The origin of the endemic African grasshopper family Lentulidae (Orthoptera: Acridoidea) and its climate-induced diversification

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Abstract
Aim: Forest relicts in the mountainous regions of Africa represent one of the most diverse ecosystems on our planet, but the processes that have generated this remarkable diversity are still poorly understood. We estimate divergence times for an endemic, flightless grasshopper family and reconstruct a potential scenario for their colonization of Africa to test the hypothesis that the diversity of these mountain-top endemics has been generated by multiple fragmentations and reconnections of tropical rain forests in parallel with climatic fluctuations.

Location: Sub-Saharan Africa.

Taxon: Lentulidae (Orthoptera).

Methods: We inferred the phylogeny of 7 genera and 28 species of the Lentulidae based on COI, 16S and Histone 3 sequences by using a Bayesian approach and we also estimated divergence dates. Based on our molecular phylogeny as well as the available information on the relationship of six additional genera and local occurrence records for 120 lentulid species across Africa, we reconstruct a potential colonization scenario for most species of this group.

Results: Our findings indicate that the forest-bound lentulids of East Africa represent a monophyletic group that originates from South Africa. We show that major splits in the phylogeny of the Lentulidae coincide with three known fragmentation events of the African rain forests (27, 16 and 9 Ma) and that lentulids subsequently diversified rapidly in parallel with the aridification and strong geological activity in East Africa.

Main conclusions: Our results corroborate the diversification patterns reported for several endemic African forest-bound animal taxa at small scales and endemic African plant taxa at larger scales, highlighting the finding that much of the biodiversity presently found in the forest relicts of the Eastern Arc Mountains biodiversity hotspot has been generated by the interplay between humid periods that allowed the spread of forest-bound lineages across Africa and periods of aridity-driven isolation of forests and their associated fauna.
1 | INTRODUCTION

The Eastern Arc Mountains of Africa are among the 25 most important global hotspots of diversity (Myers, Mittermeier, Mittermeier, Da Fonseca, & Kent, 2000). This area harbours an overwhelmingly diverse flora and fauna with a high proportion of endemic taxa (Burgess, Fjeldså, & Botterweg, 1998; Fjeldså & Lovett, 1997; Stanley, Kihaule, Howell, & Hutterer, 1998). The high degree of endemism in the Eastern Arc Mountains is generally thought to be the result of its geological age combined with a lack of major disturbance events during the last 30 Myr, a situation that has favoured the persistence of palaeoendemics (Fjeldså, Bowie, & Rahbek, 2012; Fjeldså & Lovett, 1997; Newmark, 2002; Tolley et al., 2011). However, a recent study on the endemic plant family Annonaceae suggested that multiple climate-induced periods of tropical rain forest fragmentation and reconnection (33, 16 and 8 Ma) have been major drivers of the diversification of the African flora (Couvreur, Chatrou, Sosef, & Richardson, 2008). These past climatic fluctuations restricted forest cover during arid periods, leading to the isolation of taxa, while forest expansions during humid periods seem to have allowed subsequent dispersal between refugia (in the case of the Annonaceae between the West-Central Guineo-Congolian region and the coastal and montane regions of East Africa). Similarly, studies on poorly dispersing insect taxa that are endemic to the Eastern Arc Mountains have suggested that clades occurring on different mountains are closely related and relatively young (Hemp, Heller, Warchałowska-Śliwa, & Grzywacz, 2018; Hemp, Kuechler, Kehl, Wägele, & Hemp, 2019; Mlambo, Sole, & Scholtz, 2014). Thus, the high biodiversity in the Eastern Arc Mountains might have two sources: old lineages that have persisted and more recent diversification that occurred in parallel with climatic fluctuations (Tolley et al., 2011).

In our study, we investigate the origin and the diversification of the endemic African flightless orthopteran family Lentulidae (Rowell, Hemp, & Harvey, 2015 and sources therein). Many species in this family occur in diverse landscapes across South Africa, but several species and genera can also be found in mountain ranges across East and East-central Africa (see Table S1). Morphological analyses have suggested that the Lentulidae are divided into two subfamilies, the Lentulinae and Shelforditinae, and three recently erected ungrouped genera (Cigliano, Braun, Eades, & Otte, 2019). Members of Shelforditinae and ungrouped genera are restricted to southern Africa. Most genera of the Shelforditinae include only one or two species. In contrast, the 25 genera of the subfamily Lentulinae are rather species-rich, and many of them, particularly the members of the four genera of the ‘Usambilla group’ and two other genera, Mecostibus Karsch and Mecostiboides Dirsh, are restricted to Central and East Africa (Cigliano et al., 2019). Most members of the Lentulinae are dwellers of the canopy and the herbaceous layer of the forests (Rowell, Hemp, & Harvey, 2015).

