_UL13
Uluguru, Lupangus jason
2162_UL20
3636_UL21
7714_UD03
Udzungwa, Lupangus orpheus
7713_UD03
8038_UD12
slow evolution of COI-5', 0.018 subs/s/Myr/l
8010_UD11
8.0 Ma
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

Fig. 5 – Ultrametric time tree of 11 select *Lupangus*, as obtained with BEAST using slow (0.018 subs/s/Myr/l) and fast (0.0793 subs/s/Myr/l) rates for COI-5' (Analysis **A3**). Numbers on scales above and below are million years before present for the fast and slow rates, respectively. Node bars represent 95% confidence interval of the slow rate age estimate (those for the fast rate are proportionally nearly identical and not shown). Four digit voucher numbers in terminal names precede sample codes (Table 1).

nae (Jordal 2015), Cryptorhynchinae (Riedel et al. 2016) or arguably the best studied economically important Scolytinae (reviewed in Kirkendall et al. 2015). Until now, either a few Molytinae representatives were included in broader multi-marker analyses (i.e. McKenna et al. 2009), or a larger subset of Molytinae genera was analysed using a single marker (Grebennikov 2014a,b). Each approach is limited in either coverage, or rigour, or perhaps in both and, therefore, developing of a robust Molytinae phylogeny (if indeed monophyletic, see Riedel et al. 2016) is still a pending task. The herein reported tree of Molytinae (Fig. 3) is an extended version of the barcode-only topologies from Grebennikov (2014a,b) with addition of a few terminals and of two nuclear ribosomal markers: ITS2 and 28S. The tree, even though with low support values, appears plausible, since with the exception of two *Leiosoma* Stephens, 1829, all *a priori* most closely related terminals (congeners, or closely related genera such as *Trachodes* and *Acicnemis*) predictably form strongly supported clades. The backbone resolution of the tree is, however, not much better than those obtained earlier using a single mitochondrial marker. Only a few non-anticipated clades are recovered, and some of them with low statistical support, for example Mediterranean *Aparopion* Hampe, 1861 + *Anchonidium* Bedel, 1884. Afrotropical *Aparopionella* Hustache 1939, once thought to be sister to *Typoderus* (Marshall 1953), does not group with other Typoderina, thus rejecting monophyly of the subtribe (sensu Alonso-Zarazaga & Lyal 1999). The recently described genus *Morimotodes* Grebennikov, 2014 is again (Grebennikov 2014b) recovered forming a clade with one of two included *Leiosoma*, while the latter genus is surprisingly not monophyletic. Novel is the strongly supported clade *Adexius* + (*Lepyrus* + *Plinthus*). Unexplainable is the paraphyly of three members of Entiminae with respect to *Prothrombosterus* (Molytinae). The strongly supported sister-group relationship between *Otibazo* and two poorly known Cossoninae genera suggests that the latter might be taxonomically misplaced. Overall, results of analysis **A1**, besides resolving the *Lupangus* + *Typoderus* clade, provide relatively little novel information. This can be attributed to the inadequate set of three markers (some of them, like COI and ITS2 perhaps too fast evolving) unable to resolve relatively deep phylogeny of the selected terminals.

Pre-Pleistocene vicariance best explains *Lupangus* speciation and distribution. The topology obtained in analysis **A2** with all three *Lupangus* species reciprocally monophyletic and strictly allopatric strongly suggests the simplest phylogeographical scenario of speciation through the normal ecological dispersal (Heads 2014) with subsequent subdivision into three lineages by wet forest fragmentation (= vicariance). Such interpretations are most commonly inferred for other EAM clades analysed in sufficient detail (*Kinyongia* chameleons by Tolley et al. 2011; *Trioceros* chameleons by Ceccarelli et al. 2014; *Praomys* rodents by Bryja et al. 2013; *Parepistaurus* flightless grasshoppers by Hemp et al. 2015). Timing and possible causes of this process pertaining to *Lupangus* remain, however, highly elusive due to the lack of a reliable time calibration. The herein implemented two flat evolutionary rates differ about four times (slow versus fast; see Methods and Fig. 5) and currently no information is available to allow choosing one of them. Adding to this the unavoidable uncertainty of the 95% confidence intervals (Fig. 5) deprives the herein implemented dating of most of its precision. Even though widely varying, the timing results consistently suggest that with 95% probability *Lupangus* allopatric speciation, even if estimated with the staggeringly fast evolutionary rate of 0.0793 subs/s/Myr/l, took place not later than about 1.3 Ma, and perhaps not later than 1.8 Ma (Fig. 5). These time points are in mid- and early-Pleistocene, respectively, and predate the most dramatic shrinkages and expansions of the African wet forest during the last million years (Hamilton & Taylor 1991). Application of the slow evolutionary rate (0.018 subs/s/Myr/l) pushes *Lupangus* speciation about four times deeper in the past and before the onset of the Pliocene-Pliocene climatic cycles. So widely interpreted, the results consistently suggest that the hypothesised ecological dispersal of the most recent common ancestor of *Lupangus* and its subsequent speciation through forest fragmentation took place only once and before the most pronounced dry/wet cycles of the last million years. This, in turn, suggests that during at least the last million years the local climate was not wet enough to permit forest expansion sufficiently pronounced to re-connect EAM forests into a single forested block. Such timing agrees with that estimated, for example, by Tolley et al. (2011) and much predates that of Hemp et al. (2015). Observed dating disagreements are, however, fully expected, since evolutionary history of each clade, even if most similar in dispersal capacities to many others occurring sympatrically, is expected to be fully unique through a combination of numerous stochastic evolutionary events.

