Pleistocene origin and colonization history of Lobelia columnaris Hook. f. (Campanulaceae: Lobelioideae) across sky islands of West Central Africa

Miguel Perez Perez[1] and Wen-Bin Yu[2]

[1]Northern Arizona University - Flagstaff Mountain Campus
[2]Xishuangbanna Tropical Botanical Garden

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Abstract

We aimed to infer the phylogenetic relationships of populations of Lobelia columnaris using chloroplast genomes and estimate the divergence time to reconstruct its historical colonization on the sky islands of Bioko and Cameroon. Specifically, we aim to answer the following questions: (1) What is the phylogenetic relationship among Bioko Island and Cameroon populations? (2) Are the older populations found on the older sky islands? (3) Does the colonization history reflect the age of the sky islands? We assembled novel plastomes from 20 individuals of L. columnaris from five mountain systems. The plastome data was explored with phylogenetic analyses using Maximum likelihood and Bayesian Inference. The complete plastome size varied from 164,609 bp to 165,368 bp. The populations of L. columnaris have a monophyletic origin, subdivided into three plastome-geographic clades. The plastid phylogenomic results and age of the sky islands indicate that L. columnaris colonized first along the Cameroon Volcanic Line's young sky islands. The earliest divergent event (1.54 Ma) split the population in South Bioko from those on the mainland and North Bioko. The population of South Bioko was likely isolated during cold and dry conditions in forest refugia. Presumably, the colonization history occurred during the middle-late Pleistocene from South Bioko's young sky island to North Bioko and the northern old sky islands in Cameroon. Furthermore, the central depression with lowland forest between North and South Bioko is a current geographic barrier that keeps separate the populations of Bioko from each other and the mainland populations. The Pleistocene climatic oscillations led to the divergence of the Cameroon and Bioko populations into three clades. L. columnaris colonized the older sky island in mainland Cameroon after establishing South Bioko's younger sky islands. The biogeography history was an inverse progression concerning the age of the Afromontane sky islands.

Introduction

The Pleistocene's climate and environmental oscillations caused fragmentation and expansion of lowland tropical rain forests, mountain forests, and savannas of Upper Guinea and Lower Guinea, Africa (Duminil et al. 2015). These climate oscillations shaped Afro-alpine species distribution, such as *Lobelia giberroa,* which occurs on mountains with altitudes between 3500 and 6000 m in East Africa (Kebede et al. 2007). Forest fragmentation during the Pleistocene was a driver that gave origin to allopatric speciation of plants and animals. Likewise, forest refugia and rivers contributed to current diversity patterns in West Central Africa (Nicolas et al. 2012). Moreover, during the Pliocene-Pleistocene epochs, there was substantial volcanic activity in West Central African and East Africa, such as the Eastern Arc Mountains of Tanzania and Kenya (Measey & Tolley 2011).

The Cameroon Volcanic Line (CVL) is an 1800 km SW-NE topographical feature that extends from the Gulf of Guinea to onshore central Cameroon (Adams et al. 2015). This volcanic chain was an ancient forest refuge in West Central Africa (Demenou et al. 2020). The CVL comprises plateaus and 11 dormant volcanoes,

and Mount Cameroon, a currently active volcano (Chauvel et al. 2005). Only three volcanic peaks in the CVL have elevations above 3000m (Pico Basilé, Mt. Cameroon and Mt. Oku). The geological structure of the CVL is a combination of tectonic and volcanic origins with unequal ages ranging from the middle to late Tertiary (Jesus et al. 2005). The oldest mountains are in the north, with a trend of decreasing age of volcanic activity in the southern area (Missoup et al. 2016). The marked geographical separation and isolation of the mountains are analogous to islands in the sky or sky islands. Sky islands are considered natural laboratories for studying evolutionary patterns and processes that lead to the accumulation of diversity (Cox et al. 2014). Moreover, the sky islands and sky island archipelagos of the Gulf of Guinea and West Central Africa possess an extraordinary diversity of angiosperms (Figueiredo 1994), small mammals (Missoup et al. 2016), and amphibians (Zimkus & Gvoždík 2013). This African region is part of the Guinea biodiversity hotspot (Myers et al. 2000) and is critical for the conservation of endemic species of plants and animals that inhabit the sky islands (Tropek & Konvicka 2009).

Giant lobelias may have experienced rapid diversification in East Africa mountains and subsequently dispersed to West Africa (Knox & Li 2017). *Lobelia columnaris* Hook f. (Campanulaceae: Lobelioideae) is listed as a vulnerable species in the IUCN Red List of Threatened Species 2015 (Cheek & Thulin 2015). *L . columnaris* and *L . barnsii* Exell (Mabberley 1974a) are the two giant lobelia species known from the tropical West Central Africa and Gulf of Guinea. *L . columnaris* is endemic to Bioko's mountains and highlands (Equatorial Guinea), Nigeria, and Cameroon. This giant lobelia grows in discontinuous populations between 1000m to 3000m in different ecological habitats including submontane grasslands, forest clearings transformed into grasslands overgrown with *Pteridium aquilinum* (L.) Kuhn*, and along streams and subalpine meadows.

Little is known about the origin and colonization histories of angiosperms in Bioko and Cameroon. However, a few phylogeographic studies have documented the lineages of plants and animals currently present in Bioko and Cameroon. For example, the Afromontane genus *Lynchis* had several dispersals from Ethiopia and the Western Rift Mountains and recently dispersed to West Africa (Popp et al. 2007). The hypothesis of recurrent connections over time between the West and East African mountains provides a framework to discuss the biogeographical origin among close relatives in both bioregions, like the endangered and endemic Mount Oku rat, *Lamottemys okuensis* in the CVL (Missoup et al. 2016). However, some taxa have a geographically widespread distribution, from the eastern mainland to the outlying islands of western Africa, like the endangered *Prunus africana* (Dawson & Powell 1999).

The present study's objective is to infer the phylogenetic relationships of populations of *L. columnaris* using chloroplast genomes and estimate the divergence time to reconstruct its historical colonization on the sky islands of Bioko and Cameroon. Specifically, we aim to answer the following questions: (1) What is the phylogenetic relationship among Bioko Island and Cameroon populations? (2) Are the older populations found on the older sky islands? (3) Does the colonization history reflect the age of the sky islands?

Materials & Methods

Study area

Bioko Island lies on the continental shelf and is separated from the Cameroon coast by 32 km of shallow water (60m). Bioko was separated from mainland Africa 10, 000 years ago by the rise of sea level at the end of the last glacial period (Schabetsberger et al. 2004).

A recent study of the geochemistry of volcanic rocks from Bioko dates the main three strato-volcanoes at <1.3±0.07 Ma; K/Ar (Yamgouot et al. 2016). This dating indicates that the sky islands of Bioko (South and North) are very young without difference in age. Furthermore, a central depression with a maximum elevation of 500m separates Pico Basilé (3011m) in the North and the Pico Biao-Moka (2009m) and the Gran Caldera (2260 m) in the South (Schabetsberger et al. 2004). The vegetation of Bioko is arranged in elevational rings dominated by Guineo-Congolian tropical species with Afromontane elements appearing at higher elevations (Fa et al. 2000).