In a recent study, Matenaar, Bröder, and Hochkirch (2016) presented a preliminary molecular phylogeny of the Lentulidae that included seven South African genera. They found that a separation of Lentulidae into the two above-mentioned subfamilies is not supported, as the three members of the East African genus Usambilla Sjöstedt and Rhainopomma Jago clustered with the South African genera. According to their interpretation, coastal regions of the Cape Floristic Region probably functioned as refugia during unfavourable periods, whereas orographic changes through the uplift of the Cape Fold Belt as well as oceanic regression repeatedly allowed taxa to disperse into the lowland and coastal habitats of Africa. Furthermore, Matenaar et al. (2016) hypothesized that the Lentulidae originated 70 Ma because some of the oldest lentulid genera (Betisocoides Sjöstedt, Devylidera Sjöstedt) seem to be adapted to typical fynbos plants, which originated during this time.

Here we test the hypothesis that the East African lineages of the Lentulidae originate from southern Africa and that the high diversity of this group found in the Eastern Arc Mountains has been generated by multiple tropical rain forest fragmentation and reconnection events. Specifically, we predicted that the lentulid species found in southern Africa represent older lineages than those species found in more northern regions. Furthermore, if the Lentulidae diversified in parallel with tropical rain forest connectivity variations, their stem-age should be no older than 33 Ma and any major splits in their phylogeny should roughly coincide with the major fragmentation events of endemic African tree taxa (16 and 8 Ma, Couvreur et al., 2008).

2 | MATERIALS AND METHODS

2.1 | Molecular phylogenetic analysis

2.1.1 | Samples

Specimens of lentulid species were collected in Uganda, Kenya and Tanzania, as well as in KwaZulu-Natal (South Africa) between 2007 and 2016. Specimens were identified with the keys published by Jago (1981) and were compared with material from the entomological collections of the National Museums of Kenya in Nairobi, the Natural History Museums in London, and Berlin. Specimens were preserved in 70% alcohol or dried in the field. For each specimen, the locality, collection dates and altitude were noted. For long-term preservation, insect material was transferred to 96% ethanol and stored at 4°C.
DNA was extracted from the muscle tissue of the hind femur using the QIAamp® DNeasy (QIAGEN) and the standard protocol for blood and tissue. The extracted genomic DNA was used as a template for PCR amplification with insect primers modified for Orthoptera. Primers were forward first and reverse second: 16α: 5’-CGC CTG TTT ATC AAA AAC AT-3’ and 16β: 5’-CCG GTC TGA ACT CAG ATC AGC T-3’ for the 16S rDNA (Kocher et al., 1989); H3F: 5’-ATG CTG CGT ACC AAG CAG ACG GC-3’ and H3R: 5’-ATA TCC TTG GGC ATG ATG GTG AC-3’ for the Histone H3 gene (Colgan et al., 1998); and LCO1490: 5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’ for the COI gene (Vrijenhoek, 1994). Amplification was performed under the following conditions: initial denaturation was 5 min at 94°C; 38 cycles of 45 s at 94°C, 45 s at 52°C and 80 s at 72°C; and a final extension step at 72°C for 7 min. The amplification product (2 µl) was loaded onto 1.5% agarose gels for size-fractionation in gel electrophoresis to check the amplification results (Green, Sambrook, & Sambrook, 2012). Purification of the amplification products was performed using the standard protocol of the QIAquick PCR purification kit (QIAGEN). Amplified DNA templates were sequenced at LGC Genomics GmbH. For the final editing of sequences, we used the software CODONCODE ALIGNER. For the phylogenetic analyses, we selected rapidly evolving mitochondrial genes [16S rRNA gene and the barcoding gene cytochrome oxidase subunit I (COI)] and a slowly evolving nuclear gene (Histone 3) to facilitate the recovery of both deep and recent nodes of the phylogeny.