Basal splits in two among three *Lupangus* species (Figs 4, 5) are geographically structured, rejecting panmixia. This can be fully expected for low-dispersing organisms, so the specimens of *L. orpheus* from the sample UD03 might form the sister group to the rest of the species sampled on the other side of Mt. Mwanihana only about 5 km away. Phylogeographic structure inside *L. jason* from Uluguru is even more pronounced, with the Holotype from Lupanga peak forming the deeply divergent sister to the analysed rest of the species sampled some 18 km southwards at the Bunduki village. The timing of the latter split using fast evolutionary rates suggests that with 95% probability it occurred not later than 0.5 Ma, and likely as early as 0.8 Ma (Fig. 5), which seems relatively old. It should, however, be remembered, that only the 5P fragment of the mitochondrial COI gene was used for such estimations, therefore the reported results might at least partly be linked to the phenomenon of maternal inheritance with all its known analytical advantages and shortcomings (Funk & Omland 2003).

Surprisingly and in spite of dense sampling, *Lupangus* beetles were documented in only three among nine studied EAM blocks, and in none among three volcanic and two lowland forests (Fig. 1A). Part of these absence data might be the sampling artefact of randomly failing to detect the beetles because of their highly fragmented distribution through seemingly uniform forests. This hypothesis gains support in an observation that no specimens of *L. jason* endemic to Uluguru were discovered in the densely sampled Uluguru forest adjacent to Lukwangule Plateau (samples UL01–12, see Table 1). An alternative explanation to the absence of *Lupangus* in other Tanzanian forests is that the suitable habitat was perhaps irreversibly lost due to anthropogenic changes (= human-driven extinction). Both assumptions, although not explicitly tested herein, appear unlikely, since the sampling in each forest was geographically diversified, while all sampled forests (with the exception of that on Pugu Hills, Fig. 1A) appeared large and healthy enough to support these beetles. Absence of *Lupangus* in forests of all three volcanic highlands (Fig. 1) might perhaps be linked to the relatively young age of these forests not pre-dating volcanic activities responsible for forming these highlands and commencing about 2–3 Ma (Nonnotte et al. 2008). Thus, unless otherwise demonstrated, the observed seemingly non-random distribution of *Lupangus* through 14 sampled Tanzanian forests (Fig. 1A) can most plausibly be attributed to two main factors: exceedingly low dispersal capacity coupled with highly stochastic nature of the colonizing/surviving events.

Inadequate taxonomy impedes unlocking phylogeographic potential of flightless weevils. Similarly to perhaps all presumably low-dispersing organisms, flightless weevils such as *Lupangus* are highly suitable but underutilized model organisms for testing competing phylogeographical hypotheses, particularly those pertaining to the dispersal versus vicariance dilemma. Their usefulness is compromised, however, by often acutely inadequate taxonomic knowledge (Riedel et al. 2010; Tänzler et al. 2012), making their use unpractical due to the lack of, or confusion in, their Linnaean names. In the present study, for example, the phylogeographical hypothesis is derived from a lineage entirely new to science, which, therefore, has to be first formally named and described according to the rules of the International Code of Zoological Nomenclature (ICZN 1999). This additional taxonomic burden seems, however, a much lesser hazard when compared to more numerous situations in which inadequately attributed and often synonymous historical taxonomic names making reference to organisms practically impossible (“clogging taxonomy”; Grebennikov 2016b).

Critical dependency of all biological assertions on having explicit knowledge of organisms’ branching phylogeny is a logically unavoidable requirement (Felsenstein 1985b). The taxonomic impediment might perhaps be obeyed and eventually solved though the practice of classical taxonomic Holotype-based revisions (Riedel 2011; Riedel & Tänzler 2016). This task is time consuming, cannot be significantly automated, requires high-end skills and, therefore, is becoming more expensive than the more and more democratized discovery and documentation of the inner branches on the Tree of Life (Maddison 2016). An alternative solution might be adoption, at least temporary, of a non-Linnaean DNA-based nomenclature (Ratnasingham & Hebert 2013) free of the historical taxonomic burden. Which way the biological science will choose to develop will soon be decided empirically.