Mainland Cameroon comprises several Afromontane sky islands. In the southwest Mount (Mt.) Cameroon is

the highest volcano (4085m) of West Central Africa. In the northeast, Mount Oku is another high peak with an elevation of 3011m. Likewise, Bamenda Plateau and Bambutos Mountains represent the orography of the North region. Mountains from the North Central, such as Mt. Oku and Bamenda-Banso highlands (2260m), uplifted during the Cenozoic (Oligocene to Miocene) (Missoup et al. 2016). Mainland southern volcanoes like Mt. Cameroon are the youngest with origins during the Pliocene to Pleistocene (Zimkus & Gvoždík 2013).

The vegetation is arranged in elevational bands within the montane forest and has been highly disturbed by grazing, fire, and human activities, except on Mt. Cameroon (Ineich et al. 2015).

Study plants, DNA extraction and sequencing

Lobelia columnaris is a semelparous herb or shrub up to 1 m, with curved purple corollas organized on a terminal or multi-terminal panicle. The protandrous flowers are present in the dry season and live approximately ten days (Bartos et al. 2012). The flowers produce sugared nectar that attracts birds such as the Orange-tufted sunbird (*Cinnyris bouvieri*) (Janecek et al. 2012; Riegert et al. 2011) and Hymenoptera insects. The flowers are considered bee-pollinated (Givnish et al. 2009). The fruit is a capsule with thousands of winged seeds (Mabberley 1975) dispersed by wind (Knox & Palmer 1998; Mabberley 1975).

Fieldwork was conducted in February and March of 2015 with permits from Universidad Nacional de Guinea Ecuatorial, Republic of Equatorial Guinea, and from the Ministry of Scientific Research and Innovation, Republic of Cameroon. Locality and collection data on the samples of *L. columnaris* used in this study are given in Table 1. Voucher specimens are deposited in the Philadelphia Herbarium (PH). Voucher duplicates were deposited in the National Herbarium of Cameroon (YA) and Flora of Guinea-Real Jardín Botánico Madrid (MA).

Table 1. Locality and collection data of *Lobelia columnaris*

Region	Population (# ind)	Sky Island	Elevation (m)
North Bioko	3104 (2)	Pico Basilé	2947
North Bioko	3105	Pico Basilé	2836
North Bioko	3106 (2)	Pico Basilé	2322
South Bioko	3107 (2)	Gran Caldera	1001
South Bioko	3108	Pico Biao-Moka	1582
South Bioko	3109	Pico Biao-Moka	1539
South Bioko	3110 (2)	Pico Biao-Moka	1157
Cameroon	3111	Mt. Oku	2381
Cameroon	3112 (2)	Mt. Oku	2522
Cameroon	3113	Mt. Oku	2197
Cameroon	3114	Mt. Oku	1977
Cameroon	3116	Mt. Oku	2407
Cameroon	3117	Mt. Bamenda	1798
Cameroon	3118 (2)	Mt. Bamenda	1723

DNA was extracted from silica-dried leaf samples of 20 individuals of *L. columnaris* using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle 1987). Library construction, sequence generation, and bioinformatics processing were done at the Indiana University Center for Genomics and Bioinformatics. Sequence generation was performed using Illumina NextSeq and Illumina MiSeq platforms. Additionally, we downloaded 22 plastome sequences of 22 taxa of Campanulaceae from TreeBASE no. S15797 (Knox 2014) and GenBank accessions (Knox & Li 2017).

A total of 20 individual plastid genomes of *L. columnaris* were newly sequenced and assembled. Eleven plastomes represent populations from three sky islands in Bioko (Equatorial Guinea), and nine plastid genomes are from two sky islands in Cameroon (Figure 1). Moreover, the phylogenetic tree included two

more plastome sequences of *L. columnaris* from South Bioko (Perez Perez 3103, MF061188) and another from Mt. Cameroon (Muasya 2085, MF061187) obtained from GenBank (Knox & Li 2017) .
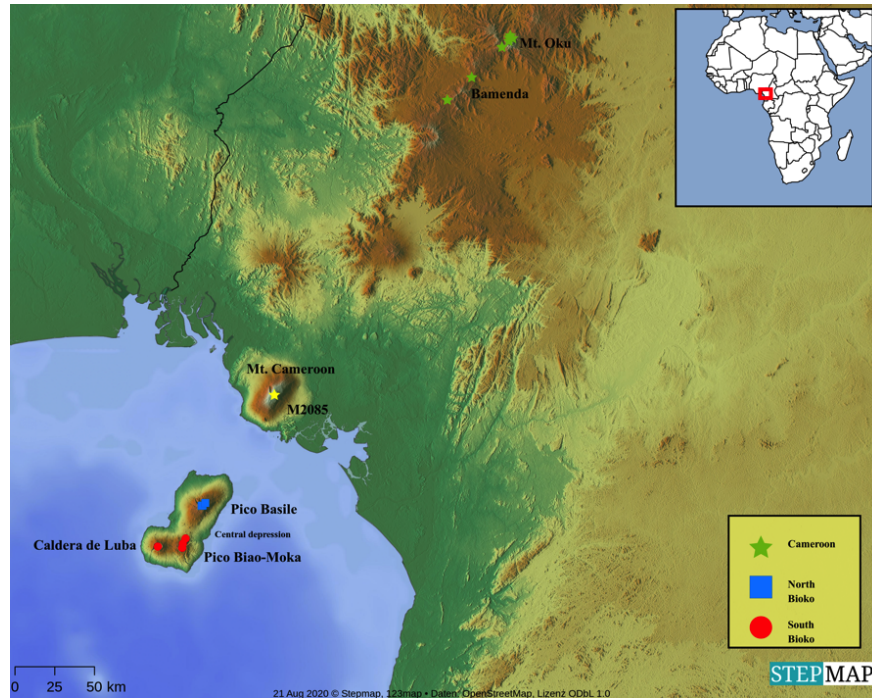

Figure 1. Geographic distribution of plastomes of *Lobelia columnaris*.

The total dataset included 44 plastomes (22 of *L. columnaris* and 22 from other Campanulaceae taxa).

Assembling and annotation of the chloroplast genomes

The raw paired-end reads were filtered and *de novo* assembled using the GetOrganelle toolkit (Jin et al. 2020). The filtered reads were assembled using the SPAdes version 3.9 using *k-mer* 21, 33, 45, 65 and 85 (Bankevich et al. 2012). To retain pure chloroplast contigs, the final "fastg" files were filtered using the "slim" script of GetOrganelle toolkit. The filtered De Brujin graph was viewed and the final sequence exported using Bandage (Wick et al. 2015). The chloroplast genome was automatically annotated using CpGAVAS (Liu et al. 2012), then adjusted using Geneious version 9.0 (Kearse et al. 2012).