We used PHYLOGENERATOR (Pearse & Purvis, 2013; accessed 1 November 2016), as well as a recent checklist of all Lentulidae and Acridoidea (catalogue of life; accessed 25 October 2016), to automatically search GenBank repositories and download additional sequences of 16S, COI and Histone 3. Sequences for 10 genera and 15 species of Acridoidea were used as the outgroup. Taxon sampling and GenBank accession numbers are given in Supporting Information (see Table S1).

2.1.3 | Alignment preparation and phylogenetic analysis

The molecular data were aligned with the MUSCLE algorithm and by hand in MEGA (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). For each alignment, we ran separate model tests in MEGA. Two different models of nucleotide substitution were selected according to the highest scores of the Bayesian information criterion (BIC; Table S2). For 16S and COI, the most complex model, GTR + I + I was selected; for Histone 3 the HKY + I model was used. To evaluate the robustness of our phylogenetic reconstruction, we compared the results between two different approaches. First, we used the best-fit substitution model to generate phylogenetic trees separately for the three regions 16S, COI and Histone 3, using a maximum likelihood, as implemented in MEGA (Tamura et al., 2013; see trees in Figures S1–S3). Bootstrap replications were set to 1,000 trees, whereas the default settings were used for all other parameters. After adjusting the taxon similarity among alignments by replacing missing sequences with blanks, we used the interface BEAUTI (Drummond, Suchard, Xie, & Rambaut, 2012) to link the three regions 16S, COI and Histone 3 and to create the xml-file for the BEAST analysis. The three alignments added up to 1,627 bp (16S: 557 bp; COI: 688 bp; Histone 3: 382 bp). Both separate clock tests (in MEGA) for each region, as well as the SD of the coefficient of variation of the multi-gene phylogeny (checked in TRACER), indicated that the assumption of rate heterogeneity can be rejected. Therefore, branch lengths were inferred with an estimated strict molecular clock for 250 million MCMC simulations (Yule birth process) using a Bayesian approach implemented in BEAST (Drummond et al., 2012). To assess the posterior distributions of the parameter estimates and to determine the burn-in—based on the point of stationarity on the log-likelihood curves as well as split-frequencies—tree statistics were sampled every 10,000th iteration and subsequently checked with the diagnostics of TRACER. To exclude an appropriate burn-in of 10% from the posterior sample, to summarize the statistics of the remaining trees and to annotate this information to the tree with the highest clade credibility, we used functions of ANNOTATOR (Drummond et al., 2012). The molecular clock was calibrated using three events of volcanism that represent the origin of Mount Hanang, Kenya and Kilimanjaro (Nonnotte et al., 2008) and hence a starting point for their colonization by ancestors of Usambilla chlorophrygana Jago and Usambilla hanangensis Hemp (normal distribution with mean ± SD =2.25 Ma ± 0.12 Ma), ancestors of Altiusambilla keniensis Hemp and Altiusambilla modicicrus Karsch (normal distribution with mean ± SD =2.50 Ma ± 0.20 Ma) and populations of A. modicicrus on Mount Kilimanjaro and Mount Meru (normal distribution with mean ± SD =1.75 Ma ± 0.12 Ma) that led to the differentiation of these taxa. Phylogenetic trees were visualized and formatted in FIGTREE (Drummond et al., 2012).

2.1.4 | Divergence time estimation of the focus nodes

To test for major splits within the phylogeny of the Lentulidae, we defined four morphologically well-supported nodes (Rowell et al., 2015) and compared their divergence time estimates. Specifically, we were interested in the time periods of the stem age of (a) the Lentulidae, (b) the Lentulinae, (c) the ‘Usambilla group’ and (d) the two most diverse genera of the east African ‘Usambilla group’. Following Couvreur et al. (2008), we considered these nodes to be significantly different if the credibility interval (95% HPD, i.e. highest probability density) if the next basal node did not overlap with the credibility interval of the following node (i.e. A ∉ [B, D], B ∉ [C, D]).

