Afro-alpine flagships revisited II: elucidating the evolutionary relationships and species boundaries in the giant senecios (*Dendrosenecio*)

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Abstract

Alpine plant radiations are common across all major mountain systems of the world, and have been regarded as the main explanation for the species diversity found within these areas. To study the mechanisms behind the origin of this diversity, it is necessary to determine phylogenetic relationships and species boundaries in radiating alpine groups. The genus *Dendrosenecio* (Asteraceae) is an iconic example of a tropical-alpine plant radiation in the East African high mountains. To this date, limited sampling of molecular markers has resulted in insufficient phylogenetic resolution and infrageneric classification, hindering a comprehensive understanding of the drivers of diversification. Here, we used Hyb-Seq and the Compositae1061 probe set to generate targeted nuclear and off-target plastid DNA data for 42 samples representing all currently accepted 11 species. We combined coalescent methods and paralogy analysis to infer phylogenetic relationships, estimate divergence times and evaluate species boundaries. Lineage differentiation in *Dendrosenecio* seems to have occurred between the Late Miocene and the Pleistocene, starting when the first high elevation habitats became available in East Africa. We retrieved four major clades corresponding to four geographically distant mountain groups, testifying the importance of allopatric speciation in the early diversification of the group. Cytonuclear discordance suggested the occurrence of historical hybridization following occasional long-distance dispersal between mountain groups. The species delimitation analysis favored 10 species, but only five were fully supported, suggesting that population level studies addressing processes such as ecological speciation and hybridization after secondary contact are needed to determine the current diversity found in the genus.

Keywords: afro-alpine, *Dendrosenecio*, evolutionary radiation, geographic speciation, long-distance dispersal, phylogenomics.

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Introduction

Evolutionary radiations, the burst of species sharing a common ancestry (Simões et al. 2016), gave rise to a major proportion of the current species diversity (Linder 2008). The numerous examples of radiations found within angiosperms (Soltis et al. 2019) highlight their importance for the evolution of plant diversity. Radiations may be primarily driven by selection (i.e., adaptive radiations), where the acquisition of a novel trait allowing access to a new niche together with ecological opportunities promotes speciation (Givnish 1997; Soulebeau et al. 2015); or by other forces (i.e., non-adaptive radiations), such as large-scale climatic shifts (Vrba 1992) or increased opportunities for allopatric speciation favored by a geographically complex region (Lieberman 2012). Alpine plant radiations, found in most mountain ranges around the world, are considered to have been recent and rapid (Hughes and Atchison 2015). They are usually associated with new habitats opened up by geotectonic changes (Linder 2008), although the processes underlying radiations often involve numerous factors (Bouchenak-Khelladi et al. 2015), necessitating tests for confluence scenarios (Donoghue and Sanderson 2015).

The tropical eastern African mountains are found along the two branches of the East African Rift System (EARS), occurring throughout the Ethiopian Highlands and as scattered peaks in East Africa. In East Africa, the western branch (Western Rift) includes the Rwenzori, Virunga, and Mitumba mountains, and the eastern branch (Eastern Rift) consists of Mount Elgon and the Cherangani Hills on the western flank and Mounts Kilimanjaro, Meru, Kenya, and the Aberdare Range on the eastern flank (Fig. 1). These mountains are predominantly of volcanic origin, with ages ranging from the Miocene to the Late Pliocene (Griffiths 1993), and they support a wide range of habitats in three altitudinal vegetation zones. The lowermost, montane forest belt is followed by an ericaceous belt that precedes the afro-alpine belt (Hedberg 1969, 1970). The majority of the afro-alpine taxa have their congeners in temperate and alpine regions elsewhere in the world (Smith and Cleef 1988; Assefa et al. 2007; Devos et al. 2010; Gehrke et al. 2016; Gizaw et al. 2016a; Kandziora et al. 2016), but some of them have their closest relatives in adjacent areas at lower elevations (Galbany-Casals et al. 2014; Nürk et al. 2015). The afro-alpine flora comprises some 77% endemic vascular plant species (Hedberg 1961; Gehrke and Linder 2014), that might be particularly vulnearable due to habitat destruction and climate change (Brochmann et al. 2021). Within this flora, only some species have originated via *in situ* radiations (e.g., *Alchemilla*, Gehrke et al. 2016; *Lobelia*, Knox and Palmer 1998; Knox and Li 2017). The evolutionary relationships among taxa of many radiating plant groups remain poorly understood, partly because their phylogenetic reconstruction is challenging due to conflicting evolutionary histories between independent loci (Fior et al. 2013; Nicholls et al. 2015; Vargas et al. 2017; Carlsen et al. 2018). Discordance among gene trees, mainly caused by evolutionary processes such as retained ancestral polymorphism (incomplete lineage sorting, ILS), hybridization, and gene duplication leading to paralogy (Degnan and Rosenberg 2009), can be pronounced in phylogenomic datasets based on hundreds of nuclear loci (Smith et al. 2020). Despite this discordance, phylogenomic approaches have recently demonstrated their potential for improving previous hypotheses about species relationships in tropical-alpine plant radiations (Vargas et al. 2017; Morales-Briones et al. 2018; Pouchon et al. 2018).

The genus *Dendrosenecio* (Hauman ex Hedberg) B.Nord. (Asteraceae) is endemic to the East African mountains and represents an iconic example of a radiating afro-alpine plant group. It consists of spectacular giant rosette plants distributed across the isolated high mountains in Kenya, Tanzania, Uganda, the Democratic Republic of the Congo and Rwanda, where they grow between 2500 and 4500 m of elevation, i.e., they span from the montane forest to the alpine belt in multiple spatially isolated mountains (Mabberley 1973). The giant dendrosenecios or ‘giant groundsels’ have been subjected to contradicting taxonomic treatments, which accepted as few as three, partly

widespread species or up to 17 species, mainly single-mountain endemics (Hedberg 1957, 1969; Mabberley 1973; Beck et al. 1982; Knox 2005). The most recent taxonomic treatment by Knox (2005) recognizes 11 species and 10 non-autonymous subspecies with a predominantly vicariant distribution across the isolated mountain peaks. The genus has been suggested to be uniformly polyploid (presumably decaploid with n = 50; Knox and Kowal 1993), and shows complex variation among and within species in altitudinal distribution, growth form and morphology. The high-altitude forms are morphologically and physiologically well adapted to the harsh climatic conditions of the afro-alpine environment, with well-developed mechanisms both to tolerate and to avoid frost (Hedberg 1964; Beck 1986; Beck et al. 1992). Taxa from the Eastern Rift mountains (Mts. Kilimanjaro, Meru, Kenya, Elgon, the Aberdare Range and the Cherangani Hills), where the genus has been hypothesized to have originated and radiated (Knox and Palmer 1995a), appear morphologically distinct, whereas the plants from the Western Rift mountains (Rwenzori, Virunga, and Mitumba Mts.) show more complex morphological variation (Hedberg 1969; Mabberley 1973).