Most of pioneering phylogeographical work utilizing flightless weevils (mainly to address the dispersal vs. vicariance dilemma) was done within the last decade focussing, predictably, on oceanic islands, such as those in Australasia near the Wallace line (Tänzler et al. 2014, 2016; Toussaint et al. 2015), Pacific Islands (Claridge et al. 2017), Macaronesia (Stüben & Astrin 2010) including the Canary Islands (Emerson et al. 2006; Faria et al. 2016; Machado et al. 2017), the Caribbean archipelago (Zhang et al. 2017), subantarctic islands (Grobler et al. 2011), Galapagos Islands (Sequeira et al. 2000, 2008) and Mauritius (Kitson et al. 2013). Attempts to extend this approach to the “sky island” faunas of the continental landmasses are few in comparison and target Europe (Meregalli et al. 2013) or Asia (Grebennikov 2014a,b, 2015b, 2016b; Grebennikov & Kolov 2016; Grebennikov & Morimoto 2016). The present work is the second DNA-based phylogeographic attempt utilizing flightless sub-Saharan weevils, following that of Grebennikov (2015a). Much of the delay in using these otherwise highly informative organisms might be apportioned to their repellently inadequate taxonomy. The latter was not based on phylogenetic principles and presently consists of poorly documented names, often synonymous at the species-, and even more so at the genus-group levels. It is symptomatic that the herein reported analysis deals with the genus entirely new to science and thus not haunted by the problem of the split identity, while two other weevil-focussed papers from the same litter-sampling program (Grebennikov 2015a, 2016a) target the previously monotypic genera. Other seemingly equally informative genera of weevils containing handfuls of named species and rediscovered in the samples (among them numerous, large and charismatic *Typoderus*) can be reported not before identity of the historical names becomes known through the type specimen examination. With relatively few names among the sub-Saharan litter weevils, this might be a manageable task, although preliminary results suggests that species- (Grebennikov 2015a) and particularly genus-level synonymy is rampant, with at least one exceedingly common and widespread “genus” having over 10 unrecognized synonyms. Similar taxonomic chaos was recently reported for flightless and phylogeographically informative *Catapionus* Schoenherr, 1842 and *Notaris* Germar, 1817 weevils in Asian highlands (Grebennikov 2016b; Grebennikov & Kolov 2016), although the main challenge there was the elusive identity of the species-, rather than that of the genus-group names. Once the taxonomic impediment is overcome one way or another (see above), flightless weevils (and indeed any among the low-dispersing and numerically abundant invertebrates) will be ready to have their phylogeographic potential fully unlocked.

Wet forests of the Eastern Arc Mountains: the gem of mainly untapped biodiversity. The Eastern Arc Mountains are arguably among the most biodiverse places on the Earth. Such an assessment is difficult to substantiate with reliable faunal and floral data, since the planet’s biodiversity is unevenly and incompletely documented. The latter statement is corroborated by the herein reported discovery of the narrowly endemic weevil genus entirely new to science. Tanzania, however, emerged as the most biodiverse continental country for chameleons (Tilbury 2010), with the majority of their diversity consisting of highly endemic species inhabiting EAM forests, and their number steadily increasing (Ceccarelli et al. 2014). Faunal surveys in EAM predominantly targeted the taxonomically better-known vertebrates (Rovero et al. 2014), and among them the relatively low-dispersing and habitat dependant amphibians and non-avian reptiles (= "herpetofauna"; Menegon et al. 2008). Invertebrates, and particularly the "cryptofauna" (Lawrence 1953; Leleup 1965) for the forest floor and the upper soil layer remain practically unknown. The most preliminary assessment of Arthropod diversity as seen when sorting the herein reported 130 litter samples suggests presence of numerically overwhelming and genetically diversified mesofauna, much of which is seemingly similar to *Lupangus* in having pronounced fine-scale phylogeographic structure. Only few among the sampled specimens were reported, mainly beetles (first apterous male of Lycidae by Bocak et al. 2014) and true bugs (*Ulugurocoris* Štys & Baňař 2013, the first Afrotropical Aenictopecheidae, by Štys & Baňař 2013; extremely sexually dimorphic *Xenocaucus* China & Usinger, 1949 assassin bugs, by Weirauch et al. 2017), and in every case new taxa and/or informative phylogeographic patters were detected. Other numerically abundant groups of the forest floor inhabitants, such as Formicidae or Acari, remain mainly unsorted and underutilized. They, and not the anthropocentrically more appealing vertebrates, constitute most of the genetic diversity in EAM and contain great and still mainly untapped potential for research on evolutionary biology.

Acknowledgements – Ignacio Ribera (Barcelona, Spain) provided continuous and reliable advice on logic and technicalities of DNA analysis; he, Jeff Skevington (Ottawa, Canada), Alexander Riedel (Kalsruhe, Germany) and Klaus-Dieter Klass (Dresden, Germany) critically read an early draft of this paper. Carmelo Andújar (London, UK) advised on the MAFFT Q-INS-i algorithm. The following colleagues provided specimens sequenced for the analysis A1: David Clarke (Memphis, USA: *Carphonotus testaceus*), Marek Wanat (Wroclaw, Poland: *Adexius scrobipennis*, *Leiosoma deflexum*, *Lepyrus palustris*, *Trachodes hispidus*), Eduard Jendek (Slovakia: Bratislava: *Acicnemis albofasciatus*, *Niphades verrucosus*), while Peribleptus from Nepal and Vietnam were provided by Olaf Jäger (Germany: Dresden) and Volker Assing (Germany, Hanover), respectively.