2.2 | Phylogeography of Lentulidae

2.2.1 | Extension of the molecular phylogeny

We combined the results of our phylogenetic analysis with information from a preliminary phylogeny of the Lentulidae published by Matenaar et al. (2016). The short segment used in their study
differed from the 16S segment we used, so directly integrating their data into our analysis was not possible. However, combining the topological information from their phylogenetic tree with our phylogenetic hypothesis allowed us to reconstruct the relationships between 13 of 50 genera and 39 internal nodes. Based on this information, 79 species could be inserted at the last common ancestor of their genus, so that the final topology included 120 of the 151 currently recognized species of Lentulidae (Rowell et al., 2015). To evaluate the uncertainty that results from polytomies in the tree (Figure S4), we repeated the ancestral area reconstruction and the calculation of the phylogenetic signal for 1,000 randomly resolved trees using the function `multi2di` of the R-package `ape` (Paradis, Claude, & Strimmer, 2004). Subsequently, for each of these trees, branch lengths of the combined phylogeny were calculated according to Grafen’s method (Grafen, 1989; i.e. the height of each node is calculated as the number of leaves of the subtree minus one) with functions of the R-package `ape` (Paradis et al., 2004).

### 2.2.2 Distributional data

To assess the distribution of lentulid species across the African continent, we compiled occurrence records from Matenaar et al. (2016) and Rowell et al. (2015) as well as location data from type material Cigliano et al. (2019, Orthoptera Species File Online accessed on 24 February 2019). Although most lentulid species are endemic to a single mountain or mountain range, some of them were recorded at several nearby locations. We therefore calculated the average coordinates of all records per species to obtain the species’ range centroids.

### 2.2.3 Phylogeographic analysis

We investigated the association of the diversification and contemporary distribution of Lentulidae using ancestral trait reconstruction as implemented in the R-package `phytools` (Revell, 2017). Variation in the mean latitude and mean longitude of the species’ range centroids were summarized to a single variable using principal component analysis (unrotated) based on a correlation matrix with functions of the R-package `psych` (Revell, 2014). We tested for a phylogenetic signal (Pagel’s Lambda; Pagel, 1999) in the first principal component of the coordinates of species’ range centroid using functions provided in the R-package `geiger` (Harmon, Weir, Brock, Glor, & Challenger, 2008). The phylogeny of the Lentulidae was mapped using the function `phylo.to.map` of the R-package `phytools` (Revell, 2017).

### 3 RESULTS

### 3.1 Molecular phylogenies of the Lentulidae

Our phylogenetic hypothesis (Figure 1) supports the monophyly of Lentulidae and its two sub-families. Species of the genus *Eremedium* Karsch, a genus occurring only in South Africa, were the basal group and the sister group to all East African Lentulidae. Basal nodes were best recovered by the Histone 3 region, whereas 16S and COI resolved the relationships of the terminals. The combination of these regions within a Bayesian phylogenetic approach resulted in high posterior probabilities across the entire phylogeny of East African Lentulidae (Figure 1). The median age of Lentulidae was 26.9 Ma (±4.8 Ma—strict clock; 12.1 Ma ± 5.2 Ma—uncorrelated log-normal clock).

Species of the closely related genera *Mecostiboides* and *Mecostibus*, the latter of which has a southern to eastern African distribution, formed sister groups with the remaining East African genera of the *‘Usambilla group’* (*Altiusambilla* Jago, *Chromousambilla* Jago, *Usambilla* and *Rhainopomma*). The East African genus *Altiusambilla* was the sister group to the remaining three East African genera. Calibration points in our analysis were the morphological and molecular sister pairs *A. modicirusc* on Mount Meru/Kilimanjaro, *A. keniensis* from Mount Kenya, and *U. hanangensis* and *U. chlorophrygana*, which separated approximately 3 Ma, when volcanic events formed these mountains. To evaluate our calibration, we included the Coptacrinae (Acrididae) clade *Parepistaurus* Karsch, which included *Parepistaurus manyara* Green *P. nairoibii* Green and *P. hanangensis* Hemp. The latter species is endemic to Mount Hanang and a sister clade of *Parepistaurus deses* Karsch and *Parepistaurus uguenoensis uguenoensis* Hemp, which are endemic to Mount Kilimanjaro and the North Pare Mountains respectively. The ages of the splits coincided with those found for the genus *Altiusambilla* (Figure 1).