Phylogenetic analyses

The whole chloroplast genome matrix was aligned using MAFFT version 7.1 (Yamada et al. 2016). Only one inverted repeat region was used in the phylogenetic analyses. Two matrixes were prepared, one including all gaps, and another removed gaps using trimAl (Capella-Gutiérrez et al. 2009) by "-gt 0.4 -st 0.001 -cons 40". Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used to reconstruct phylogenetic trees. No nucleotide positions were excluded from analyses. The ML tree analyses and bootstrap estimation of clade support were conducted with RAxML version 8.2.10 (Stamatakis et al. 2008). These analyses used the GTR substitution model with gamma-distributed rate heterogeneity among sites and the proportion of invariable sites estimated from the data. Support values for the node and clade were calculated from 1000 bootstrap replicates. Bootstrap support (BS) [?] 70 is considered as well supported (Hillis & Bull 1993). The BI analyses were performed using MrBayes version 3.2.6 (Ronquist & Huelsenbeck 2003), with DNA substitution models selected for each gene partition by the Bayesian information criterion (BIC) using jModeltest version 2.1.10 (Darriba et al. 2012; Guindon & Gascuel 2003). Markov Chain Monte Carlo (MCMC) analyses were run in MrBayes for 10,000,000 generations, with two simultaneous runs, and each

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run comprising four incrementally heated chains. The BI analyses were started with a random tree and sampled every 1000 generations. The number of generations for the three datasets was sufficient because the average standard deviation of split frequencies for the datasets was lower than 0.005, and Potential Scale Reduction Factor (PSRF) of Convergence Diagnostic (Gelman & Rubin 1992) for the datasets was 1.00. The first 25% of the trees were discarded as burn-in, and the remaining trees were used to generate a majority-rule consensus tree. Posterior probability values (PP) [?] 0.95 were considered as well supported (Alfaro et al. 2003; Erixon et al. 2003; Kolaczkowski & Thornton 2007). Both ML and BI analyses, as well as jModeltest, were performed at the CIPRES Science Gateway (http://www.phylo.org).

Estimation of divergent times and phylogeographical history

Dating analyses were conducted using Markov Chain Monte Carlo (MCMC) methods in BEAST version 2.4 (Bouckaert et al. 2014), which was performed at the CIPRES Science Gateway (http://www.phylo.org). The setting parameters in BEAUti, we chose "BEAST model test" for "Site model", "Relaxed Clock Log Normal" for "Clock model", and "Yule Model" for speciation. Meanwhile, we selected two crown nodes for calibrations from published data using the CladeAge package (Matschiner et al. 2016). (1) The *Lobelia thuliniana – L. columnaris* clade was 8.0 Mya (sigma 1.0, offset: 0.0), and (2)*Lobelia* laxiflora - *L. columnaris* clade was 21 Mya (sigma 2.0, offset: 0.0) (Chen et al. 2016; Knox 2014). For reconstructing the phylogeographical history of *L. columnaris* , a plastome phylogeny of the *L. thuliniana – L. columnaris* clade including 22 samples of *L. columnaris* and five outgroups was estimated in BEAST2 with "beast-classic package" of BEAUti. For each dataset, MCMC ran 200,000,000 generations and sampled every 20,000 generations. The first 5,000 generations were removed as "Pre Burnin". Log output of the BEAST analysis was evaluated using Tracer version 1.6. Effective sample sizes (ESS) of all parameters were more than 200, indicating that the estimations were confident. Maximum clade credibility (MCC) tree was generated using TreeAnnotator by setting "Mean heights" for the "Node heights". The MCC tree was visualized using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Characteristics of plastomes

The plastome of *L. columnaris* is circular and quadripartite by having a large single copy (LSC), a small single copy (SSC), and two inverted repeated (IR) regions. The complete plastome size of varied from 164,609 bp to 165,368 bp, with LSC from 82,421 bp to 82,904 bp, SSC from 7,950 bp to 8025 bp, and IRs from 37,028 bp to 37636 bp (Figure 2). The GC content of the whole plastome was 39.2 %. The plastome contained 117 unique genes, including 80 recognized and three predicted protein-coding regions (CDS), 30 tRNA, and 4 ribosomal RNA (rRNA) genes. The IR regions had 14 CDS, seven tRNA, and four rRNA genes.
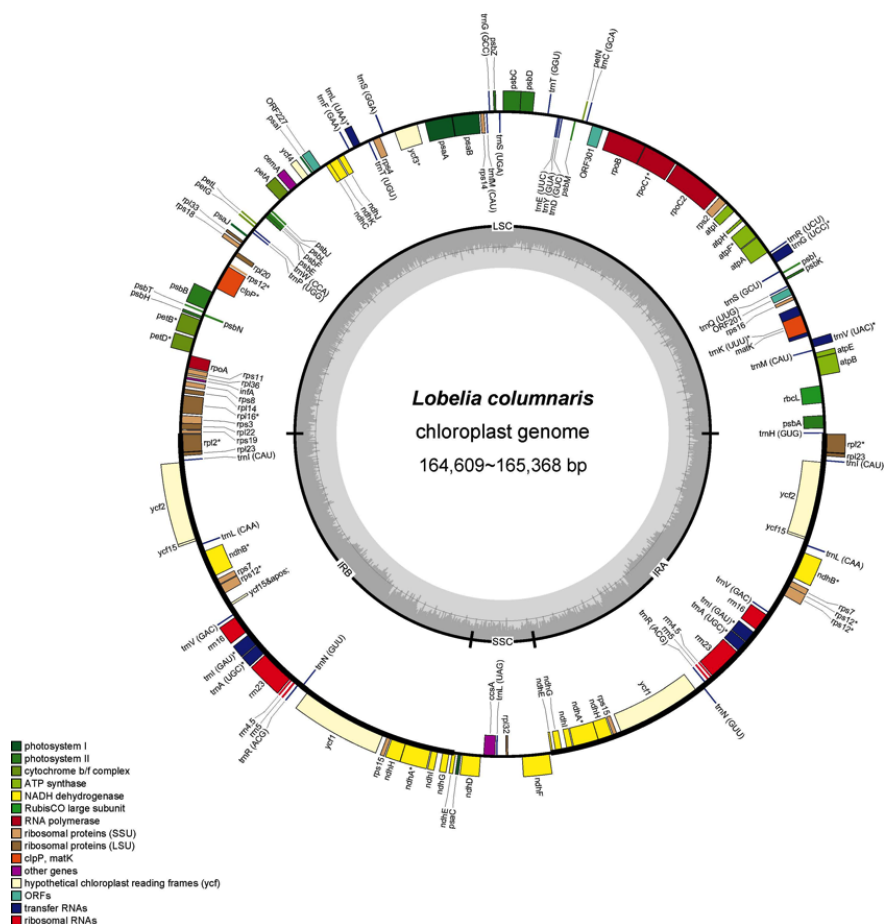
Figure 2. Map of newly sequenced plastome of *Lobelia columnaris* .