The placement of *Dendrosenecio* as a distinct genus within the tribe Senecioneae was confirmed based on evidence from plastid DNA (cpDNA) restriction-site variation (Knox and Palmer 1995b) as well as Sanger-sequenced nuclear and cpDNA regions (Pelser et al. 2007, 2010). The current species diversity is thought to have originated relatively recently, based on the poorly supported phylogenetic tree and little variation found in cpDNA (Knox and Palmer 1995a). This view has recently been strengthened by the finding of a shallow genetic structure based on amplified fragment length polymorphism (AFLP) data (Tusiime et al. 2020). A hypothesis of parallel adaptation to alpine habitats from montane forest ancestors in different mountains was suggested by Hedberg (1970) and Mabberley (1973), whereas Knox and Palmer (1995a) suggested that the radiation started at high altitudes in the geologically very young Mt. Kilimanjaro, followed by dispersal to other mountain tops and repeated diversification in each mountain. Tusiime et al. (2020) used AFLPs to test a parallel adaptation hypothesis versus a hypothesis of a single origin of adaptations, and found that geography rather than habitat structures genetic variation as predicted by the parallel adaptation hypothesis. Four main genetic groups corresponding to the four main groups of mountains were identified. They also found evidence for admixture in several populations, suggesting recent secondary contact after rare long-distance dispersals among mountains (Tusiime et al. 2020). However, AFLP markers cannot be used for dating and are not well suited for resolution of phylogenetic relationships.

In this study, we re-evaluate the phylogenetic relationships in *Dendrosenecio* and provide a time frame for the evolutionary history of this group, for which allopatric speciation, parallel adaptation, and secondary contact following long-distance dispersal between mountains have been suggested as important drivers of diversification. We generated hundreds of nuclear markers and cpDNA data using Hyb-Seq and the Asteraceae conserved orthologous set (Compositae1061; Mandel et al. 2014) and used phylogenomic approaches that account for ILS and paralogy to assess the evolutionary history of the genus. Our objectives were to (1) test if the radiation hypothesis suggested by Knox and Palmer (1995a) is supported by nuclear DNA data, (2) determine the role of geographic isolation in the differentiation of lineages, including testing whether the four genetic groups identified by Tusiime et al. (2020) correspond to supported clades, and if so, establishing their phylogenetic relationships, (3) test the boundaries between currently accepted species, and (4) compare nuclear and cpDNA phylogenies to provide further insights into hypotheses on secondary contact and hybridization.

Materials and Methods

Study group

Following Knox's (2005) taxonomic treatment, the high-altitude *Dendrosenecio* (sub)species that typically are confined to the afro-alpine zone (~3200-4500 m) are *D. kilimanjari* subsp. *cottonii* (Mt. Kilimanjaro), *D. keniodendron* (Mt. Kenya and Aberdare Range), *D. keniensis* (Mt. Kenya), *D. elgonensis* subsp. *barbatipes* (Mt. Elgon), *D. adnivalis* subsp. *adnivalis* and subsp. *friesiorum* (Rwenzori Mts) and *D. erici-rosenii* subsp. *alticola* (Virunga Mts). Two (sub)species are found both in the afro-alpine zone and in the sub-alpine ericaceous zone (~3000-3900 m), *D. kilimanjari* subsp. *kilimanjari* (Mt. Kilimanjaro) and *D. brassiciformis* (Aberdare Range), and two typically occur at intermediate altitude in the sub-alpine ericaceous zone (~3000-3200 m), *D. meruensis* (Mt. Meru) and *D. cheranganiensis* subsp. *dalei* (Cherangani Hills). Two taxa are generally confined to the lower-altitudinal afro-montane zone (<3000 m), *D. johnstonii* (Mt. Kilimanjaro) and *D. cheranganiensis* subsp. *cheranganiensis* (Cherangani Hills). Three (sub)species occur in all three altitudinal zones, *D. battiscombei* (Mt. Kenya and Aberdare Range), *D. elgonensis* subsp. *elgonensis* (Mt. Elgon) and *D. erici-rosenii* subsp. *erici-rosenii* (Rwenzori, Virunga, and Mitumba Mts.).

The high-altitude *Dendrosenecio* plants typically have large, modified stems, thick leaves that are densely pubescent on the lower surface, and massive leaf rosettes which fold up and close during the night particularly at the juvenile stage, whereas the low-altitude plants tend to have thin stems, sparsely pubescent leaves, and scantily leaved rosettes (Hedberg 1957, 1964; Mabberley 1973). The arborescent species (e.g., *D. johnstonii* and *D. keniodendron*) are erect with the main stem growing up to 10 m and often branching high above the ground, whereas the prostrate ones (e.g., *D. keniensis* and *D. brassiciformis*) are short and branch close to the ground (Hedberg 1964; Mabberley 1973; Beck 1986).

Sampling and DNA sequencing

We conducted several field expeditions to eight tropical eastern African mountains in Kenya, Uganda and Tanzania in 2008/09 and 2019/20 and sampled all 11 currently accepted species of *Dendrosenecio* (Knox 2005) across almost their entire range, including all eight single-mountain endemics and the three species reported from more than a single mountain (Table 1). Leaf tissue was collected and dried in silica-gel. Representative herbarium vouchers were pressed and deposited at the Natural History Museum, University of Oslo Norway (O), as well as at scientific institutions in the country of collection, i.e., the East African Herbarium, National Museum of Kenya (EA), the Makerere University Herbarium Uganda (MHU), and the National Herbarium of Tanzania, Arusha (NHT).

For the present study, we selected 41 *Dendrosenecio* samples from different populations, including at least two representative samples for each of the 11 accepted species and all but one of the 10 described subspecies. One extra sample from one randomly chosen population of *D. elgonensis* was included as a technical replicate (the replicates belonged to different individuals within the same population due to material availability). In addition, four closely related species within Senecioneae were included as outgroup taxa (Pelser et al. 2010), making a final dataset of 46 samples (see Supplementary Information Table S1 for accession numbers and Table S2 for voucher information).

Genomic DNA was extracted using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany). Approximately 300 to 800 ng extracted DNA was sheared in 50 µl ddH_2O using an M220 Focused-ultrasonicator (Covaris, Woburn, Massachusetts, USA) with the program for fragmentation to 300-500 base pairs (bp). Library preparation was performed following the NEBNext Ultra II DNA Library Prep Kit protocol for Illumina (New England Biolabs, Ipswich, Massachusetts, USA) with the following modifications. Half volumes of samples and NEBNext chemicals were used, an additional cleanup step after the adapter ligation was done using a QIAquick Purification Kit (QIAGEN, Venlo, Netherlands), and size selection to approximately 400 to 600 bp was performed using Pippin Prep

and the 2% agarose gel cassette (Sagescience, Beverly, Massachusetts, USA). Amplification of the ligated, size-selected fragments was done using NEBNext Multiplex Oligos for Illumina Dual Index Primers Set 1 (New England Biolabs) and KAPA HiFi HotStart ReadyMix PCR Kit (Kappa Bioscience, Oslo, Norway). Purification of enriched PCR products was done twice using Agencourt AMPure XP beads (Beckman Coulter , Danvers, Massachusetts, USA) at a 0.75 volume ratio.