We identified three significant splitting events of lineages within the Lentulidae. The first split, represents the origin of the Lentulidae (Figure 2, node A; 95% HPD: 32.8–21.9 Ma, median: 26.9 Ma); the second, the origin of the ‘Usambilla group’ (Figure 2, node C; 95% HPD: 19.3–12.1 Ma, median: 15.9 Ma); and the third, the origin of the two most diverse genera of the east African ‘Usambilla group’ (Figure 2, node D; 95% HPD: 11.5–7.6 Ma, median: 9.5 Ma). However, our results did not support the hypothesis that the split between the Lentulinae and the Shelforditinae represents a significant splitting event as its HPD (Figure 2, node B; 95% HPD: 27.1–18.0 Ma, median: 22.36 Ma) overlapped with that of nodes ‘A’ and ‘C’. Because this node is phylogenetically well-supported (100% posterior probability), the likely reason for lack of support of a significant splitting event is the rather large uncertainty of the age estimates of the basal splits that result from the terminal position of our calibration points.

### 3.2 Phylogeographic reconstruction

Our phylogeographic analysis showed that older clades of Lentulidae, such as the genera *Devyleria*, *Leatettix* Dirsh and *Uvarovidium* Dirsh, have more southern distributions than younger clades, such as the genera of the ‘Usambilla group’. This pattern is reflected by a
very strong phylogenetic signal in the first principal component of the coordinates of species' range centroids (mean Lambda = 0.90, $P < 0.001$; see Figure S5).

3.3 | Taxonomic remarks

Our multi-locus phylogenetic reconstruction supported the recently debated monophyly of the two lentilid subfamilies Lentulinae and Shelforditinae (Figure 2; cf. Matenaar et al., 2016), while also underscoring the differences in the internal genital morphology between the groups. The Shelforditinae are characterized by an attenuated or divided endophallus, whereas Lentulinae have simple rod-like endophalli, as detailed in Lentula or Altisambilla (Ritchie, 1982).

By providing the first molecular phylogeny for species of the genera Mecostibus and Mecostiboides, we found that this ‘Mecostibus group’ is not monophyletic. Mecostibus minor and Mecostiboides physalus formed an inner group with Mecostibus rubripes and Mecostibus sellatus as sister taxa. In addition, the inclusion of the latter species into the genus Mecostibus was poorly supported. The status of this phylogenetically recovered separate
genus calls, in fact, for a systematic revision, as it is consistent with certain morphological differences between the two groups: *M. sellatus* is a large species with a very verrucose cuticula and conspicuous bumps on the pronotum, whereas both *M. minor* (Bruner) and *M. rubripes* Dirsh as well as *M. physalus* Dirsh are much smaller species that share a shiny smooth integument and lack bumps on the pronotum.

**FIGURE 2** Dated phylogenetic reconstruction of the relationships between 7 genera and 27 species of the orthopteran family Lentulidae from a Bayesian multi-gene analysis of the COI, 16S and Histone 3 region of 125 specimens. Sequences for 15 additional species of Acridoidae were used as the outgroup (grey branches). Branch lengths and median node ages (node bars indicate 95% confidence intervals) represent averages across 250 million independent runs annotated to the tree with the highest clade credibility after excluding an appropriate burn-in of 10%. In addition, median node ages (black tick marks) and 95% confidence intervals of four hypothesized main splits in the Lentulidae are shown above the scale (the origin of Lentulidae—node A, red bar; the origin of the Lentulinae—node B, yellow bar; the origin of the forest-bound ‘Usambilla group’—node C, green bar; and the origin of the two most species-rich genera of the ‘Usambilla group’—node D, blue bar). Median node ages (black tick marks) and 95% confidence intervals of species-level splits within the Lentulidae are indicated as grey bars. Node ages were estimated by calibrating a strict molecular clock with the formation of Mount Kilimanjaro and Mount Meru that separated sister species pairs and populations (red triangles).