Phylogenetic relationships

Our phylogenetic results using maximum likelihood (ML) and Bayesian inference (BI) generated identical tree topologies. We recovered a monophyletic relationship among the populations of *L. columnaris* from Bioko and Cameroon. However, we distinguished three highly supported clades in the phylogenetic tree. A common ancestor closely relates the mainland Cameroon clades (Mt. Cameroon, Bamenda, and Mt. Oku) with North Bioko (Pico Basilé) sister to these clades. The clade of southern Bioko (Pico Biao-Moka and Gran Caldera) is distinct and sister to the previous subclades (Figure 3).
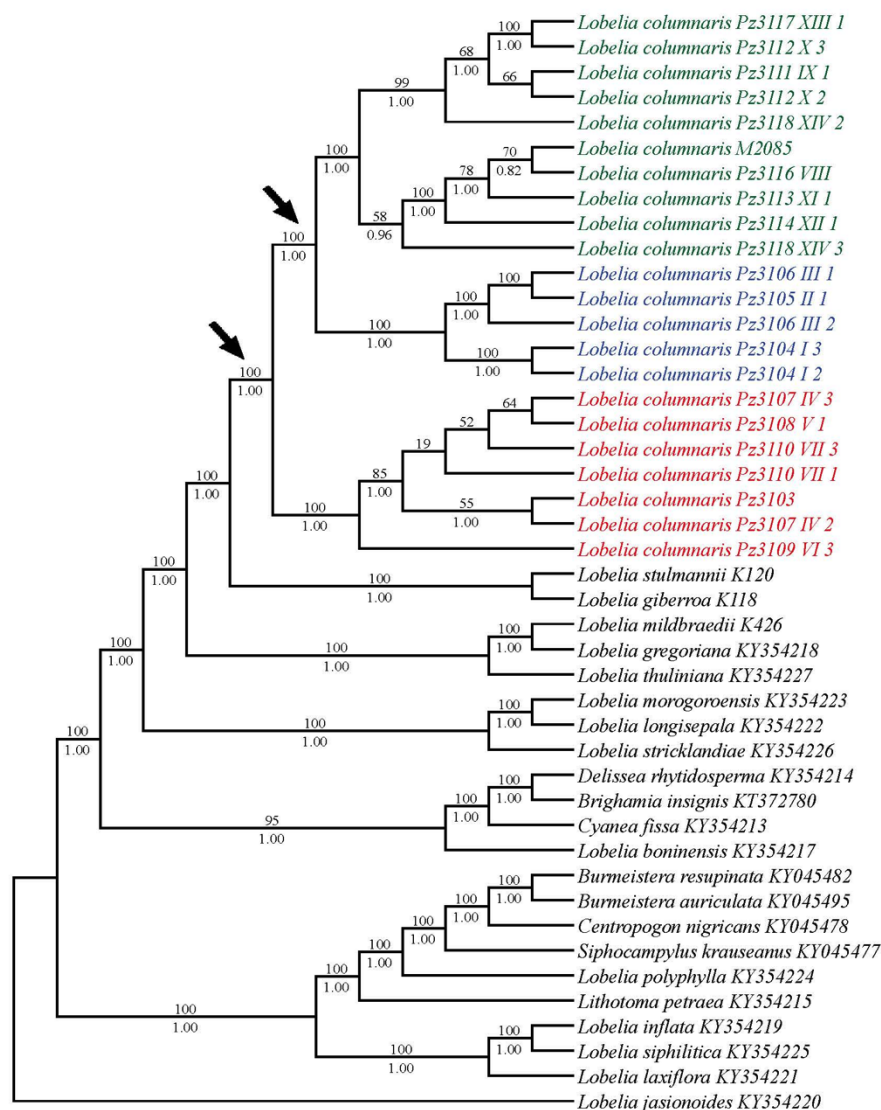
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Figure 3. Phylogenomic tree of *Lobelia columnaris* .

Reconstruction of the phylogeographical history

The geographic distribution of the plastid genomes shows the distant relationship between the populations from South Bioko and the other populations in this study. In contrast, populations in the three sky islands of mainland Cameroon are more closely related. Each pie graph shows the proportion of plastome sequences from each geographic sky island system (Figure 4).
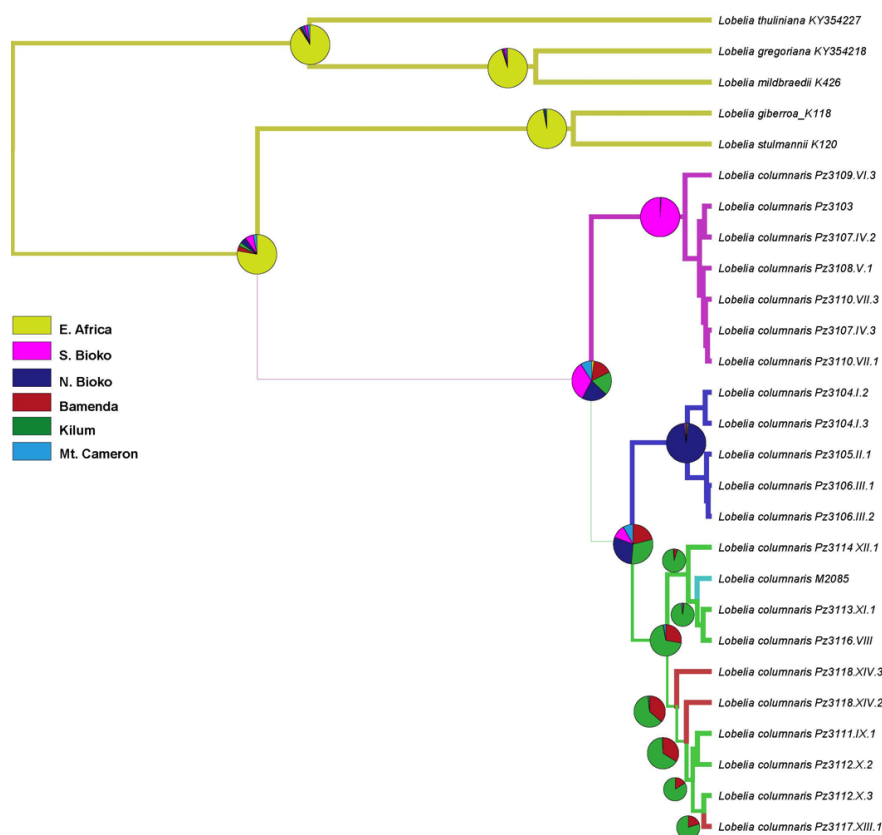
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Figure 4. Geographic and phylogenomic relationship of plastomes of

Lobelia columnaris.