The solution hybridization was done using the myBaits Expert Compositae-1061 target capture kit (Arbor Biosciences, Ann Arbor, Michigan, USA). The enrichment followed the myBaits manual v.4.01 with 800 ng DNA in 7 µl for a set of 24 samples and 12 cycles of PCR enrichment. Concentration of DNA was measured using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Target-enriched libraries were mixed with unenriched libraries at a 1.5:1 ratio to increase the proportion of off-target cpDNA. Most samples were sequenced on an Illumina (San Diego, California, USA) NovaSeq 6000 at IAB (Olomouc, Czech Republic) and a few were sequenced on an Illumina MiSeq at BIOCEV (Vestec, Czech Republic); in both cases 150 bp paired-end reads were obtained.

Data processing and analysis of targeted nuclear loci

For the analysis of the nuclear loci, we considered it important to account for paralogy, given the paleopolyploid origin of Asteraceae (Barker et al. 2008, 2016) and the evidence pointing to a high ploidy level of *Dendrosenecio* species (Knox and Kowal 1993). To achieve this, ParalogWizard (available at: https://github.com/rufimov/ParalogWizard; Ufimov et al. unpublished) was followed, which allows paralog detection and phylogenetic utilization by discriminating between orthologs and paralogs. The divergence threshold between orthologs and paralogs is selected based on pairwise sequence similarity of *de novo* assembled exonic contigs. Paralogs are then used in addition to orthologs for constructing the species tree.

In a pre-processing step, raw reads were trimmed using Trimmomatic v.0.39 (Bolger et al. 2014) and duplicates removed with BBMap v.38.42 (Bushnell 2014) using the sets of scripts 'hybseq_1' and 'hybseq_2' (available at: https://github.com/V-Z/hybseq-scripts). To increase locus recovery, the Compositae1061 probe set was bioinformatically customized by assembling contigs *de novo* using Compositae1061 as target file for initial read mapping in HybPiper v.1.3.1 (Johnson et al. 2016) and running ParalogWizard scripts 1b and 2b with default settings and paralogy flag turned off (i.e., paralogs=no). Outgroup taxa were specified in the "blocklist" to exclude them and generate a target file containing only *Dendrosenecio* sequences. Subsequently, the assembly was repeated using the newly generated *Dendrosenecio* target file in HybPiper. The newly assembled contigs were analyzed for paralogy following the ParalogWizard workflow using default settings and outgroup taxa as "blocklist". To assess paralogy, the minimum and maximum sequence divergence threshold between exonic contigs of each locus was set based on the pairwise distance histogram (see Figure S1). In this histogram, the first peak comprises the "main" exonic copies of the paralogous loci, whereas the second peak resembles the more divergent ("secondary" thereafter) exonic copies, which are the result of whole genome duplication events. Values for the mean of the second peak ± the standard deviation were chosen as the divergence threshold for considering exonic copies as paralogous. To separate the exonic contigs to the respective orthologous and paralogous copy, the contigs were matched to a newly generated reference containing one main and one secondary copy per exon using BLAT v.34 (Kent 2002) with a minimum identity of 80%. Gene alignments and gene and species tree reconstruction followed the HybPhyloMaker (Fér and Schmickl 2018) workflow. Alignments were built with MAFFT v.7.029 (Katoh and Toh 2008) and filtered using ≤70% missing data per accession at each locus and ≥75% accession presence per locus as threshold values. Gene trees were estimated using RAxML

v.8.4.2 (Stamatakis 2014) with the general-time reversible (GTR) substitution model with a gamma distributed rate variation among sites "GTRGAMMA" and 500 bootstrap replicates. An ASTRAL multi-allele species tree (where accessions from the same species were forced into a single tip; Rabiee et al. 2019) and an ASTRAL species tree (where all accessions were treated as terminal taxa) were generated using ASTRAL v.5.7.4 (Zhang et al. 2018), support values were computed as local posterior probabilities based on gene tree quartet frequencies (Sayyari and Mirarab 2016). Considering that the average bootstrap support of individual gene trees was below 47.3%, a common feature in phylogenomic datasets (Molloy and Warnow 2018), branches with bootstrap support <30% were collapsed to account for gene tree estimation error (Sayyari and Mirarab 2016)**.** An additional ASTRAL species tree ("reduced species tree") was generated with a subset of data where potentially admixed accessions were excluded (see criteria below), with accessions treated as terminal taxa. To evaluate the reliability of the phylogenies generated with ASTRAL, a concatenated nuclear phylogeny was also generated. Loci were concatenated using AMAS v.1.0 (Borowiec 2016), and the best substitution model for each partition was calculated using ModelTest-NG v.0.1.6 (Flouri et al. 2015; Darriba et al. 2020) based on Akaike Information Criterion (AIC) values. A maximum likelihood tree was then generated using RAxML-NG v.8 (Kozlov et al. 2019) with the best model for each partition and bootstopping, employing a maximum of 1000 bootstrap replicates (transfer bootstrap expectation; Lemoine et al. 2018). Bootstopping converged after 400 replicates.

To avoid strong levels of admixture biasing phylogenetic inference in methods accounting only for ILS, such as ASTRAL, phylogenetic networks were constructed, which visualize the conflict between gene trees and detect potentially admixed accessions. For this, a quartet-based supernetwork approach, where edge lengths correspond to quartet frequencies, was taken using SuperQ (Grünewald et al. 2007, 2013) available in SPECTRE v.1.1.5 (Bastkowski et al. 2018) with the balanced edge-weight objective function and JOptimizer for optimization. For comparison, a distance-based network was generated using Neighbor-Net (Bryant and Moulton 2004) available in SplitsTree v.4.16.2 (Huson et al. 2008). Potentially admixed accessions were determined to be those forming mixed groups (i.e., accessions belonging to different mountain group lineages in the nuclear phylogeny) or showing a "misplacement" in the network (i.e., isolated accessions with numerous interconnected branches or groups of accessions emerging directly from the center of the network). Admixture was not explored further, as the sample selection was designed for phylogenetic reconstruction, while informative admixture tests will require a population-level sampling.

