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1 **Young, disturbed, unsaturated: accelerating species accumulation in the enigmatic tropical alpine**
2 **flora on the African sky islands**

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22

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25 phylogenetic trees and analysis settings are available via the Open Data Framework, [osf.io/brmjq.](https://osf.io/brmjq/) including
26 reference to the respective original publications on which alignments and settings were based and respective
27 initial publications are cited. Code used for this study is also available via the Open Data Framework
28 (osf.io/brmjq).

29

30 **This PDF file includes:**

31 Main Text

32 Figures 1 to 3

33 **Abstract**

34 Tropical alpine floras are renowned for high endemism, spectacular giant rosette plants testifying to
35 convergent adaptation to harsh climates with nightly frosts, and recruitment dominated by long-distance
36 dispersal from remote areas. In contrast to the larger, more recent (late Miocene onwards) and contiguous
37 expanses of tropical alpine habitat in South America, the tropical alpine flora in Africa is extremely
38 fragmented across small patches on distant mountains of variable age (Oligocene onwards). How this has
39 affected the colonization and diversification history of the highly endemic but species-poor afroalpine flora is
40 not well known. Here we infer phylogenetic relationships of ~20% of its species using novel genome
41 skimming data and published matrices and infer a timeframe for species origins in the afroalpine region
42 using fossil-calibrated molecular clocks. Although some of the mountains are old, and although stem node
43 ages may substantially predate colonization, most lineages appear to have colonized the afroalpine during
44 the last 5 or 10 My. The accumulation of species increased exponentially towards the present. Taken
45 together with recent reports of extremely low intra-population genetic diversity and recent inter-mountain
46 population divergence, this points to a young, unsaturated, and dynamic island scenario. Habitat disturbance
47 caused by the Pleistocene climate oscillations likely induced cycles of colonization, speciation, extinction,
48 and recolonization. This study contributes to our understanding of differences in the histories of recruitment
49 on different tropical sky islands and on oceanic islands, providing insight into the general processes shaping
50 their remarkable floras.

51 **Significance Statement**

52 Resilience is required to withstand or mitigate the effect of human-induced climate change. Today whole
53 ecosystems are affected by climate change, but our understanding of their evolution and natural response is
54 limited, often restricted to individual populations or species. The enigmatic flora on the tops of the African sky
55 islands is isolated and unique, showing striking adaptations to the harsh tropical alpine conditions. Here we
56 analyze genome data from a large fraction of afroalpine plants and show that this remarkable flora has a
57 dynamic history with frequent colonizations and extinctions, most likely caused by previous natural climate
58 changes during the ice age cycles. The flora will be particularly vulnerable to human-induced climate
59 warming reducing alpine habitat into successively smaller areas.

60

61 **Main text**

62 **Introduction**

63 The enigmatic floras restricted to high mountains in the tropics provide a unique opportunity to study the
64 timing and impact of dispersal, establishment, and diversification, allowing us insight into the evolution of
65 whole floras. These unique, often startlingly rich floras contrast starkly in growth forms and species
66 compositions with those found in the tropical climes closer to sea level (1, 2). Across the tropics, the upper
67 elevations of the high mountains in South America, Southeast Asia, and Africa (3) experience similar harsh
68 environmental conditions: year-round high insolation and extreme diurnal rather than seasonal variation in
69 temperature (4). The archetypical dwarf shrub forms (e.g., *Alchemilla*, *Lachemilla*) and giant rosette forms
70 (e.g., *Dendrosenecio*, *Espeletia*) typically found in these habitats provide classic examples of convergent
71 adaptations independently derived in distantly related plant groups (5, 6).

72 Whereas most mountains that harbor tropical alpine floras are the result of recent uplift (late Miocene, 11.6
73 million years ago [Ma] onwards; (7, 8)), the high mountains in tropical Africa vary in age from about 40 Ma to
74 1 Ma (9), with the origins of volcanoes in the rift zone of East Africa and Ethiopia likely resulting in isolated
75 alpine conditions from the early Miocene (23 Ma) onwards (10). This places maximum bounds on the ages of
76 present-day species communities but leaves open questions as to the rate with which plants colonized the
77 emerging high-elevation niches and the relative roles of dispersal leading to successful establishment, *in situ*
78 diversification, and extinction in generating present day species diversity and endemism (11, 12).