**4 | DISCUSSION**

Our study aimed to reconstruct the origin of the endemic African grasshopper family Lentulidae and determine the timing of its diversification to understand the role of climatic fluctuations at evolutionary time-scales for shaping the insect diversity of the Eastern Arc Mountains. Our results show that the East African lentulid species are
descendants of the subfamily Lentulidae that originated from ancestors in South Africa. Our phylogeographic reconstruction indicates that the high diversity of the East African Lentulidae resulted from two main colonization waves of the forest-bound ancestors of the ‘Mecostibus group’ and the ‘Usambilla group’. Particularly, the latter group, to which most species in the Eastern Arc Mountains belong, rapidly diversified in parallel with periods of high geological activity between 9 and 3 Ma. Thus, whereas high concentrations of endemic species are often interpreted to be the result of historical climatic stability (reviewed in Harrison & Noss, 2017), our analyses show that multiple climate-induced fragmentations and reconnections have been an important driver of the diversification of lineages and the distributions of endemic taxa over evolutionary time-scales. In line with these findings, recent studies have suggested that intermediate levels of climatic stability favour diversity because of the persistence of both old and recent radiations (Cowling et al., 2015), and that low climate stability during the last 21,000 years can also positively affect species richness and endemism (Pinkert et al., in revision).

### 4.1 The origin of the Lentulidae

In line with the age of the oldest fossil record of the superfamily Acridoidea that includes all taxa considered in our analysis (Scudder, 1885), our independent divergence estimation suggests that the Lentulidae are much younger than previously thought [27 (33–22) Myr cf. 85 (113–57) Myr, as suggested by Song et al. 2015]. Its two subfamilies separated approximately 22 Ma at the end of the Oligocene. The lentulid species of East Africa stem from forest-bound lineages that colonized this region in two waves (Figure 3). In a first wave, ancestors of the genus Mecostibus and the monotypic genus Mecostiboides seem to have spread towards the more northerly regions of Africa in parallel with a period of forests’ expansion in Africa (Couvreur et al., 2008). This event is also thought to be responsible for a spread of forest-bound taxa in chameleons (Tolley et al., 2011) and some scarabaeid genera (Daniel, Sole, Davis, Strümpher, & Scholtz, 2020). Mecostibus and Mecostiboides form the most basal of the East African lentulid genera, and their distributions are predominately restricted to coastal and inland Tanzania (Figure 1). The only exception from this pattern is M. sellatus Uvarov, which clusters as the species of the 'Usambilla group' and is widespread in savanna habitats in Africa (but see Results for remarks of the systematic status of this species). In the second wave, approximately 15 Ma, the ancestors of the genera collectively called the ‘Usambilla group’ (Altiusambilla, Chromousambilla, Usambilla and Rhainopomma) seem to have reached East Africa (Figures 2 and 3). A latter lineage split approximately 9 Ma, resulting in the two most diverse East African lentulid genera: Rhainopomma and Usambilla. A broadly similar diversification pattern has been reported for the African tree family Annonaceae, with East African endemics (stem age: approximately 33 Myr) having split up approximately 17 and 8 Ma (Figure 2; Couvreur et al., 2008). Together, with the strong association of the East African lentulid lineages with forest habitats, these results suggest that the expansion of forests during relatively humid periods might have created corridors that allowed the flightless, forest-bound ancestors of ‘Mecostibus group’ and of ‘Usambilla group’ to colonize East Africa.

### 4.2 Radiations of the East African Lentulidae

Previous studies have shown that more species and higher concentrations of endemic species are generally found in regions that experienced low climate change since the last glacial maximum (Araújo et al., 2008; Harrison & Noss, 2017; Pinkert et al., 2018). However, the influences of climatic fluctuations and of high geological activity over longer scales of time are still poorly understood (but see Cowling et al., 2015). Our results suggest that not only most splits within the main lineages of Lentulidae but also the rapid diversification of East African lentulids coincide with periods of high climatic and geological instability. Thus, particularly in the highly diverse East African ‘Usambilla group’, many species-level splits occurred during a period of aridity between 8 and 5 Ma (Figure 2). This arid period is characterized by the uplift of the Tanganyikan Plateau in the East African Rift, which has played a crucial role in the aridification of (Sepulchre et al., 2006) and the extension of savannas in eastern Africa (Jacobs, 2004). In addition, the regional effect of volcanic activity between 5 and 3 Ma in East Africa and the resulting climatic fluctuations (Sepulchre et al., 2006; Trauth, Maslin, Deino, & Strecker, 2005) are reflected by several splits at the species level in the ‘Usambilla group’ (Figure 2), indicating that the diversity of the Eastern Arc Mountains stems from young endemics rather than from high concentrations of palaeoendemics.