Data processing and analysis of off-target cpDNA data

For cpDNA analysis, the set of scripts included in HybPhyloMaker was used. Sequences from five Senecioneae species were retrieved from GenBank and included as additional outgroup taxa (see Table S1 for accession numbers). The complete chloroplast genome of *Dendrosenecio kilimanjari* (accession number NC_037957) was retrieved from GenBank to serve as reference for read mapping. The inverted repeat region was removed and the sequence split to individual coding and non-coding regions to later generate a 'pseudoreference' with stretches of 400 Ns separating the cpDNA regions. Raw reads were trimmed using Trimmomatic v.0.33 and duplicates removed with FastUniq v.1.1 (Xu et al. 2012). Mapping of reads to the pseudoreference was done using BWA v.0.7.15 (Li and Durbin 2009) and kindel v.0.1.4 (Constantinides and Robertson 2017) with a 0.51 threshold for consensus variant calling. The same sequence identity and filtering settings used in the HybPhyloMaker step of the analysis of the targeted nuclear loci were applied, except for the manual removal of two accessions (DE_001_1 and DE_061_1) that showed >15% missing data after the filtering had been applied. Coding and non-coding regions were used to generate a concatenated plastid phylogeny, the

same methodology employed for constructing the concatenated nuclear phylogeny was applied, with the exception that bootstopping converged after 300 replicates.

Divergence times estimation

To calculate divergence times, we employed a molecular dating approach based on penalized likelihood using treePL (Sanderson 2002; Smith and O'Meara 2012). We used a previously dated Asteraceae-wide phylogeny based on *ndh*F, *rbc*L and *trn*LF (Kandziora et al. unpublished) for calibrating the *Dendrosenecio* nuclear-based species phylogeny (ASTRAL species tree with all accessions as terminal taxa). The Asteraceae-wide phylogeny was dated using an uncorrelated lognormal clock as implemented in BEAST v.2.6.2 (Bouckaert et al. 2014), based on two fossil calibration points (Barreda et al. 2012, 2015) and restricting the age of the root to 73–101 million years ago (Mya) (maximum age of Asterales according to Beaulieu et al. 2013). The Asteraceae phylogeny does not completely agree with the phylogenetic relationships shown by Panero et al. (2014), despite using a starting tree, but age estimates are congruent with other age estimates (Senecioneae, Kandziora et al. 2017; *Euryops*, Devos et al. 2010). The 95% confidence interval of the highest posterior density (HPD) values of the crown age of *Dendrosenecio* (16.77 -2.07 Mya) was then used as a secondary minimum and maximum calibration point to date the nuclear *Dendrosenecio* phylogeny as calculated using ASTRAL v.5.7.4 with the bootstrapping option to obtain confidence intervals for the molecular dating. To infer the confidence interval, we conducted a molecular dating of 99 bootstrap phylogenies and combined them using TreeAnnotator. Automatic estimation of the optimal parameters for cross-validation was done before running treePL using a wrapper script (https://github.com/tongjial/treepl_wrapper).

Species delimitation

Boundaries between species were evaluated using BPP v.4.3.8 (Yang and Rannala 2014), a Bayesian approach that allows the use of multilocus data for conducting both species delimitation and species tree inference analysis (i.e., unguided species delimitation). We used the data subset that excluded potentially admixed accessions (for details on these accessions, see phylogenetic networks results below) to avoid a strong violation of the assumption of no migration (i.e., significant ongoing gene flow between species; Flouri et al. 2018).

To test boundaries between currently accepted species as well as the existence of independent lineages in each mountain, 13 entities were predefined by coding the accessions according to taxonomy and mountain of origin. A total of 34 accessions were included, and the species tree topology obtained with ASTRAL was used as a starting species tree. Three replicates of 299 randomly sampled alignments (25% of all loci) and both reversible-jump MCMC algorithms (0 and 1) available in BPP were used to increase computational efficiency and evaluate the convergence between runs. The default species tree model prior 1, that assigns equal probabilities to rooted species trees, was chosen. A diffuse prior was used for both population size (θ) and divergence time (τ) parameters, setting alpha = 3 and adjusting beta to fit the mean to empirical values (i.e., proportion of variable sites and crown age for *Dendrosenecio*). The 'invgamma' v.1.1 package (Kahle and Stamey 2017) in R v.3.5 (R Core Team 2018) was used for visualization of prior distributions. The inverse-gamma (IG) distribution for θ was IG(3, 0.08) with a mean of 0.04, which corresponds to the proportion of variable sites estimated from our nuclear dataset. Based on a median crown age for *Dendrosenecio* of 7.89 Mya (value from the Asteraceae-wide phylogeny used for calibration in the divergence time estimation) and a substitution rate of 5×10^{-9} (approximate rate for plant nuclear DNA; Lowe et al. 2004), the value for τ was set to IG(3, 0.079) with a mean of 0.039. The GTR nucleotide substitution model was employed. To account for heterogeneous

mutation rate across loci, the re-scaling option for the locusrate parameter was chosen with IG(0, 0; where 0 means ∞), following suggestions from the BPP documentation for cases where there are no fossil calibrations in the tree. The concentration parameter was set to 2, as recommended in the BPP documentation for exonic data, and a hierarchical prior was selected. A molecular clock with independent rates across loci was implemented with a gamma (G) distribution G(10, 100), with shape parameter = 1 and selecting a hierarchical prior, to avoid that variance across loci violated the molecular clock. The option for automatic fine-tuning of the MCMC was used for running 2.7 million iterations at a sampling frequency of five and discarding 200,000 samples as burn-in. The adequacy of step-length acceptance proportions in all parameters was checked for all replicates.

Results

Sequence data processing

The customized reference used for paralog detection contained a total of 848 loci. Sequence pairs, belonging to the same exon, with divergence values between 8.03% and 19.95% (Figure S1) were flagged as paralogous. An average of 333 (40%) paralogous loci was detected for all accessions, but values were higher for *Dendrosenecio* compared to outgroup taxa, with 342 (41%) and 244 loci (29%), respectively (Table S3). The splitting of paralogous loci led to an increase in the total number of loci that were finally analyzed to 1197 in total. At least 344,913 reads were mapped for each accession, and alignment length for each locus averaged 244 bp (ranging from 84 to 735 bp; Table 2). The mean number of variable sites was 29 (12%), and loci averaged 13.1 (5%) parsimony-informative sites. Percentage of missing data was below 60% across loci, and missing data values were lower for *Dendrosenecio* accessions than for outgroup taxa, with averages of 2% and 5%, respectively. In the case of off-target cpDNA data, 149 coding and non-coding regions were analyzed. Over 5301 reads were mapped for each accession, with an average length of 742 bp for all alignments (length range 201 to 6669 bp; Table 2). cpDNA regions averaged 35.4 (5%) variable sites and 12 (2%) parsimony informative sites. Percentage of missing data was below 7% across loci and under 9% for all samples.