79

80 These factors can be expected to differ markedly between tropical mountainous regions that differ in age,
81 spatial extent, and degree of connectivity through time. The rich flora of the high Andes extends thousands
82 of kilometers through the Americas, with the potential for north-south exchange with ‘flickering connectivity’
83 across wide areas caused by climatic change (13). By contrast, the much less species-rich afroalpine flora
84 (Figure 1a) is typically restricted to solitary mountains isolated from each other by vast expanses of tropical
85 lowland vegetation (14). These lowland barriers, in place since the origins of the mountains, largely persisted
86 throughout the glaciations, despite the eight times greater than present day area of afroalpine habitat
87 inferred for the Last Glacial Maximum (14). Population genetic studies of several afroalpine plant species
88 have shown strong inter-mountain isolation of populations with genetic exchange limited to rare long-
89 distance dispersal events rather than ecological connectivity (15). The pattern of recruitment to the floras on
90 such isolated continental ‘sky islands’ might be most similar to that on oceanic islands such as Hawaii (11).

91

92 **Figure 1.** Geographic distribution and recruitment to the afroalpine flora (>3500 m). A) Major mountains and
93 mountain regions in eastern Africa. The Great Rift Valley is shown as stippled lines. B) Numbers of
94 afroalpine colonization (orange) and *in situ* diversification (olive) events per 0.5 Ma time bin. Colonizations,
95 here represented by stem nodes, must be interpreted as maximum age estimates for events that are more
96 recent to an unknown degree. C) Stem age estimates of afroalpine species in relation to mountain orogeny
97 (gray bars; Gehrke et al. (7)). Species are colored according to family. Local endemics (species restricted to
98 one or two mountains) are mapped to individual mountains; the remaining species are classified as ‘eastern
99 African endemics’ (‘endemicEA’) or as ‘widespread’. Age estimates in A) and B) represent both Bayesian
100 median node ages of individual seed plant clades.

101

102 The key to inferring the sequence and timing of the dispersal and speciation events that founded a biome is
103 comprehensively sampled time-calibrated phylogenetic trees. The afroalpine flora is dominated by genera
104 that are well known from their numerous species distributed across both northern and southern temperate
105 regions. Case studies (e.g., *Alchemilla*, *Carex*, *Helichrysum* and *Ranunculus* (15)) have begun to paint a
106 picture of multiple independent colonizations from the late Miocene c. 10 Ma onwards, particularly from
107 remote northern as well as southern temperate areas, followed by limited *in situ* diversification (15). A deeper
108 understanding of the process of recruitment to tropical alpine floras requires a broad comparison of multiple
109 independent groups (12), but past comparisons in the afroalpine region (11) have been limited by the state of
110 phylogenetic knowledge across the flora. Here, we address this limitation, analyzing multiple lineages from
111 the afroalpine flora based on new field-collected material and data from high throughput sequencing.

112

113 **Results**

114 We collected new material during field expeditions to all major mountains in East Africa and Ethiopia and
115 gathered novel sequence data for 92 species belonging to 33 genera and 13 families (SI Appendix 1, Table
116 S1). The species were selected to represent 1) both local and regional endemics as well as more
117 widespread species, 2) species-rich as well as species-poor lineages in the afroalpine flora, 3) different
118 habitats, and 4) those not previously included in phylogenetic studies. We used genome skimming (16) to
119 retrieve high copy chloroplast genomes and nuclear ribosomal cistron, obtaining 1.8-9.8 million reads per
120 sample (NCBI Sequence Read Archive (SRA) database under the BioProject ID PRJNA766027).

121 We used an analytical pipeline to update alignments from published analyses of 1) >35,000 species of seed
122 plants represented by two chloroplast markers (17) and 2) individual seed plant clades with chloroplast
123 and/or nuclear sequence data (6, 18–29); SI Appendix S1 Table S3). Our approach (SI Appendix 1) allowed
124 us to supplement the new data with similar sequences from GenBank, thus increasing the number of
125 afroalpine species to a total of 102 (19 of which were represented with two or more accessions, SI Appendix
126 1, Table S2-S4), representing 20% of the 515 species, 18% of the 90 genera and 26% of the 50 families
127 known from the flora (9) plus non-afroalpine outgroups (SI Appendix 1 Table S3). To infer time windows for
128 colonization of the afroalpine region and *in situ* diversification within it, we used molecular dating analyses, of
129 which most were calibrated using fossil evidence. We summarized ages for stem and crown nodes of
130 afroalpine species and clades. To test the robustness of the results we compared these two datasets and
131 two different molecular dating methods: seed plant-wide analyses with penalized likelihood (PL), and
132 individual seed plant clades with both PL and Bayesian statistics (the latter incorporating phylogenetic
133 uncertainty; matrices and phylogenetic trees in text are available via the Open Data Framework (28) and
134 figure format phylogenies are presented in SI Appendix 2). Thus, we obtained up to three stem age
135 estimates for each of the 102 afroalpine species (SI Appendix 1, Table S2), depending on differences in
136 sampling across datasets, with each approach delivering 91 to 93 dated nodes (representing common
137 ancestors of afroalpine species/populations and their closest relatives).
138