Our results resemble the diversification pattern of the Eastern Arc mountain endemics of the two flightless tettigoniid genera Altihoratosphaga Hemp and Monticolaria Sjöstedt (Orthoptera, Tettigonioidea, subfamily Phaneropterinae, Voje, Hemp, Flagstad, Saetre, & Stenseth, 2009) and the flightless acridid genus Parepistaurus (Orthoptera, Acrididae: subfamily Coptacrinai, Hemp, Kehl, Schultz, Wägele, & Hemp, 2015). Indeed, by incorporating sequences of the latter into our analysis, we were able to confirm that splits between the two Altiusambilla species (used for dating the phylogeny, 3.0 Myr) occurred at a similar time as those of the Parepistaurus species (estimated based on the molecular clock, 3.3 Myr). The two youngest genera of the ‘Usambilla group’, Rhainopomma and Usambilla, separated approximately 9 Ma (Figure 2). Almost all members of these two species-rich genera are dwellers of the herb layer of the sub-montane to montane forests in the Eastern Arc Mountains, and when projected onto a map, their molecular relationships (Figure 3) showed that they probably speciated when populations became isolated on the Eastern Arc Mountains during arid periods.

### 4.3 Limitations and extensions

Compared with the stem-age of the Lentulidae provided by a recent molecular phylogeny of the Orthoptera (Song et al., 2015) our
divergence time estimate was at least 24 Myr lower. This difference may have two reasons. First, the estimates provided by Song et al. (2015) are based on hundreds of mitochondrial loci (complete genomic sequences). This high information content is essential to resolve basal phylogenetic relationships, but mitochondrial genes exhibit different substitution rates that introduce a large scatter in estimates derived from a single overall molecular clock model (in their case 113–57 Myr). In addition, due to their high saturation for older divergences and the common focus on older calibration points, dates derived from mitochondrial data are typically overestimated and systematically biased towards calibrations points for basal nodes (Zheng, Peng, Kuro-o, & Zeng, 2011). Here it is important to note that despite being based on only three loci, our divergence time estimation also included a nuclear marker and these data provided independent support for the oldest definitive record of the superfamily Acridoidea (dated to 38–34 Myr; also used in Song et al. (2015)] as well as for previously reported divergence time estimates of splits within the genus Parepistaurus (Hemp et al., 2015). Second, arguably due to the major cost- and time-effort of complete genomic sequencing, the phylogeny by Song et al. (2015) only includes one representative of the Lentulidae. Our results underline the complementarity of genomic and readily available single-locus

Figure 3 Map showing the range centroids (black triangles) of 120 of the 151 extant lentulid species across the African continent and their phylogenetic relationship. Branch colours indicate the position of each species in the phylogenetic tree. To evaluate the uncertainty in the phylogeny (polytomies), the original tree topology was randomly resolved, and the analysis was repeated for 250 alternative phylogenies (one of them is displayed in the lower right corner). The genera Leatettix and Uvarovidium are Shelforditinae (2 of the total 10 recognized genera) and the remaining genera are Lentulinae (12 of the total 22 recognized genera). Of the four genera for which the subfamily association is currently unclear, two occur in western and two in eastern South Africa.

Rhainopomma
Chromousambilla
Altusambilla
Mecostitus & Mecostiboides
Eremidium
Lentula
Basutacris
Gymnidium
Betiscoides
Leatettix
Uvarovidium
Devylderia
data as well as the potential of integrating both types of information. Further studies may therefore expand the backbone phylogeny of the Orthoptera with additional nuclear loci, complete genomic sequences and younger effective calibrations points to improve the accuracy of divergence estimates of recent nodes (e.g. family-level). However, the most cost- and time-effective alternative to merely extending available phylogenies is probably the use of hybrid phylogenetic analyses that allow integrating both complete genomic and single-locus data (e.g. Zhou et al., 2016) and we expect that future orthopteran research will greatly benefit from the data complementarity that hybrid phylogenetic approaches can exploit.