Phylogenetic analyses based on the targeted nuclear loci

Both nuclear species trees generated with ASTRAL (Figs. 2a, 2c and S2) supported *Dendrosenecio* as a monophyletic group comprising two fully supported lineages: one consisting of all three species from the mountains in Tanzania (Kilimanjaro/Meru), and one comprising the eight species from the remaining mountains. The latter group further split into three distinct, strongly supported clades (BS ≥95%), each of them corresponding to a separate mountain group (Kenya/Aberdare, Elgon/Cherangani and Rwenzori/Virunga). Additional splits within these four clades were consistent with geography, and most species occurring on a single mountain formed distinct lineages, with the exception of *D. keniensis* and *D. adnivalis*. Relationships between species from the Kilimanjaro/Meru clade were unresolved (BS <90%), but the Mt. Kilimanjaro endemic *D. johnstonii* and the Mt. Meru endemic species *D. meruensis* were strongly supported. Within the Kenya/Aberdare clade, *D. battiscombei*, from Mt. Kenya and the Aberdare Range, and the Aberdare Range endemic species *D. brassiciformis* formed two fully supported clades, whereas *D. keniensis* and *D. keniodendron*, from Mt. Kenya, formed two strongly supported lineages with one potentially admixed *D. keniensis* accession (see below) nested within the *D. keniodendron* lineage. The Elgon/Cherangani clade showed a split between *D. elgonensis* on Mt. Elgon (fully supported) and *D. cheranganiensis* from the Cherangani Hills, which showed full support and cytonuclear incongruence (see cpDNA results below). The Rwenzori/Virunga clade showed no clear separation between *D. erici-rosenii* and *D. adnivalis*. The "reduced species tree", where potentially admixed accessions

were excluded, did not show any major changes in topology and support (see Figure S3) and the species tree based on locus concatenation was congruent with those obtained with ASTRAL (Figure S4).

The phylogenetic networks were mostly congruent with the species trees. Four major groups corresponding to the same four mountain groups were obtained. Most accessions of the same species grouped together, with the exception of eight accessions that were identified as potentially admixed. *D. keniensis* (DE_051_X), *D. elgonensis* (DE_040_1, DE_011_2), and *D. erici-rosenii* (DE_061_1) clearly formed mixed groups, and *D. battiscombei* (DE_047_1, DE_50_1) and *D. erici-rosenii* (DE_032_5) showed "misplacement" (see Methods) in the SuperQ network (Fig. 3a). Additionally, *D. meruensis* (DE_058_1) and *D. erici-rosenii* (DE_032_5, DE_061_1) displayed "misplacement" in the Neighbor-Net network (Fig. 3b). Interestingly, the two *D. elgonensis* samples from the same population (DE_011_2, DE_011_10) showed a distant placement in the networks, while having a similar number of mapped exons and missing data values.

Phylogenetic analysis based on the off-target cpDNA

The phylogenetic analysis of cpDNA also found full support for the monophyly of *Dendrosenecio* and for the basal split differentiating the Kilimanjaro/Meru clade from a sister clade comprising the remaining species (Figs. 2b, 2d). Subsequent splits within these two clades were largely incongruent with those obtained in the nuclear phylogeny. Within the Kilimanjaro/Meru clade, *D. meruensis* and *D. johnstonii* were nested within the *D. kilimanjari* lineage, whereas within the latter clade, the previously defined mountain groups (Kenya/Aberdare, Elgon/Cherangani and Rwenzori/Virunga) were not recovered as monophyletic lineages. The species from the Elgon/Cherangani mountain group were recovered in two separate lineages, revealing a cytonuclear discordance pattern. The first of these lineages formed a fully supported clade in which *D. cheranganiensis* was sister to *D. battiscombei* and one *D. brassiciformis* accession from the Aberdare Range. The second lineage was unresolved and showed *D. elgonensis* and representatives of *D. erici-rosenii* from the Virunga Mts. in sister relationship. The accessions corresponding to *D. erici-rosenii* from the Rwenzori Mts. formed a strongly supported clade together with *D. adnivalis.* The remaining species from the Kenya/Aberdare mountain group formed one lineage comprising three strongly supported clades. Two corresponded to *D. keniode*ndron and *D. keniensis,* and the third contained representatives of *D. battiscombei* occurring on Mt. Kenya and the remaining accessions of *D. brassiciformis.*

Divergence times estimation

The crown age estimated for *Dendrosenecio* was 10.6 Mya, with the 95% HPD interval ranging from 4.6 to 16.8 Mya (Fig. 4). The four main clades showed ages spanning from the Late Miocene to the Pleistocene. The Kenya/Aberdare clade was estimated to be the oldest (median = 8.1 Mya, HPD: 11.2-0.4). The Elgon/Cherangani clade was found to have originated later (median = 5.6 Mya, HPD: 7.4-0.3), and the clades from Kilimanjaro/Meru (median = 4.2 Mya, HPD: 6.0-0.3) and Rwenzori/Virunga (median = 3.2 Mya, HPD: 6.6-0.1) were inferred to be the youngest. Whenever possible to estimate, the age of the most recent common ancestor of the species was younger than the time estimates for the orogeny of the mountains where they occur.

Species delimitation

The replicate analyses performed using BPP showed consistent results among data subsets for both algorithms employed, thus we considered convergence issues to be unlikely. All species delimitation replicates found the highest

support for a model with 10 species with a posterior probability (PP) ≥0.49 (Table 3). The highest supported alternative result showed 11 species in most replicates with support ≤0.14 PP (Table S4). Posterior probability for individual species found five of the currently accepted species to be fully supported, and the remaining six showed mean PP values between 0.99 and 0.1 (Tables 3 and S4). *Dendrosenecio erici-rosenii* and *D. adnivalis* from the Western Rift mountains (Rwenzori/Virunga) showed higher support as a single species (mean PP ≥0.72) than as distinct ones. The representatives of *D. battiscombei* occurring on different mountains (Mt. Kenya and the Aberdare Range) were better supported as the same species (mean PP = 0.83) than as different ones (mean PP ≤0.17). The tree inference analysis, which tested for changes in the starting species tree topology used as prior for the unguided species delimitation, found highest support for two species tree hypotheses. These topologies were congruent with the nuclear phylogenies obtained with ASTRAL and RAxML-NG (Figures 2a, S2 and S4) with the exception of the relationships with the Kilimanjaro/Meru clade (Figure S5).

Discussion

Poorly resolved phylogenies for plant groups that have recently radiated, like those found in tropical-alpine areas, are common, but resolution and support may be improved using phylogenomic approaches such as genome skimming and especially Hyb-Seq (Vargas et al. 2017; Morales-Briones et al. 2018; Pouchon et al. 2018). Whole genome duplication events in the relatively recent history of such plant groups might present additional challenges to phylogenetic reconstruction. The combination of coalescent-based phylogenetic methods (Maddison and Knowles 2006) and the identification and utilization of paralogs for phylogenetic reconstruction allows accounting for ILS and gene duplication as potential sources of gene tree discordance. In this study, we refined the phylogenetic hypothesis of one of the most iconic tropical-alpine plant groups, the afro-alpine Asteraceae genus *Dendrosenecio*, and we were able to determine species relationships more reliably compared to previous studies (Hedberg 1970; Mabberley 1973; Knox and Palmer 1995a; Tusiime et al. 2020), estimate divergence times and test species boundaries. We found that the *Dendrosenecio* lineage might have started to diversify as soon as high elevation habitats were available in East Africa, and that geographic speciation likely played an important role in the early diversification of the group. In addition, we detected that historical hybridization and recent secondary contact following long-distance dispersal as well as ecological speciation probably contributed to the current diversity found in this genus.