139 Our phylogenies revealed that the closest relatives of afroalpine lineages were geographically distant, nested
140 within wider temperate clades, from which we assumed that afroalpine colonization occurred after
141 divergence of afroalpine lineages from their most recent common ancestors. We found evidence for
142 divergent lineages (supported non-monophyly) within some afroalpine species (SI Appendix 1, Table S4),
143 and more independent dispersals into the afroalpine region than expected if each genus only dispersed once
144 (SI Appendix 1, Table S5). Each of the genera *Lobelia*, *Ranunculus*, and *Swertia*, for example, colonized the
145 afroalpine region at least twice independently.

146 The molecular dating results enabled us to place the assemblage of multiple afroalpine plant lineages into a
147 unified timeframe (Figure 2). Stem node ages may thus substantially predate dispersals (31), but we
148 nevertheless found a range of ages concentrated towards the present (Figure 1b and c). Of a total of 43
149 stem nodes of species and clades representing the maximum bounds for times of colonization of the
150 afroalpine, 58%, 68% and 83% fell within the last 5 million years (My; individual seed plant clades BEAST2
151 and PL, and seed-plant wide PL) and 72%, 89% and 88% within in the last 10 My. None were consistently
152 older than 15 My (SI Appendix 1, Table S6). Similar to Rabosky et al. (32), we found no obvious relationship
153 between the timing of arrival in the afroalpine region and subsequent *in situ* diversification, as illustrated by
154 the diversifications of both early- and late-arriving lineages of *Lobelia* and of *Swertia* (SI Appendix 1, Figure
155 S1).

156

157 **Figure 2.** Seed plant-wide dated phylogeny based on our new data combined with the original matrix of
158 Janssens et al. (17) with afroalpine species indicated in red type and those of the original dataset further
159 indicated with a red colored star. Clades not including afroalpine species are pruned to crown clade nodes
160 to increase readability. Afroalpine clades supported by posterior probability ≥ 0.95 in the BEAST2 analyses
161 of the individual seed plant clades are indicated with blue stars. Circles indicate time in million years before
162 present. Photographs show examples of afroalpine species: A) *Viola eminii*, B) *Swertia crassiuscula*, C)
163 *Lobelia telekii* and D) *Euryops browneii* (photographs: B. Gehrke, available under a creative commons CC-
164 BY-NC license at <https://www.inaturalist.org/>).

165

166 We found that most afroalpine lineages diverged from their non-afroalpine relatives after the end of the major
167 mountain uplifting processes (Figure 1b, the number of colonization events). More than 80% of those
168 included in the analyses of individual seed plant clades diverged within the last 5 My, with a maximum of
169 only seven of our 102 sampled species dating back to the early phases of afroalpine habitat existence (SI
170 Appendix 1, Table S7). Those with narrow distributions typically arrived or diverged from their sister lineages
171 after the final uplift of the mountain(s) to which they are currently restricted (Figure 1c). The distribution of
172 node ages within independent afroalpine lineages, as summarized across independent clades, showed
173 increase towards the present (Figure 1b and c). Species accumulation increased exponentially over time:
174 fitting our data to an exponential distribution revealed high R^2 values (individual clades BEAST2: 0.98;
175 individual clades treePL: 0.97; seed plant wide treePL: 0.96; Figure 3). The data showed distinct changes in
176 accumulation rates through time that were not apparent in modeled curves (Figure 3 and SI Appendix 1,
177 Figure S2). According to an exponential diversification model, we would only expect a change in rates close
178 to the present, but we also detected changes that occurred earlier. Depending on the dating method,
179 changes began around 6 to 5 Ma, and became more frequent within the last 2.5 Ma (Figure 3).