Events of divergence times

Three important divergent events are noticeable on the genomic-dating tree. The first event is dated 5.58 Ma, marking the divergence between the ancestor of *L. columnaris* and giant lobelias from East Africa (Figure 5). The second occurred approximately 1.54 Ma and marked the separation of populations in West Central Africa and North Bioko from South Bioko. Finally, the third event is estimated to be 0.99 Ma displaying the split of North Bioko and mainland Cameroon populations.
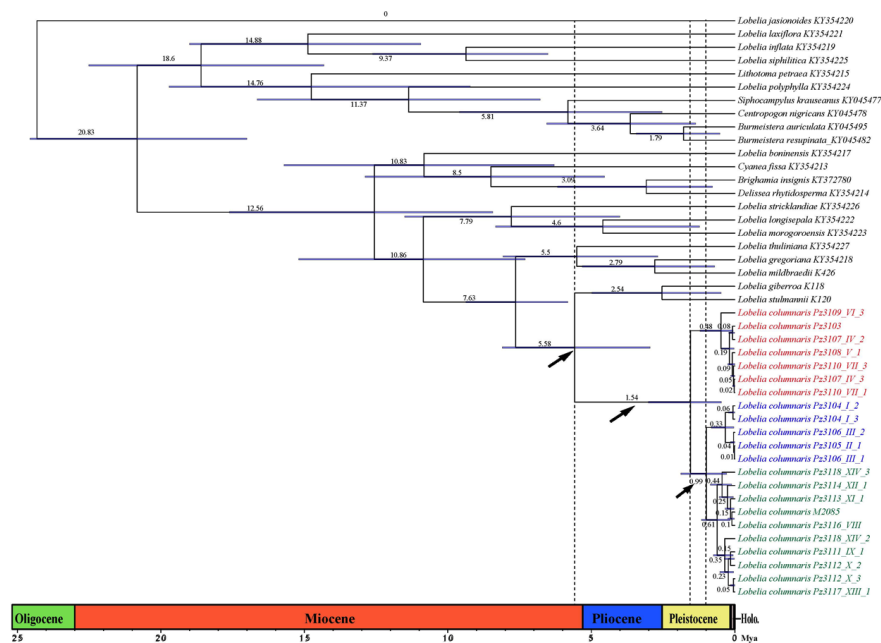
Figure 5. MCC tree with 95% HPD confident intervals for phylogenomic relationships and estimation of divergence times obtained in BEAST.

Discussion *Phylogenetic relationship of populations*

The ancestor of *L. columnaris* spread from East Africa via the Congo's Basin to establish in West-Central Africa approximately 5.5 Ma (Knox & Li 2017). The genealogical relationships among populations of *L. columnaris* in the present day were resolved with the analyses of plastomes. However, populations within South Bioko's subclade do not have high bootstrap values or posterior probabilities, possibly because these populations diverged recently, and the plastome may not be the right marker to show a robust phylogenetic resolution at this fine scale.

We hypothesized that the populations on Bioko shared the same common ancestor. However, our results refute the hypothesis. Our outcome clearly shows an early colonization event in South Bioko. A second divergence separated the populations of North Bioko and Cameroon. According to this result, Bioko's two populations are not a monophyletic group suggesting that the evolutionary history of the island populations is more complex.

We found a phylogeographic pattern with three clades distributed on the six sky islands. Remarkably, the two clades located in Bioko do not share a more recent common ancestor. The third clade in Cameroon shares a more recent common ancestor with populations in North Bioko.

Historical colonization

Sky island's age

The Hawaiian archipelago is well documented for the correlation between age of the islands and their colonization and radiation of various plants' lineages, such as the Hawaiian lobeliads (Givnish et al. 2009). In contrast, the West-Central Africa sky archipelago does not have a simple chronological age from east to west (Suh et al. 2008). Nevertheless, the six sky islands that we studied have a clear progression. Their estimated ages run from old (West-Central Cameroon) to young mountains (Bioko and Mt. Cameroon). Our plastome phylogeographic interpretation indicated that *L. columnaris* colonized the older sky islands in mainland Cameroon after establishing South Bioko's younger sky islands. Moreover, the South Bioko colonization predated North Bioko.

Overall, this result contradicts our sky island age hypothesis because Cameroon's sky islands with estimated ages of 3.0, 22.0, and 31.0 Ma are older than Bioko's sky islands (ca. 1.3 Ma) (Table 2). Our results suggest a biogeography history with an inverse progression concerning the age of the Afromontane sky islands.

Table 2. Elevation and estimated age of six sky islands of the Cameroon Volcanic Line

Sky Island	Elevation (m)	Age (Myr)	Reference
Pico Biao-Moka	2009	1.3	Yamgouot et al., 2016
Gran Caldera	2260	1.3	Yamgouot et al., 2016
Pico Basilé	3011	1.3	Yamgouot et al., 2016
Mt. Cameroon	4085	3	Suh et al., 2008
Mt. Bamenda	2621	22-21	Zimkus & Gvozdik, 2013
Mt. Oku	3011	31-22	Suh et al., 2008

Perhaps, South Bioko populations are in a basal position on the ML/BI/Beast trees because the plastomes provide a skewed view of the biogeographic history of *L. columnaris* , or there is sampling error because we sequenced only 1-2 individuals per populations. Furthermore, we do not have population data from Nigeria, and we did not find more populations in Cameroon, possibly for the local extinction of populations caused by the intense human transformation of Afromontane habitat on the sky islands.

Population differentiation

Microevolutionary processes could be an alternative explanation for the colonization history of *L. columnaris* in the Afromontane sky islands of West Central Africa. The early split in *L. columnaris* populations between the mainland and South Bioko possibly occurred via a founder event and genetic bottleneck or subsequent genetic drift so that South Bioko plastomes are very distinct relative to the other plastome groups. Furthermore, the two early splits (Figure 3) suggest two independent colonization events of *L. columnaris* on the island. Under this scenario, the colonization history was likely from mainland Cameroon's old sky islands to the southern young sky islands in Bioko.

This explanation should be developed with population genetic analyses to explore the correlation between geographic distance and genetic differentiation of populations and find which evolutionary forces are responsible for population differentiation in Bioko.

Missing links

The lack of Nigerian populations of *L. columnaris* in the phylogenomic reconstruction likely is causing missing links between the island and mainland populations. Future analyses with samples from Nigeria will provide a better interpretation of colonization history.

Afromontane forest expansion and contraction

Eastern giant lobelias evolved in Afromontane forests at elevation ranges from 1000 to 2500m. Through frost tolerant adaptations, some taxa colonized the inhospitable Afroalpine elevations from 3000 to 5000m (Hedberg 1969). We found that middle elevations are the most likely habitat for *L. columnaris* . Only Pico Basilé's (North Bioko) population extends its range from middle elevation up to >3000m. At this altitude, the habitat is composed of shrubs and subalpine meadows.