### 4.4 Implications for conservation

Our results confirm those of small-scale studies on other forest-bound animals (Hemp et al., 2018, 2015; Hemp, Kuechler, Kehl, Waegele, & Hemp, 2019; Portillo et al., 2018; Tolley, Townsend, & Vences, 2013; Voje et al., 2009), and they resemble the diversification pattern of the endemic African floras (Couvreur et al., 2008; Nyman, Linder, Peña, Malm, & Wahlberg, 2012; Pokorny et al., 2015), highlighting that the remarkable diversity today found in the forests of the Eastern Arc Mountains has been generated by several climate-induced isolation events. Understanding such diversification and distribution patterns of the biodiversity in mountainous regions of Africa is not only an important theoretical issue, but also has crucial implications for conservation. The similarities between our results and the diversification pattern reported for tree taxa (Couvreur et al., 2008) underscores the assumption that the phylogenetic diversity of tree species can serve as a surrogate for the phylogenetic diversity of a broad spectrum of the biodiversity found in forests. Moreover, the majority of lentulid species and probably many other insect taxa are endemic to small forest relics on only one or two mountains. Therefore, our study highlights these forest patches as areas of high importance for conservation because they promoted the diversification of young lineages of taxa endemic to East Africa through a dynamic history of habitat fragmentations and reconstructions. However, although the Eastern Arc Mountains have long been recognized as a highly threatened diversity hotspot (Myers et al., 2000) the pressure on these unique forest ecosystems has even increased during the last two decades (Ahrends et al., 2010; Williams, 2013). Many fast-growing cities of Africa are adjacent to the established protected areas, which has increased human encroachment, with disturbances, illegal hunting and illegal tree cutting at an alarming rate (Burgess et al., 2007; Williams, 2013). Therefore, our results reinforce the need for stricter protection of the existing protected areas as centres of speciation and potential arcs of evolutionary history, as well as stress the need for programmes that can help us developing effective alternatives in the face of the demands of a rapidly increasing human population in this region. Furthermore, given the buffering and refugial function of these forests, their high fragmentation and the low dispersal ability of many forest-bound taxa, new conservation areas might enhance the connectivity among forest patches along elevational gradients, to enable range shifts of species along corridors of suitable habitat (Saura et al., 2018).

### 5 Conclusion

Our molecular phylogenetic study on the endemic African grasshopper family Lentulidae suggests that this family originated in South Africa, probably in the Cape Region, approximately 27 Ma, which coincides with the phylogeographical history of several taxa (i.e. the Cape Region origin of East African taxa, reviewed in Linder et al., 2012). Ancestors of the subfamily Lentulinae seem to have colonized East and Central Africa via forested areas of mountains and mountain ranges. Our phylogeographic analysis shows that older clades of Lentulidae have a more southern distribution than younger clades, especially those of the ‘Usambilla group’. This pattern is reflected by a strong and significant phylogenetic signal in the first principal component of the coordinates of the species’ range centroids. Splits within the East African taxa coincide with the beginning of aridification in Africa approximately 8 Ma and a successive spread of dry savanna habitats triggered diversification at the genus level in the ‘Usambilla group’. The latter genera, which comprise most of the species in the Eastern Arc Mountain diversity hotspot, seem to have rapidly speciated in parallel with the progressive conversion of the African rainforest to savannas and with the climatic fluctuations imposed by high geological activity (Sepulchre et al., 2006; Trauth et al., 2005). Thus, whereas the climate stability, particularly long periods of humidity, seem to have facilitated major dispersal events of lineages of the Lentulidae, our results indicate that the high biodiversity and degree of endemism in the Eastern Arc Mountains is the result of climatic fluctuations in this area.

### ACKNOWLEDGEMENTS

We acknowledge support from the Deutsche Forschungsgemeinschaft and thank the Tanzania Commission for Science and Technology and the Tanzania Wildlife Research Institute for permitting this research (no. 2016-102-ER-96-44). We thank the National Museum of Kenya, Nairobi for continuous support and collaboration. We also thank Neil and Tanza Crouch for making it possible to collect lentulids in KwaZulu-Natal, South Africa. Part of the research received support from the Synthesys Project http://www.synthesys.info/, which is financed by the European Community Research Infrastructure Action under the FP6 ‘Structuring the European Research Area Programme’ and enabled C.H. to visit various entomological collections in Europe.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in ‘DRYAD’ (https://doi.org/10.5061/dryad.c59zwr34f). Sequence data are openly available at GenBank (www.ncbi.nlm.nih.gov/genbank/).

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Author contributions: C.H. and S.P. conceived the ideas; C.H. wrote the first draft; C.H. and S.P. collected the data; C.S. and S.P. analysed the data; and R.B. and S.P. led the writing; all authors contributed significantly to the revisions.

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