Origin and early dispersal in *Dendrosenecio*

The current species diversity in *Dendrosenecio* has been suggested to be the result of a recent and rapid radiation across the East African high mountains (Knox and Palmer 1995a; Tusiime et al. 2020), as reported for other alpine plant groups (Hughes and Atchison 2015). The short internal branch lengths obtained in our nuclear and cpDNA phylogenies (Fig. 2) could be indicative of such radiation (Degnan and Rosenberg 2009; Naciri and Linder 2015), but given the uncertainty of our divergence time estimation it is difficult to determine how recently it occurred. Geological prerequisites (i.e., mountains with at least 3200-3500 m altitude) for the afro-alpine flora could have existed in East Africa since the Middle Miocene (Hedberg 1970; Gehrke and Linder 2014; Wichura et al. 2015) and even though alpine-like climates in the area may have been absent at that time, the global climatic cooling that started during thatsame period (~15 Mya; Couvreur et al. 2020) could have favored the development of such climatic conditions. Thus, our estimated age of 10.6 Mya (HPD 16.8-4.6 Mya, Fig. 4) for the most recent common ancestor of *Dendrosenecio*, between the Late Miocene and Late Pliocene, suggests that the genus started to diversify during a

period when alpine-like habitats already might have been available in East Africa. The limited sampling of outgroup taxa and the lack of sufficient calibration points did not provide sufficient certainty to determine a reasonable stem age for *Dendrosenecio*, thus its time of origin remains uncertain. The four main clades recovered in the nuclear phylogeny (Fig. 2a) suggest that adaptation to different elevations occurred independently in the different mountain groups, supporting the parallel adaptation scenario that has been suggested (Hedberg 1970; Mabberley 1973; Knox and Palmer 1995a; Tusiime et al. 2020). However, the lack of resolution within these clades does not allow for extending this hypothesis further. What can be concluded from our results is that the hypothesis of initial high altitude adaptation on Mt. Kilimanjaro, followed by dispersal across mountain tops and subsequent radiation (Knox and Palmer 1995a) is unlikely, in particular when also considering the relatively young volcanic origin estimated for Mt. Kilimanjaro (2.5-1.9 Mya; Nonnotte et al. 2008) in comparison to other East African mountains (e.g., Mt. Elgon 23-12 Mya, Rwenzori Mts. 8-3 Mya, Aberdare Range 6.5–5 Mya, Mt. Kenya 5-2.5 Mya; Gehrke and Linder 2014).

The mountains currently occupied by *Dendrosenecio* started to rise in the Miocene and continued throughout the Pliocene-Pleistocene (Hamilton and Taylor 1991; Chorowicz 2005). As in other mountainous regions (Luebert and Weigend 2014; Favre et al. 2015), these geological processes may have provided new evolutionary opportunities and facilitated migration by increasing habitat availability and landscape heterogeneity. The availability of new habitats resulting from volcanic activity in the EARS could have enhanced the expansion of ancestral populations across the mountains during the early evolutionary history of *Dendrosenecio.* Given our results, such connection between mountain groups, possibly through early long-distance dispersal, would have occurred mainly around the Miocene-Pliocene. During a later period, continuous isolation might have strengthened reproductive barriers, thereby promoting divergence between taxa from different mountain groups.

The role of geographic isolation in species differentiation

Our nuclear phylogenetic analysis revealed that closely related species usually occur within the same mountain(s), and the four main clades detected clearly correspond to the four spatially distant mountain groups (Fig. 2a), in accordance with the AFLP pattern detected by Tusiime et al. (2020). This geographic pattern could be a consequence of reduced gene flow between these isolated mountain groups during the early diversification of lineages in the genus. Geographic divergence is considered as one of the main drivers of diversification in mountain systems (Hughes and Atchison 2015; Boucher et al. 2016; Wallis et al. 2016; Schneeweiss et al. 2017), and geological changes during the Late Neogene associated with the genesis of the EARS could have increased the opportunities for allopatric speciation and favored a geographic radiation (sensu Lieberman 2012; Simões et al. 2016) in *Dendrosenecio.* In addition, the estimated age for the ancestral population of each species suggests that they diversified within their respective mountain of origin, reaching other mountains only in a few cases (e.g., *D. battiscombei* and *D. erici-rosenii*).

The most recent taxonomic classification with 11 accepted species (Knox 2005) first divides species according to their geographic distribution and then splits them further. Our species delimitation results supported a model with 10 species, but only five of them were fully supported and the remaining five showed PP values above 0.50 (Table 3). The interpretation of these results should be done cautiously considering that molecular species delimitation should be combined with additional evidence (e.g., morphological, ecological; Yang and Rannala 2010), and that species delimitation under the multispecies coalescent model could be diagnosing genetic structure and not true species (Sukumaran and Knowles 2017). Although the true number of species of *Dendrosenecio* cannot be fully determined at this stage, our results highlight the importance of geography in diversification of species/populations. Interestingly, the

dendrosenecios in the Western Rift mountains (Rwenzori/Virunga) showed highest support as a single species. Moreover, the plants referred to *D. erici-rosenii* from the Rwenzori Mts. seem to be more closely related to *D. adnivalis* than to those referred to *D. erici-rosenii* from the Virunga Mts., as shown by the cpDNA phylogeny (Fig. 2b) and the PP per entity obtained in the species delimitation analysis (Table S4). However, determining if this is evidence of ongoing speciation or hybridization needs to be further investigated. Another interesting finding is the consistent support for *D. keniensis* and *D. keniodendron* as distinct species (Table 3), possibly forming one monophyletic lineage based on the nuclear phylogeny (Fig. 2a). Considering that these two species grow sympatrically on Mt. Kenya and occupy distinct habitats (*D. keniensis* on water-saturated soils, *D. keniodendron* on well-drained soils), it seems likely that they originated through ecological speciation. Studying the role of ecological speciation within a single mountain in the case of these two species and in similar cases (i.e., *D. johnstonii* and *D. kilimanjari* or *D. brassiciformis* and *D. battiscombei*), would help to determine the contribution of ecological processes to the diversification of afro-alpine plant groups.