References

Alonso-Zarazaga M.A., Lyal C.H.C. 1999. A world catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) (excepting Scolytidae and Platypodidae). Entomopraxis, Barcelona, 315 pp.

Andújar C., Faille A., Pérez-González S., Zaballos J.P., Vogler A.P., Ribera I. 2016. Gondwanian relicts and oceanic dispersal in a cosmopolitan radiation of euedaphic ground beetles. Molecular Phylogenetics and Evolution, 99: 235–246, DOI: 10.1016/j.ympev.2016.03.013

Andújar C., Serrano J., Gómez-Zurita J. 2012. Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. BMC Evolutionary Biology, 12: 40, DOI: 10.1186/1471-2148-12-40

Bauzà-Ribot M.M., Juan C., Nardi F., Oromí P., Pons J., Jaume D. 2012. Mitogenomic phylogenetic analysis supports continental-scale vicariance in subterranean thalassoid crustaceans. Current Biology, 22(21): 2069–2074, DOI: 10.1016/j.cub.2012.09.012

Bocak L., Grebennikov V.V., Sklenarova K. 2014. *Cautires apterus*, a new species and the first record of wingless male Lycidae (Coleoptera) discovered in the North Pare mountains, Tanzania. Annales Zoologici, 64: 1–7, DOI: 10.3161/000345414X680500

Brower A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from mitochondrial DNA evolution. PNAS, 91: 6491–6495.

Bryja J., Mikula O., Patzenhauerová H., Oguge N.O., Šumbera R., Verheyen E. 2014. The role of dispersal and vicariance in the Pleistocene history of an East African mountain rodent, *Praomys delectorum*. Journal of Biogeography, 41: 196–208, DOI: 10.1111/jbi.12195

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

Claridge E.M., Gillespie R.G., Brewer M.S., Roderick G.K. 2017. Stepping-stones across space and time: repeated radiation of Pacific flightless broad-nosed weevils (Coleoptera: Curculionidae: Entiminae: Rhyncogonus). Journal of Biogeography, 44: 784–796, DOI:10.1111/jbi.12901

deMenocal P.B. 2004. African climate change and faunal evolution during the Pliocene–Pleistocene. Earth and Planetary Science Letters, 220: 3–24, DOI: 10.1016/S0012-821X(04)00003-2

de Queiroz A. 2014. The Monkey's Voyage: how Improbable Journeys Shaped the History of Life. Basic Books, New York, 368 pp.

Dimitrov D., Nogués-Bravo D., Scharff N. 2012. Why do tropical mountains support exceptionally high biodiversity? The Eastern Arc Mountains and the drivers of *Saintpaulia* diversity. PLoS ONE, 7: e48908, DOI:10.1371/journal.pone.0048908

Downie D.A., Donaldson J.S., Oberprieler R.G. 2008. Molecular systematics and evolution in an African cycad-weevil interaction: Amorphocerini (Coleoptera: Curculionidae: Molytinae) weevils on *Encephalartos*. Molecular Phylogenetics and Evolution, 47: 102–116, DOI: 10.1016/j.ympev.2008.01.023

Drummond A.J., Suchard M.A., Xie D., Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution, 29: 1969–1973, DOI: 10.1093/molbev/mss075

Emerson B.C., Forgie S., Goodacre S., Oromí P. 2006. Testing phylogeographic predictions on an active volcanic island: *Brachyderes rugatus* (Coleoptera: Curculionidae) on La Palma (Canary Islands). Molecular Ecology, 15: 449–458, DOI: 10.1111/j.1365-294X.2005.02786.x

Faria C.M.A., Machado A., Amorim I.R., Gage M.J.G., Borges P.A.V., Emerson B.C. 2016. Evidence for multiple founding lineages and genetic admixture in the evolution of species within an oceanic island weevil (Coleoptera, Curculionidae) super-radiation. Journal of Biogeography, 43: 178–191, DOI: 10.1111/jbi.12606

Felsenstein J. 1985a. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783–791, DOI: 10.2307/2408678

Felsenstein J. 1985b. Phylogenies and the comparative method. The American Naturalist, 125: 1–15, DOI: 10.1086/284325

Finch J., Leng M.J., Marchant R. 2009. Late Quaternary vegetation dynamics in a biodiversity hotspot, the Uluguru. Quaternary Research, 72: 111–122, DOI: 10.1016/j.yqres.2009.02.005

Fjeldså J., Lovett J.C. 1997. Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. Biodiversity and Conservation, 5: 325–346, DOI: 10.1023/A:1018356506390

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294–299.

Funk D.J., Omland K.E. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics, 34: 397–423, DOI: 10.1146/annurev.ecolsys.34.011802.132421

Grebennikov V.V. 2014a. DNA barcode and phylogeography of six new high altitude wingless *Niphadomimus* (Coleoptera: Curculionidae: Molytinae) from Southwest China. Zootaxa, 3838: 151–173, DOI: 10.11646/zootaxa.3838.2.1

Grebennikov V.V. 2014b. *Morimotodes*, a new genus for two minute wingless litter species from southwest China and Taiwan with an illustrated overview of Molytina and Plinthina genera (Coleoptera: Curculionidae: Molytini). Bonn Zoological Bulletin, 63: 123–147.