The paleoclimatic scenarios of the Afromontane vegetation during the Pleistocene in West Central Africa are not well known. However, it is known that climatic fluctuations changed the past vegetation patterns dramatically. With pollen analysis is possible to reconstruct historical processes (Kadu et al. 2011) like the presence of forest bridges that connected sky islands and highlands (Kebede et al. 2007) and, as a result, facilitated periodic expansions of the Afromontane forest through lowland elevations (Mairal et al. 2017). This dynamic allowed species to disperse and to be isolated in adjacent mountains (Zimkus & Gvoždík 2013).

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Under this scenario, *L. columnaris* probably had changes in population size during the Pleistocene, mainly caused by repeated climatic oscillations and Afromontane fragmentation. Also, environmental fluctuations caused the constriction of populations into forest refugia. Later, populations expanded during warm and humid environmental alternations (Gao et al. 2015). Therefore, the current disjunction of *L. columnaris* in sky islands probably was a Pleistocene product of retraction and isolation of Afromontane forest and continuous post-Pleistocene dispersal to empty niches.

Although not explored in the study, active volcanism and land use have to be considered in a holistic view of the current distribution of this giant lobelia.

Pleistocene refugia

The entire Cameroon line is considered a Pleistocene forest refuge for its distinctive flora. Forest refugia are also supported because of the high genetic diversity detected in different trees (Pineiro et al. 2017) such as the genus *Greenwayodendron* (Migliore et al. 2018), and other flowering plants. For example, *Arabis alpina* (L.) survived the Pleistocene oscillations of temperature and drought in refugia. Once the environmental conditions changed during the interglacial periods, *A. alpina* colonized or recolonized new sky islands in East-Central Africa (Assefa et al. 2007).

It is possible that the populations of *L. columnaris* were dynamically isolated and expanded within and between the sky islands of West Central Africa. However, Pico Biao-Moka in South Bioko might be a Pleistocene forest refuge to Afromontane plant species.

Geographical barriers

The forest contraction that occurred during the Pleistocene radically changed the landscape. Additional landscape changes occurred in the region at the end of the last glacial period (ca. 10,000 years ago) when a rise in sea level isolated Bioko from the African mainland (Jones 1994). A shallow channel separates Bioko from the Cameroon coast by 32 km (Schabetsberger et al. 2004). We do not have evidence of recent dispersal and gene flow between North Bioko and mainland Cameroon.

The central depression with lowland forest (0-500 masl) between North and South Bioko (Schabetsberger et al. 2004) exemplifies another geographic barrier that probably maintains reproductive isolation between the populations of *L. columnaris* in Bioko Island. The central depression consists of lowland forest vegetation.

The expansion, colonization, and recolonization of *L. columnaris* was possible by wind dispersal. Wind played an essential role in dispersing tiny seeds altitudinally and latitudinally across sky islands. In Cameroon, high ridges act as a natural forest corridor connecting sky islands, and facilitating dispersal and gene flow among contemporaneous populations (Smith et al. 2000).

Ecology and conservation

The type of ecological habitat may have a possible effect on the morphology of *L. columnaris* (Mabberley 1974b). This observation has to be developed in future studies. Our study observed that populations grow in a mosaic of ecological habitats, latitudinal, and elevational gradients. Indeed, we observed phenotypic variation in some traits, for example, in plant height, size of the inflorescence, leaves, and flower colour. *L. columnaris* is smaller in height, inflorescence, and leaf length at a higher elevation (approx. 3000m) and lower elevation (1000m). Elevations between 2000 to 2600m, the variation is spectacular with greater height, inflorescence number, colour, and leaf length.

The populations of *L. columnaris* in mainland Cameroon are at high risk of local extirpation because of excessive anthropogenic pressure on montane forest fragments. Only for the populations on Mt. Cameroon, which is a National Park, have conservation protection plans. The scenario in Bioko for this giant lobelia and the Afromontane forest is better. Bioko has an active conservation procedure in two of the three sky islands (Müller & Pócs 2007). Moreover, South Bioko is undisturbed because of low human population density and supports the highest numbers of plant and animal species on the island (see Jones 1994).

Study limits

Our results are limited by several factors. This preliminary study was conducted with few individuals (1-2) for each population, and as such should be considered a baseline for future studies. The available sample sizes for every population is ten individuals. We hope to conduct future studies with all the individual samples.

Social instability was the main reason no samples of *L. columnaris* were collected in Nigeria. Future fieldwork in Nigeria to find and collect more populations will expand the sampling effort and allow a more robust phylogeographic study. Populations from Mt. Cameroon were under-collected because it was challenging to get the permits to work in the National Park. Finally, this study presents a partial history with the analysis of a non-recombinant marker. A more robust analysis should include nuclear genomic sequences.

Conclusions

The plastome analysis provided an informative phylogenetic reconstruction but partial colonization reconstruction of *Lobelia columnaris* . Overall, three groups are in West-Central Africa.

According to our phylogenomic calibration, the ancestor of *L. columnaris* arrived in West Central Africa (ca. 1.5 Ma) during the Cameroon line's youngest volcanoes' uplift. The Pleistocene climatic oscillations led to the divergence of the Cameroon and Bioko populations into three clades. Likely, South Bioko was forest refugia during the interglacial periods. Here, we show that the biogeographic history of *L. columnaris* does not follow the progression of ages of our sky islands.

In more recent times, Bioko's central depression likely functions as a geographic barrier to further isolate the population groups discovered in our analysis (South Bioko versus North Bioko-Cameroon). Moreover, grazing, burning, and deforestation are threatening Afromontane forest patches with *L. columnaris* in mainland Cameroon.

Data accessibility We are working the data storage from the plastomes generated in this study. Data will be deposited in GeneBank

References

Adams, A. N., Wiens, D. A, Nyblade, A. A., Euler, G. G., Shore, P. J., & Tibi, R. (2015). Lithospheric instability and the source of the Cameroon volcanic line: Evidence from Rayleigh wave phase velocity tomography. *Journal of Geophysical Research: Solid Earth,120* , 1708–1727. doi:10.1002/2014jb011580

Alfaro, M. E., Zoller, S., & Lutzoni, F. (2003). Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution, 20* , 255–266. doi:10.1093/molbev/msg028

Assefa, A., Ehrich, D., Taberlet, P., Nemomissa, S., & Brochmann, C. (2007). Pleistocene colonization of afro-alpine 'sky islands' by the arctic-alpine Arabis alpina. *Heredity (Edinb), 99* , 133–142. doi:10.1038/sj.hdy.6800974

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., . . . Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology, 19* , 455–477. doi:10.1089/cmb.2012.0021

Bartos, M., Janecek, S., Padysakova, E., Patacova, E., Altman, J., Pesata, M., Kantorova, J., & Tropek, R. (2012). Nectar properties of the sunbird-pollinated plant Impatiens sakeriana: A comparison with six other co-flowering species. *South African Journal of Botany ,78* , 63–74. doi:10.1016/j.sajb.2011.05.015