Possible secondary contact following dispersal

Our phylogenetic analysis of cpDNA recovered a different mountain grouping than the one supported by the nuclear phylogeny (Fig. 2a, 2b). The most striking cytonuclear discordance involves *D. cheranganiensis* from the Cherangani Hills and *D. battiscombei* from the Aberdare Range, which were recovered in sister relationship in the cpDNA phylogeny but in different clades in the nuclear phylogeny. Cytonuclear discordance has been shown to be a sign of introgression in other Asteraceae lineages (Vargas et al. 2017; Lee-Yaw et al. 2019), and it might explain the weak support (BS = 57%) obtained for *D. cheranganiensis* when branches showing low bootstrap support in the individual gene trees were not collapsed (results not shown). This improved resolution could be indicating that, despite geographic isolation, long-distance dispersal might have enhanced occasional gene flow between populations from different mountain groups. However, specific testing and a more extensive population sampling are needed to evaluate the role of historical hybridization in *Dendrosenecio* and determine if introgression is the source of cytonuclear discordance.

During the Pleistocene, the climatic oscillations increased also in tropical Africa and caused recurrent glacial–interglacial cycles (Hamilton and Taylor 1991), which might have promoted recent diversification in *Dendrosenecio*. During glacial periods the afro-alpine vegetation expanded downward, occupying about eight times its current area (Chala et al. 2017). This expansion of distribution ranges might have reduced the distances between habitats from adjacent mountains, favoring dispersal through migration corridors for afro-montane species (the montane forest bridge hypothesis; Hedberg 1969; Kebede et al. 2007), and also facilitating long-distance dispersal for those adapted to alpine conditions. Increased opportunities for migration through forest bridges could explain the distribution of *D. battiscombei*, which occurs in the afro-montane zone of adjacent mountains in central Kenya (Kenya/Aberdare), and that of *D. erici-rosenii*, which occurs at lower altitudes in the Western Rift mountains (Rwenzori/Virunga). A continuous expansion and contraction of ranges could have favored connectivity-isolation cycles, increasing the opportunities for allopatric differentiation as well as for secondary contact (Flantua and Hooghiemstra 2018; Muellner-Riehl 2019), giving rise to hybrid species of *Dendrosenecio* as suggested by Tusiime et al. (2020). The formation of species-mixed groups in the SuperQ network (Fig. 3a) suggests that recent long-distance dispersal could have facilitated secondary contact and hybridization between species from different mountain groups, as inferred for other afro-alpine taxa (Gizaw et al. 2016b). Furthermore, the distant placement of the two *D. elgonensis* accessions from the same

population in the phylogenetic networks could indicate that different degrees of admixture are present within populations.

Although the climatic oscillations during the Pleistocene have been proposed to shape plant diversity in alpine areas (Kadereit et al. 2004; Winkworth et al. 2005; Madriñán et al. 2013; Roquet et al. 2013; Kadereit 2017), determining their role in the radiation of certain afro-alpine groups such as *Dendrosenecio* remains difficult due to the confluence of numerous factors (i.e., topographic, climatic and ecological). Only in multidisciplinary approaches that combine phylogenomics with population genomics and ecological modeling, it may be possible to fully disentangle the processes behind the diversification of tropical-alpine plant groups.

Fig. 1 Map showing the study area. Mountain names are shown in bold. The gray scale indicates elevation. Fault lines of the western and eastern branches of the Great Rift Valley are marked with stippled lines.

Fig. 2 Nuclear and plastid DNA phylogenies of *Dendrosenecio*. Each tip corresponds to one accession. Species names for accessions are followed by abbreviated geographic origin. Numbers along branches are bootstrap support. Samples that were considered admixed are indicated by asterisks (*), and samples not included in the plastid phylogeny due to poor quality (see Methods) are denoted with black squares (□). Colors correspond to the four main clades recovered in the nuclear phylogeny; these clades are also denoted with letters A to D. The map shows the four geographically distant mountain groups colored according to these four clades; elevation is indicated by the gray scale, and fault lines in the western and eastern branches of the Great Rift Valley are marked with stippled lines. **(a)** *Dendrosenecio* clade from the nuclear phylogeny generated using ASTRAL. **(b)** *Dendrosenecio* clade from the plastid phylogeny generated using RaxML-NG with clades colored according to their placement in the nuclear phylogeny to show (in)congruence. **(c)** Entire nuclear phylogeny showing outgroup taxa. **(d)** Entire plastid phylogeny showing outgroup taxa. Abbreviations: Ki, Mt. Kilimanjaro; Me, Mt. Meru; Ke, Mt. Kenya; Ab, Aberdare Range; El, Mt. Elgon; Ch, Cherangani Hills; Rw, Rwenzori Mts.; Vi, Virunga Mts.

Fig. 3 Phylogenetic networks of *Dendrosenecio* based on nuclear DNA data. Species names for accessions are followed by abbreviated geographic origin. Samples that were considered admixed are indicated by asterisks. Colors represent the four mountain groups and the four main clades recovered in the nuclear DNA phylogeny; these clades are also denoted with letters A to D. **(a)** SuperQ network. **(b)** Neighbor-Net network. Abbreviations: Ki, Mt. Kilimanjaro; Me, Mt. Meru; Ke, Mt. Kenya; Ab, Aberdare Range; El, Mt. Elgon; Ch, Cherangani Hills; Rw, Rwenzori Mts.; Vi, Virunga Mts.

Fig. 4 Dated phylogeny of *Dendrosenecio* based on nuclear DNA data. Species names for accessions are followed by abbreviated geographic origin. Numbers along branches are bootstrap support (above) and median age (below). The 95% confidence interval of the highest posterior density is shown by horizontal bars. Colors represent the four mountain groups and the four main clades recovered in the nuclear DNA phylogeny; these clades are also denoted with letters A to D. Abbreviations: Ki, Mt. Kilimanjaro; Me, Mt. Meru; Ke, Mt. Kenya; Ab, Aberdare Range; El, Mt. Elgon; Ch, Cherangani Hills; Rw, Rwenzori Mts.; Vi, Virunga Mts.; Mya, Million years ago.

Table 1 *Dendrosenecio* samples included in the study. Geographic origin and altitude where samples were collected are provided. Abbreviations: Mt., mountain.