Grebennikov V.V. 2015a. Wingless *Paocryptorrhinus* (Coleoptera: Curculionidae) rediscovered in Tanzania: synonymy, four new species and a mtDNA phylogeography. Bonn Zoological Bulletin, 64: 1–15.

Grebennikov V.V. 2015b. Neglected *Trichalophus* (Coleoptera: Curculionidae): DNA barcode and phylogeography of high-altitude flightless weevils rediscovered in Southwest China. Bonn Zoological Bulletin, 64: 59–76.

Grebennikov V.V. 2016a. The genus *Prothrombosternus* (Coleoptera: Curculionidae: Molytinae) rediscovered: a male from Rubeho Mountains, Tanzania. Zootaxa, 4171: 170–174, DOI: 10.11646/zootaxa.4171.1.7

Grebennikov V.V. 2016b. Flightless *Catapionus* (Coleoptera: Curculionidae: Entiminae) in Southwest China survive the Holocene trapped on mountaintops: new species, unknown phylogeny and clogging taxonomy. Zootaxa, 4205: 243–254, DOI: http://dx.doi.org/10.11646/zootaxa.4205.3.4

Grebennikov V.V., Bulirsch P., Magrini P. 2017. Four new species, DNA barcode library and pre-Pliocene speciation of the euedaphic Afromontane Clivinini genera *Trilophidius* and *Antireicheia* (Coleoptera: Carabidae, Scaritinae). Fragmenta entomologica, 49 (1): 1–11.

Grebennikov V.V., Kolov S.V. 2016. Flightless *Notaris* (Coleoptera: Curculionidae: Brachycerinae: Erirhinini) in Southwest China: monophyly, mtDNA phylogeography and evolution of habitat associations. Zootaxa, 4105: 557–574, DOI: http://dx.doi.org/10.11646/zootaxa.4105.6.3

Grebennikov V.V., Morimoto K. 2016. Flightless litter-dwelling *Cotasterosoma* (Coleoptera: Curculionidae: Cossoninae) found outside of Japan, with mtDNA phylogeography of a new species from Southwest China. Zootaxa, 4179: 133–138, DOI: 10.11646/zootaxa.4179.1.11

Grobler G.C., Bastos A.D.S., Chimimba C.T., Chown S.L. 2011. Inter-island dispersal of flightless *Bothrometopus huntleyi* (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Island archipelago. Antarctic Science, 23: 225–234, DOI: 10.1017/S0954102011000113

Hamilton A.C., Taylor D. 1991. History of climate and forests in tropical Africa during the last 8 million years. Climatic Change, 19: 65–78, DOI: 10.1007/978-94-017-3608-4_8

Heads M. 2014. Biogeography of Australasia. A Molecular Analysis. Cambridge University Press, Cambridge, 503 pp.

Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R. 2003a. Biological identification through DNA barcoding. Proceedings of the Royal Society of London B: Biological Sciences, 270: 313–321, DOI: 10.1098/rspb.2002.2218

Hebert P.D.N., Ratnasingham S., deWaard J.R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society B: Biological Sciences, 270: S96–S99, DOI 10.1098/rsbl.2003.0025

Heiss E., Grebennikov V.V. 2016. Monophyly, review, six new species and DNA barcode of micropterous Afromontane *Afropictinus* (Heteroptera: Aradidae). Zootaxa, 4208: 149–160, DOI: http://dx.doi.org/10.11646/zootaxa.4208.2.3

Hemp C., Grzywacz B., Warchałowska-Śliwa E., Hemp A. 2016. Topography and climatic fluctuations boosting speciation: biogeography and a molecular phylogeny of the East African genera *Afroanthracites* Hemp & Ingrisch and *Afroagraecia* Ingrisch & Hemp (Orthoptera, Tettigoniidae, Conocephalinae, Agraeciini). Organisms Diversity and Evolution, 16: 211–223, DOI: 10.1007/s13127-015-0244-4

Hemp C., Kehl S., Schultz O., Wägele J.W., Hemp A. 2015. Climatic fluctuations and orogenesis as motors for speciation in East Africa: case study on *Parepistaurus* Karsch, 1896 (Orthoptera). Systematic Entomology, 41: 17–34, DOI: 10.1111/syen.12092

ICZN 1999. International Code of Zoological Nomenclature, 4th edition. The International Trust for Zoological Nomenclature, London, 306 pp.

Ivanova N.V., deWaard J.R., Hebert P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, 6: 998–1002, DOI: 10.1111/j.1471-8286.2006.01428.x

Ivanova N.V., deWaard J.R., Hajibabaei M., Hebert P.D.N. no date. Protocols for high-volumeDNA barcode analysis. Available at http://www.barcodeoflife.org/sites/default/files/Protocols_for_High_Volume_DNA_Barcode_Analysis.pdf.