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C-H., Xie, D., Suchard, M. A.,. . . Drummond, A. J. (2014). BEAST 2: a software patform for bayesian evolutionary analysis. *PLOS Computational Biology, 10* , e1003537. doi:10.1371/journal.pcbi.1003537

Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics, 25* , 1972–1973.

doi:10.1093/bioinformatics/btp348

Chauvel, C., Dia, A. N., Bulourde, M., Chabaux, F., Durand, S., Ildefonse, P., Gerard, M.,...Ngounouno, I. (2005). Do decades of tropical rainfall affect the chemical compositions of basaltic lava flows in Mount Cameroon? *Journal of Volcanology and Geothermal Research, 141* , 195–223. doi:10.1016/j.jvolgeores.2004.10.008

Cheek, M., & Thulin, M. (2015). Lobelia columnaris. The IUCN red list of threatened species 2015. doi:10.2305/IUCN.UK.2015-4.RLTS.T39505A2928168.en

Chen, L-Y., Wang, Q-F., & Renner, S. S. (2016). East Asian Lobelioideae and ancient divergence of a giant rosette Lobelia in Himalayan Bhutan.*Taxon, 65* , 293–304. doi:10.12705/652.6

Cox, S. C., Prys-Jones, R. P., Habel, J. C., Amakobe, B. A., & Day, J. J. (2014). Niche divergence promotes rapid diversification of East African sky island white-eyes (Aves: Zosteropidae). *Molecular Ecology, 23* , 4103–4118. doi:10.1111/mec.12840

Darriba, D., Taboada, G. L., Doallo, R., Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods, 9* , 772. doi: 10.1038/nmeth.2109

Dawson, I. K., & Powell, W. (1999). Genetic variation in the Afromontane tree Prunus africana, an endangered medicinal species.*Molecular Ecology 8* , 151–156. doi: 10.1046/j.1365-294X.1999.00518.x

Demenou, B. B., Migliore, J., Heuertz, M., Monthe, F. K., Ojeda, D. I., Wieringa, J. J., Dauby, G.,...Hardy, O. J. (2020). Plastome phylogeography in two African rain forest legume trees reveals that Dahomey Gap populations originate from the Cameroon volcanic line.*Mol Phylogenet Evol, 150* , 106854. doi:10.1016/j.ympev.2020.106854

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, 19* , 11–15.

Duminil, J., Mona, S., Mardulyn, P., Doumenge, C., Walmacq, F., Doucet, J-L., & Hardy, O. J. (2015). Late Pleistocene molecular dating of past population fragmentation and demographic changes in African rain forest tree species supports the forest refuge hypothesis. *Journal of Biogeography, 42* , 1443–1454. doi:10.1111/jbi.12510

Erixon, P., Svennblad, B., Britton, T., & Oxelman, B. (2003). Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology, 52* , 665–673. doi:10.1080/10635150390235485

Fa, J. E., Garcia-Yuste, J. E., & Castelo, R. (2000). Bushmeat markets on Bioko Island as a measure of hunting pressure. *Conservation Biology, 14* , 1602–1613. doi: 10.1111/j.1523-1739.2000.99067.x

Figueiredo, E. (1994). Diversity and enemism of angiosperms in the Gulf of Guinea islands. *Biodiversity and Conservation, 3* , 785–793.

Gao, Y. D., Zhang, Y., Gao, X. F., & Zhu, Z. M. (2015). Pleistocene glaciations, demographic expansion and subsequent isolation promoted morphological heterogeneity: A phylogeographic study of the alpine Rosa sericea complex (Rosaceae). *Scientific Reports, 5* , 11698. doi:10.1038/srep11698

Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science, 7* , 457–472. doi:10.1214/ss/1177011136

Givnish, T. J., Millam, K. C., Mast, A. R., Paterson, T. B., Theim, T. J., Hipp, A. L., Henss, J. M., . . . Sytsma, K. J. (2009). Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society B: Biological Sciences, 276* , 407–416. doi:10.1098/rspb.2008.1204

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood.*Systematic Biology, 52* , 696–704. Doi doi:10.1080/10635150390235520

Hedberg, O. (1969). Evolution and speciation in a tropical high mountain flora. *Biological Journal of the Linnean Society of London, 1*, 135–148. doi: 10.1111/j.1095-8312.1969.tb01816.x

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis.*Systematic Biology, 42* , 182–192. doi:10.1093/sysbio/42.2.182

Ineich, I., Lebreton, M., Lhermitte-Vallarino, N., & Chirio, L. (2015). The reptiles of the summits of Mount Oku and the Bamenda Highlands, Cameroon.pdf. *Amphibian & Reptile Conservation, 9* , 15–38.

Janecek, S., Riegert, J., Sedlacek, O., Bartos, M., Horak, D., Reif, J., Padysakova, E.,. . . Dolezal, J. 2012. Food selection by avian floral visitors: an important aspect of plant-flower visitor interactions in West Africa. *Biological Journal of the Linnean Society*107:355–367. doi:10.1111/j.1095-8312.2012.01943.x

Jesus, J., Brehm, A., & Harris, D. J. (2005). Phylogenetic relationships of Hemidactylus geckos from the Gulf of Guinea islands: patterns of natural colonizations and anthropogenic introductions estimated from mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution, 34* , 480–485. doi:10.1016/j.ympev.2004.11.006

Jin, J-J., Yu, W-B., Yang, J-B., Song, Y., dePamphilis, C. W., Yi, T-S., & Li, D-Z. (2020). GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology,*Article 21. doi:10.1186/s13059-020-02154-5

Jones, P. T. (1994). Biodiversity in the Gulf of Guinea: An overview.*Biodiversity and Conservation, 3* , 772–784.

Kadu, C. A. C., Schueler, S., Konrad, H., Muluvi, G. M. M., Eyog-Matig, O., Muchugi, A., Williams, V. L., . . . Geburek, T. (2011). Phylogeography of the Afromontane Prunus africana reveals a former migration corridor between East and West African highlands.*Molecular Ecology, 20* , 165–178. doi:10.1111/j.1365-294X.2010.04931.x

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S.,. . . Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28* , 1647–1649. doi:10.1093/bioinformatics/bts199

Kebede, M., Ehrich, D., Taberlet, P., Nemomissa, S., & Brochmann, C. (2007). Phylogeography and conservation genetics of a giant lobelia (Lobelia giberroa) in Ethiopian and Tropical East African mountains.*Molecular Ecology, 16* , 1233–1243. doi 10.1111/J.1365-294x.2007.03232.X

Knox, E. B. (2014). The dynamic history of plastid genomes in the Campanulaceae sensu lato is unique among angiosperms. *Proceedings of the National Academy of Sciences of the United States of America,111* , 11097–11102.