Sample id	Taxa	Mountain/Mt. range	Altitude (m)
DE_023_3	*Dendrosenecio adnivalis*	Rwenzori Mts.	3440
DE_025_5	*Dendrosenecio adnivalis*	Rwenzori Mts.	3900
DE_034_3	*Dendrosenecio adnivalis* subsp. *adnivalis*	Rwenzori Mts.	4153
DE_009_5	*Dendrosenecio battiscombei*	Aberdare Mts.	3865
DE_010_3	*Dendrosenecio battiscombei*	Aberdare Mts.	3215
DE_015_10	*Dendrosenecio battiscombei*	Mount Kenya	3300
DE_047_1	*Dendrosenecio battiscombei*	Aberdare Mts.	3898
DE_050_1	*Dendrosenecio battiscombei*	Mount Kenya	4009
DE_006_3	*Dendrosenecio brassiciformis*	Aberdare Mts.	3865
DE_007_4	*Dendrosenecio brassiciformis*	Aberdare Mts.	3898
DE_008_1	*Dendrosenecio brassiciformis*	Aberdare Mts.	3906
DE_004_5	*Dendrosenecio cheranganiensis* subsp. *cheranganiensis*	Cherangani Hills	3184
DE_005_2	*Dendrosenecio cheranganiensis* subsp. *dalei*	Cherangani Hills	3184
DE_011_2	*Dendrosenecio elgonensis*	Mount Elgon	4222
DE_011_10	*Dendrosenecio elgonensis*	Mount Elgon	4222
DE_040_1	*Dendrosenecio elgonensis* subsp. *babatipes*	Mount Elgon	3330
DE_001_1	*Dendrosenecio elgonensis* subsp. *babatipes*	Mount Elgon	3293
DE_002_5	*Dendrosenecio elgonensis* subsp. *babatipes*	Mount Elgon	4040
DE_003_2	*Dendrosenecio elgonensis* subsp. *elgonensis*	Mount Elgon	3629
DE_012_6	*Dendrosenecio elgonensis*	Mount Elgon	3935
DE_032_5	*Dendrosenecio erici-rosenii* subsp. *alticola*	Virunga Mts.	4126
DE_033_4	*Dendrosenecio erici-rosenii* subsp. *alticola*	Virunga Mts.	4019
DE_061_1	*Dendrosenecio erici-rosenii* subsp. *alticola*	Virunga Mts.	4126
DE_024_4	*Dendrosenecio erici-rosenii*	Rwenzori Mts.	3581
DE_026_4	*Dendrosenecio erici-rosenii*	Rwenzori Mts.	4126
DE_031_3	*Dendrosenecio erici-rosenii* subsp. *erici-rosenii*	Rwenzori Mts.	3550
DE_021_4	*Dendrosenecio johnstonii*	Mount Kilimanjaro	2932
DE_022_3	*Dendrosenecio johnstonii*	Mount Kilimanjaro	2569
DE_014_1	*Dendrosenecio keniensis*	Mount Kenya	4243
DE_017_7	*Dendrosenecio keniensis*	Mount Kenya	4197
DE_051_X	*Dendrosenecio keniensis*	Mount Kenya	3472
DE_013_10	*Dendrosenecio keniodendron*	Mount Kenya	4243
DE_016_1	*Dendrosenecio keniodendron*	Mount Kenya	4182
DE_049_3	*Dendrosenecio keniodendron*	Mount Kenya	3696
DE_019_4	*Dendrosenecio kilimanjari*	Mount Kilimanjaro	4387
DE_020_2	*Dendrosenecio kilimanjari* subsp. *cottonii*	Mount Kilimanjaro	4112
DE_027_3	*Dendrosenecio kilimanjari* subsp. *cottonii*	Mount Kilimanjaro	4390
DE_028_2	*Dendrosenecio kilimanjari* subsp. *cottonii*	Mount Kilimanjaro	3817
DE_018_7	*Dendrosenecio kilimanjari*	Mount Kilimanjaro	3513
DE_029_3	*Dendrosenecio meruensis*	Mount Meru	3608
DE_030_5	*Dendrosenecio meruensis*	Mount Meru	3116
DE_058_1	*Dendrosenecio meruensis*	Mount Meru	3321

Table 2 Summary statistics obtained in the processing of nuclear and plastid DNA data. Minimum, maximum and mean values are provided. Abbreviations: bp, base pairs; PI sites, parsimony informative sites.

Nuclear DNA data (1197 loci)	**Alignment length (bp)**	**Number of variable sites**	**Proportion of variable sites**	**Number of PI sites**	**Proportion of PI sites**	**Missing data percentage**
minimum	84	1	0.01	0	0	0
maximum	735	183	0.4	69	0.2	58.7
mean	244	29	0.1	13.1	0.1	2.2
Plastid DNA data (149 loci)						
minimum	201	1	0.004	0	0	0
maximum	6669	425	0.1	143	0.1	6.9
mean	742	35.4	0.05	12.0	0.02	0.8

Table 3 Summary of results obtained in the species delimitation analysis using BPP. The highest supported result for each replicate analysis (subsample and reversible-jump MCMC algorithms 0 and 1) is shown. The posterior probability values for the overall number of delimited species and for each individual species are provided. The main clades recovered in the nuclear DNA phylogeny are indicated at the top of the table. Abbreviations: sp. No., species number; PP, posterior probability; D. kil, *Dendrosenecio kilimanjari*; D. joh, *Dendrosenecio johnstonii*; D. mer, *Dendrosenecio meruensis*; D. ked, *Dendrosenecio keniodendron*; D. ken, *Dendrosenecio keniensis*; D. bat, *Dendrosenecio battiscombei*; D. bra, *Dendrosenecio brassiciformis*; D. elg, *Dendrosenecio elgonensis*; D. che, *Dendrosenecio cheranganiensis*; D. eri, *Dendrosenecio erici-rosenii*; D. adn, *Dendrosenecio adnivalis.*

			Kilimanjaro/Meru			**Kenya/Aberdare**				**Elgon/Cherangani**		**Rwenzori/Virunga**
Algorithm 0	**sp. No.**	**PP**	**D. kil**	**D. joh**	**D. mer**	**D. ked**	**D. ken**	**D. bat**	**D. bra**	**D. elg**	**D. che**	**D. eri+D. adn**
Subsample 1	10	0.50	1	0.77	0.77	1	1	0.79	1	1	1	0.82
Subsample 2	10	0.67	0.99	0.99	1	1	1	0.83	1	1	1	0.81
Subsample 3	10	0.49	0.99	0.99	1	1	1	0.87	1	1	1	0.56
mean		0.55	0.99	0.92	0.92	1	1	0.83	1	1	1	0.73
Algorithm 1	**sp. No.**	**PP**	**D. kil**	**D. joh**	**D. mer**	**D. ked**	**D. ken**	**D. bat**	**D. bra**	**D. elg**	**D. che**	**D. eri+D. adn**
Subsample 1	10	0.49	1	0.80	0.80	1	1	0.79	1	1	1	0.77
Subsample 2	10	0.68	0.99	0.99	1	1	1	0.83	1	1	1	0.83
Subsample 3	10	0.52	1	0.99	1	1	1	0.87	1	1	1	0.60
mean		0.56	1	0.93	0.93	1	1	0.83	1	1	1	0.73

Declarations

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Conflicts of interest/Competing interests The authors declare that they have no conflict of interest.

Availability of data and material Raw reads that were generated for the current study are available under NCBI SRA BioProject PRJNA729901 (see Supplementary Information Table S1 for accession numbers).

Code availability Not applicable

Authors' contributions RS, JMG, AG, FK and CB conceived and designed the research. AG, DC, LG, AZ, GE, CB and FK performed the fieldwork and curated the plant material. LS performed DNA extractions and library preparation. JMG processed the data, performed phylogenetic and species delimitation analyses and led the manuscript preparation together with AG. MK performed the divergence times analysis. RS supervised the analyses and the manuscript preparation. CB, FK and RS facilitated the project by logistic and infrastructure support. All authors contributed to the text.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

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