Ji Y.-J., Zhang D.-X., He L.-J. 2003. Evolutionary conservation and versatility of a new set of primers for amplifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. Molecular Ecology Notes, 3: 581–585, DOI: 10.1046/j.1471-8286.2003.00519.x

Jordal B.H. 2015. Molecular phylogeny and biogeography of the weevil subfamily Platypodinae reveals evolutionarily conserved range patterns. Molecular Phylogenetics and Evolution, 92: 294–307, DOI: 10.1016/j.ympev.2015.05.028

Katoh K., Misawa K., Kuma K., Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30: 3059–3066, DOI: https://doi.org/10.1093/nar/gkf436

Katoh K., Toh H. 2008a. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics, 9: 286–298, DOI: https://doi.org/10.1093/bib/bbn013

Katoh K., Toh H. 2008b. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinformatics, 9: 212, DOI: 10.1186/1471-2105-9-212

Kirkendall L.R., Biedermann P.H.W., Jordal B.H. 2015. Evolution and diversity of bark and ambrosia beetles, pp. 85–156. In: Vega F.E., Hofstetter R.W. (eds), Bark beetles: biology and ecology of native and invasive species. Academic Press, Boston, MA, DOI: 10.1016/B978-0-12-417156-5.00003-4

Kitson J.J.N., Warren B.H., Florens F.B.V., Baider C., Strasberg

D., Emerson B.C. 2013. Molecular characterization of trophic ecology within an island radiation of insect herbivores (Curculionidae: Entiminae: *Cratopus*). Molecular Ecology, 22: 5441–5455, DOI: 10.1111/mec.12477

Kumar S., Stecher G., Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870–1874, DOI: https://doi.org/10.1093/molbev/msw054

Lawrence R.F. 1953. The Biology of the Cryptic Fauna of Forests. With Special Reference to the Indigenous Forests of South Africa. A.A. Balkema, Cape Town, 408 pp.

Leleup N. 1965. La Faune Entomologique Cryptique de l'Afrique Intertropicale. Annales du Musée Royal de l'Afrique Centrale, Serie in-8 °, Sciences zoologiques, 141: ix+186 pp.

Lindqvist G., Albert V.A. 1999. Phylogeny and conservation of African violets (*Saintpaulia*: Gesneriaceae): new findings based on nuclear ribosomal 5S non-transcribed spacer sequences. Kew Bulletin, 54: 363–377, DOI: 10.2307/4115813

Loader S.P., Ceccarelli F.S., Menegon M., Howell K.M., Kassahun R., Mengistu A.A., Saber S.A., Gebresenbet F., de Sá R., Davenport T.R.B., Larson J.G., Müller H., Wilkinson M., Gower D.J. 2014. Persistence and stability of Eastern Afromontane forests: evidence from brevicipitid frogs. Journal of Biogeography, 41: 1781–1792, DOI: 10.1111/jbi.12331

Loader, S.P., Wilkinson, M., Cotton, J.A., Measey, J., Menegon., M., Howell, K.M., Müller, H., Gower, D.J. (2011) Molecular phylogenetics of *Boulengerula* (Amphibia: Gymnophiona: Caeciliidae) and implications for taxonomy, biogeography and conservation. Herpetological Journal, 21: 5–16.

Lovett J.C., Wasser S.K. (eds) 1993. Biogeography and Ecology of the Rain Forests of Eastern Africa. Cambridge University Press, Cambridge, 351 pp.

Lyal C.H. 2014. 3.7.7 Molytinae Schoenherr, 1823, pp. 529–570. In: Leschen, R.A.B., Beutel, R.G. (eds), Handbook of zoology, Arthropoda: Insecta: Coleoptera. Volume 3: morphology and systematics (Phytophaga). Walter de Gruyter, Berlin.

Machado A., Rodríguez-Expósito E., López M., Hernández M. 2017. Phylogenetic analysis of the genus *Laparocerus*, with comments on colonisation and diversification in Macaronesia (Coleoptera, Curculionidae, Entiminae). Zookeys, 651: 1–77, DOI: 10.3897/zookeys.651.10097

Maddison D. 2016. The rapidly changing landscape of insect phylogenetics. Current Opinion in Insect Science, 18: 77–82, DOI: 10.1016/j.cois.2016.09.007

Maddison W.P., Maddison D.R. 2011. Mesquite: a modular system for evolutionary analysis. Version 3.11. Program and documentation, available at http://mesquiteproject.org.

Maley J. 1996. The African rain forest — main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary, pp. 31–73. In: Alexander, I.J., Swaine, M.D., Watling, R. (eds.), Essays on the ecology of the Guinea-Congo rain forest. Proceedings of the Royal Society of Edinburgh, 104B.

Marshall G.A.K. 1953. On a collection of Curculionidae (Coleoptera) from Angola. Publicaçôes Culturais da Companhia de Diamantes de Angola, 16: 98–119.