Knox, E. B., Li, C. (2017). The East Asian origin of the giant lobelias.*American Journal of Botany, 104* , 924–938. doi:10.3732/ajb.1700025

Knox, E. B., & Palmer, J. D. (1998). Chloroplast DNA evidence on the origin and radiation of giant Lobelias in eastern Africa.*Systematic Botany, 23*, 109–149.

Kolaczkowski, B., & Thornton, J. W. (2007). Effects of branch length uncertainty on Bayesian posterior probabilities for phylogenetic hypotheses. *Molecular Biology and Evolution, 24* , 2108–2118. doi:10.1093/molbev/msm141

Liu, C., Shi, L., Zhu, Y., Chen, H., Zhang, J., Lin, X., Guan, X. (2012). CpGAVAS, an integrated web server for the annotation, visualization, analysis, and GenBank submission of completely sequenced chloroplast genome sequences. *BMC Genomics, 13* , 715.

Mabberley, D. J. (1974a). Branching in Pachycaul Senecios: The Durian Theory and the evolution of angiospermous trees and herbs. *New Phytologist, 73* , 967–975.

Mabberley, D. J. (1974b). The Pachycaul Lobelias of Africa and St. Helena. *Kew Bulletin* , *29* ,535–584.

Mabberley, D. J. (1975). The Giant Lobelias: Pachycauly, Biogeography, Ornithophily and Continental Drift. *New Phytologist, 74* , 365–374.

Mairal, M., Sanmartin, I., Herrero, A., Pokorny, L., Vargas, P., Aldasoro, J. J., & Alarcon, M. (2017). Geographic barriers and Pleistocene climate change shaped patterns of genetic variation in the Eastern Afromontane biodiversity hotspot. *Scientific Reports,7* , 45749. doi:10.1038/srep45749

Matschiner, M., Musilová, Z., Barth, J. M. I., Starostová, Z., Salzburger, W., Steel, M., & Bouckaert, R. (2016). Bayesian node dating based on probabilities of fossil sampling supports trans-Atlantic dispersal of cichlid fishes. *bioRxiv* . doi:10.1101/038455

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* , e26606. doi:10.1371/journal.pone.0026606

Migliore, J., Kaymak, E., Mariac, C., Couvreur, T. L. P., Lissambou, B. J., Piñeiro, R., & Hardy O. J. (2018). Pre-Pleistocene origin of phylogeographical breaks in African rain forest trees: New insights from Greenway-odendron(Annonaceae) phylogenomics. *Journal of Biogeography, 46* , 212–223.doi:10.1111/jbi.13476

Missoup, A. D., Nicolas, V., Eiseb, S., Chung, E. K., & Denys, C. (2016). Phylogenetic position of the endemic Mount Oku rat,Lamottemys okuensis(Rodentia: Muridae), based on molecular and morphological data.*Zoological Journal of the Linnean Society, 177* , 209–226. doi:10.1111/zoj.12361

Muller, F., & Pocs, T. (2007). A contribution to the knowledge of epiphyllous bryophytes of Bioko Island (Equatorial Guinea), including additional remarks on non-epiphyllous species. *Journal of Bryology, 29* , 81–94. doi:10.1179/174328207X186803

Myers, N., Mittermeier, R. A, Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature,403* , 853–858. doi: 10.1038/35002501

Nicolas, V., Missoup, A-D., Colyn, M., Cruaud, C., & Denys, C. (2012). West-Central African Pleistocene lowland forest evolution revealed by the phylogeography of Missonne's soft -furred mouse. *African Zoology, 47* , 100–112.

Pineiro, R., Dauby, G., Kaymak, E., Hardy, O. J. (2017). Pleistocene population expansions of shade-tolerant trees indicate fragmentation of the African rainforest during the Ice Ages. *Proceedings of the Royal Society B: Biological Sciences, 284* . doi:10.1098/rspb.2017.1800

Popp, M., Gizaw, A., Nemomissa, S., Suda, J., & Brochmann, C. (2007). Colonization and diversification in the African 'sky islands' by Eurasian Lychnis L. (Caryophyllaceae). *Journal of Biogeography,35* , 1016–1029. doi:10.1111/j.1365-2699.2008.01902.x

Riegert, J., Fainova, D., Antczak, M., Sedlaček, O., Hořák, D., Reif, J., & Pešata, M. (2011). Food niche differentiation in two syntopic sunbird species: A case study from the Cameroon mountains. *Journal of Ornithology, 152* , 819–825. doi:10.1007/s10336-011-0650-0

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,19* , 1572–1574. doi:10.1093/bioinformatics/btg180

Schabetsberger, R., Drozdowski, G., Drozdowski, I., Jersabek, C. D., & Rott, E. (2004). Limmnological aspects of two tropical crater lakes (Lago Biao and Lago Loreto) on the island of Bioko (Equatorial Guinea).*Hydrobiologia, 524* , 79–90.

Smith, T. B., Holder, K., Girman, D., O'Keefe, K., Larison, B., & Chan, Y. (2000). Comparative avian phylogeography of Cameroon and Equatorial Guinea mountains: implications for conservation. *Molecular Ecology, 9* , 1505–1506. doi: 10.1046/j.1365-294x.2000.01032.x

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology,57* , 758–771. doi:10.1080/10635150802429642

Suh, C. E., Luhr, J. F., & Njome, M. S. (2008). Olivine-hosted glass inclusions from Scoriae erupted in 1954-2000 at Mount Cameroon volcano, West Africa. *Journal of Volcanology and Geothermal Research,169* , 1–33. doi:10.1016/J.Jvolgeores.2007.07.004

Tropek, R., & Konvicka, M. (2009). Forest eternal? Endemic butterflies of the Bamenda highlands, Cameroon, avoid close-canopy forest.*African Journal of Ecology, 48* , 428–437. doi:10.1111/j.1365-2028.2009.01129.x

Wick, R. R., Schultz, M. B., Zobel, J., & Holt, K. E. (2015). Bandage: interactive visualization of de novo genome assemblies.*Bioinformatics, 31* , 3350–3352. doi:10.1093/bioinformatics/btv383

Yamada, K. D., Tomii, K., & Katoh, K. (2016). Application of the MAFFT sequence alignment program to large data—reexamination of the usefulness of chained guide trees. *Bioinformatics, 32* , 3246–3251. doi:10.1093/bioinformatics/btw412

Yamgouot, F. N., Déruelle, B., Gbambié Mbowou, I. B., Ngounouno, I., & Demaiffe, D. (2016). Geochemistry of the volcanic rocks from Bioko Island ("Cameroon Hot Line"): Evidence for plume-lithosphere interaction. *Geoscience Frontiers, 7* , 743–757. doi:10.1016/j.gsf.2015.06.003

Zimkus, B. M., & Gvoždík, V. (2013). Sky Islands of the Cameroon Volcanic Line: a diversification hot spot for puddle frogs (Phrynobatrachidae:Phrynobatrachus). *Zoologica Scripta ,42* , 591–611. doi:10.1111/zsc.12029