Measey G.J., Tolley K.A. 2011. Sequential fragmentation of Pleistocene forests in an East Africa biodiversity hotspot: chameleons as a model to track forest history. PLoS ONE, 6(10): e26606, DOI: 10.1371/journal.pone.0026606

Menegon M., Doggart N., Owen N. 2008. The Nguru mountains of Tanzania, an outstanding hotspot of herpetofaunal diversity. Acta Herpetologica, 3: 107–127, DOI: 10.13128/Acta_Herpetol-2678

Menegon, M., Loader, S.P., Marsden, S.J., Branch, W.R., Davenport, T.R.B., Ursenbacher, U. 2014. The genus *Atheris* (Serpentes: Viperidae) in East Africa: Phylogeny and the role of rifting and climate in shaping the current pattern of species diversity. Molecular Phylogenetics and Evolution, 79: 12–22, DOI: 10.1016/j.ympev.2014.06.007

Meregalli M., Menardo F., Klass K.-D., Cervella P. 2013. Phylogeny of the *Saxifraga*-associated species of *Dichotrachelus* (Insecta: Coleoptera: Curculionidae), with remarks on their radiation in the Alps. Arthropod Systematics and Phylogeny, 71: 43–68.

McKay B.D., Zink R.M. 2015. Sisyphean evolution in Darwin's finches. Biological Review, 90: 689–698, DOI: 10.1111/brv.12127

McKenna D.D., Sequeira A.S., Marvaldi A.E., Farrell B.D. 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. PNAS, 106: 7083–7088, DOI: 10.1073/pnas.0810618106

Miller M., Pfeiffer W., Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, pp. 1–8.

Mitterboeck T.F., Adamowicz S.J. 2013. Flight loss linked to faster molecular evolution in insects. Proceeding of the Royal Society B, 280: 20131128, DOI: 10.1098/rspb.2013.1128

Möller M., Cronk Q.C.B. 1997. Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. American Journal of Botany, 84: 956–965.

Mumbi C.T., Marchant R., Hooghiemstra H., Wooller M.J. 2008. Late Quaternary vegetation reconstruction from the Eastern Arc Mountains, Tanzania. Quaternary Research, 69: 326–341, DOI: 10.1016/j.yqres.2007.10.012

Murienne J., Benavides L.R., Prendini L., Hormiga G., Giribet G. 2013. Forest refugia in Western and Central Africa as 'museums' of Mesozoic biodiversity. Biology Letters, 9: 20120932, DOI: 10.1098/rsbl.2012.0932

Nonnotte P., Guillou H., Le Gall B., Benoit M., Cotten J., Scaillet S. 2008. New K–Ar age determinations of Kilimanjaro volcano in the North Tanzanian diverging rift, East Africa. Journal of Volcanology and Geothermal Research, 173: 99–112, DOI: 10.1016/j.jvolgeores.2007.12.042

Oberprieler R.G., Marvaldi A.E., Anderson R.S. 2007. Weevils, weevils, weevils everywhere. Zootaxa, 1668: 491–520.

Papadopoulou A., Anastasiou I., Vogler A.P. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. Molecular Biology and Evolution, 27: 1659–1672, DOI: 10.1093/molbev/msq051

Peck S.B. 1990. Eyeless arthropods of the Galapagos Island, Ecuador: composition and origin of the cryptozoic fauna of a young, tropical oceanic archipelago. Biotropica, 22: 366–381, DOI: 10.2307/2388554

Ratnasingham S., Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes, 7: 355–364, DOI: 10.1111/j.1471-8286.2007.01678.x

Ratnasingham S., Hebert P.D.N. 2013. A DNA-based registry for all animal species: the barcode index number (BIN) system. PLoS ONE, 8(8): e66213, DOI: 10.1371/journal.pone.0066213

Riedel A. 2011. The weevil genus *Trigonopterus* Fauvel (Coleoptera, Curculionidae) and its synonyms - a taxonomic study on the species tied to its genus-group names. Zootaxa, 2977: 1–49.

Riedel A., Daawia D., Balke M. 2010. Deep cox1 divergence and hyperdiversity of *Trigonopterus* weevils in a New Guinea mountain range (Coleoptera, Curculionidae). Zoologica Scripta, 39: 63–74, DOI: 10.1111/j.1463-6409.2009.00404.x

Riedel A., Sagata K., Suhardjono Y.R., Tänzler R., Balke M.

2013. Integrative taxonomy on the fast track - towards more sustainability in biodiversity research. Frontiers in Zoology, 10: 15, DOI: 10.1186/1742-9994-10-15

Riedel A., Tänzler R. 2016. Revision of the Australian species of the weevil genus *Trigonopterus* Fauvel. ZooKeys, 556: 97–162, DOI: 10.3897/zookeys.556.6126

Riedel A., Tänzler R., Pons J., Suhardjono Y.R., Balke M. 2016. Large-scale molecular phylogeny of Cryptorhynchinae (Coleoptera, Curculionidae) from multiple genes suggests American origin and later Australian radiation. Systematic Entomology, 41: 492–503, DOI: 10.1111/syen.12170

Rovero F., Menegon M., Fjeldså J., Collett L., Doggart N., Leonard C., Norton G., Owen N., Perkin A., Spitale D., Ahrends A., Burgess N.D. 2014. Targeted vertebrate surveys enhance the faunal importance and improve explanatory models within the Eastern Arc Mountains of Kenya and Tanzania. Diversity and Distributions, 20: 1438–1449, DOI: 10.1111/ddi.12246

Saux C., Fisher B.L., Spicer G.S. 2004. Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). Molecular Phylogenetics and Evolution, 33: 457–468, DOI: 10.1016/j.ympev.2004.06.017

Sequeira A.S., Lanteri A.A., Scataglini M.A., Confalonieri V.A., Farrell B.D. 2000. Are flightless *Galapaganus* weevils older than the Galápagos Islands they inhabit? Heredity, 85: 20–29, DOI: 10.1046/j.1365-2540.2000.00690.x

Sequeira A.S., Sijapati M., Lanteri A.A., Roque Albelo L. 2008. Nuclear and mitochondrial sequences confirm complex colonization patterns and clear species boundaries for flightless weevils in the Galápagos archipelago. Philosophical Transactions of the Royal Society B, 363: 3439–3451, DOI: 10.1098/rstb.2008.0109

Shorthouse D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. Program and documentation available at http://www.simplemappr.net.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22: 2688–2690.

Stanley, W.T., Hutterer, R., Giarla, T.C., Esselstyn, J.A. 2015. Phylogeny, phylogeography and geographical variation in the *Crocidura monax* (Soricidae) species complex from the montane islands of Tanzania, with descriptions of three new species. Zoological Journal of the Linnean Society, 174: 185–215, DOI: 10.1111/zoj.12230

Stüben P.E., Astrin J.J. 2010. Molecular phylogeny in endemic weevils: revision of the genera of Macaronesian Cryptorhynchinae (Coleoptera: Curculionidae). Zoological Journal of the Linnean Society, 160: 40–84, DOI: 10.1111/j.1096-3642.2009.00609.x

Štys P., Baňař P. 2013. Eastern Arc Mountains in Tanzania: Hic sunt Aenictopecheidae. The first genus and species of Afrotropical Aenictopecheidae (Hemiptera: Heteroptera: Enicocephalomorpha). European Journal of Entomology, 110: 677–688.

Tänzler R., Sagata K., Surbakti S., Balke M., Riedel A. 2012. DNA barcoding for community ecology - how to tackle a hyperdiverse, mostly undescribed Melanesian fauna. PLoS One, 7: e28832, DOI: 10.1371/journal.pone.0028832

Tänzler R., Toussaint E.F.A., Suhardjono Y.R., Balke M., Riedel A. 2014. Multiple transgressions of Wallace‘s Line explain diversity of flightless *Trigonopterus* weevils on Bali. Proceedings of the Royal Society of London B: Biological Sciences, 28: 20132528, DOI: 10.1098/rspb.2013.2528

Tänzler R., Van Dam M.H., Toussaint E.F.A., Suhardjono Y.R., Balke M., Riedel A. 2016. Macroevolution of hyperdiverse flightless beetles reflects the complex geological history of the Sunda Arc. Scientific Reports, 6: 18793, DOI: 10.1038/srep18793

Tilbury C.R. 2010. Chameleons of Africa - an Atlas. Including the Chameleons of Europe, the Middle East and Asia. Edition Chimaira, Frankfurt am Main, 831 pp.

Tolley K.A., Tilbury C.R., Measey G.J., Menegon M., Branch W.R., Matthee C.A. 2011. Ancient forest fragmentation or recent radiation? Testing refugial speciationmodels in an African biodiversity hotspot. Journal of Biogeography, 38: 1748–1760, DOI: 10.1111/j.1365-2699.2011.02529.x

Toussaint E.F.A., Tänzler R., Rahmadi C., Balke M., Riedel A. 2015. Biogeography of Australasian flightless weevils (Curculionidae, Celeuthetini) suggests permeability of Lydekker‘s and Wallace‘s Lines. Zoologica Scripta, 44: 632–644, DOI: 10.1111/zsc.12127

Vences M., Guayasamin J.M., Miralles A., De la Riva I. 2013. To name or not to name: criteria to promote economy of change in Linnaean classification schemes. Zootaxa, 3636: 201–244, DOI: 10.11646/zootaxa.3636.2.1

Voss E. 1965. Mission zoologique de l'I.R.S.A.C. en Afrique Orientale (P. Basilewsky et N. Leleup, 1957) Résultats scientifiques. Cinquième partie. Coleoptera Curculionidae II (Schluss). Annales du Musée Royal de l'Afrique Centrale, Tervuren (Série in 8°, Zoologie), 138: 293–377.

Ward P. 2011. Integrating molecular phylogenetic results into ant taxonomy (Hymenoptera: Formicidae). Myrmecological News, 15: 21–29.

Weirauch C., Forthman M., Grebennikov V., Baňař P. 2017. From Eastern Arc Mountains to extreme sexual dimorphism: systematics of the enigmatic assassin bug tribe Xenocaucini (Reduviidae: Tribelocephalinae). Organisms Diversity and Evolution, 17: 421–445, DOI: 10.1007/s13127-016-0314-2

Zhang G., Basharat U., Matzke N., Franz N.M. 2017. Model selection in statistical historical biogeography of Neotropical insects – the *Exophthalmus* genus complex (Curculionidae: Entiminae). Molecular Phylogenetics and Evolution, 109: 226–239, DOI: 10.1016/j.ympev.2016.12.039