180

181 **Figure 3.** Species accumulation over time in the afroalpine region based on historical lineage diversity esti-
182 mates and relative branching times obtained from the time-calibrated phylogenies. These plots differ from
183 standard lineage through time plots in that species accumulation in a region results from colonization events
184 in addition to regional diversification of multiple independent lineages. Row 1: Number of colonization/diversi-
185 fication events over time. The black dotted line marks a constant rate model and the black dashed line an
186 exponential model. Row 2: Change in number of colonization/diversification events over time calculated as a
187 difference quotient (see text). The red line shows the maximum difference of change in a constant model.
188 Row 3: number of colonization/ diversification events per 0.5-million-year intervals. Age estimates were sub-
189 sampled to 50% 200 times. Results for our three different dating methods are shown in A). Simulated results
190 are shown in B) (exponential model) and C) (constant model), with the left columns representing 200 itera-
191 tions (one highlighted in blue), and right columns a single iteration subsampled to 50%.

192

193 Discussion

194 Although the final uplift of some of the tropical African high mountains date back to the Oligocene (>30 Ma),
195 and although our stem node ages constitute a considerably conservative estimate for estimating the timing of
196 colonization of afroalpine plant lineages, we found that most lineages must have colonized the afroalpine
197 during the last 5 or 10 My. Based on estimated ages of 20% of the afroalpine flora, we show that the
198 assembly of this flora accelerated towards the present, with a rate of species accumulation within the flora
199 increasing exponentially (Figure 3). This suggests that the afroalpine region remains below its carrying
200 capacity for species diversity.

201 We might expect colonization events to accumulate steadily over the time that habitat is available, or even to
202 tail off towards the present as niches are filled. Our finding of concentrations of both afroalpine colonization
203 and *in situ* diversification events towards the present (Figure 1b) appears, therefore, to point to a substantial
204 impact of extinction on the composition of the flora. The pattern we observe is consistent with the extremely
205 low intra-population genetic diversity and recent inter-mountain population divergence reported for many
206 individual afroalpine species (15). Palynological and macrocharcoal records from the Bale Mountains in
207 Ethiopia show a marked reduction of afroalpine communities around 12.5 thousand years before present
208 (Ka) (33) and paleoenvironmental data from lake cores imply a major climatic incursion in tropical East Africa
209 around 11.5 Ka (34), suggesting climate induced extinction of afroalpine plants after the last glaciation. Our
210 results thus provide evidence from across the flora in line with the emerging view of the afroalpine flora as
211 the product of cycles of colonization, speciation, extinction, and recolonization induced by the Pleistocene
212 climate oscillations, resulting in unsaturated, fragile communities of species depauperated by frequent
213 genetic bottlenecks (15).

214 The occurrence of several spikes in species accumulation through the last 7 Ma (Figure 3A row B) suggests
215 that net species accumulation across lineages within the flora may have been impacted by common factors
216 such as climate change and mountain uplifting. The precision of our age estimates is not, however, sufficient
217 to address the effect on *in situ* diversification in more detail. Future analyses could narrow down such
218 estimates through a combination of denser taxon sampling, coalescence-based dating of species (35), and

219 dating the evolution of key adaptive traits (36), and could incorporate model-based biogeographic analysis to
220 assess lag times between origins of habitat, colonizations, and *in situ* diversification in individual clades (37).

221 How does the recruitment of the afroalpine flora compare to that of other tropical alpine floras, and to that of
222 comparably isolated oceanic island floras? The stages of mountain building that pushed colonizable
223 substrate out of the tropical climate zone were mostly recent in South America and South East Asia, taking
224 place from the mid-Miocene onwards (7, 8). Global cooling cycles that further contributed to shaping current
225 day occurrence patterns of tropical-alpine plants intensified since the start of the Quaternary glaciations
226 about 2.6 million years ago (38). This is consistent with recent dating studies from the tropical alpine flora in
227 South America (39). Here we show that most of the afroalpine flora also dates to the Plio-Pleistocene (Figure
228 1), in spite of the much more variable ages of the individual African high mountains, with only few earlier-
229 arriving lineages.

230 Thus, endemic alpine species diversity is recent in origin across the tropics, making these regions
231 evolutionary cradles of biological diversity (12, 39). The Andean tropical alpine flora is however much richer
232 in species than the afroalpine flora and includes hyper-diverse clades such as *Calceolaria*, *Espeletia* s.s.,
233 *Lupinus* and *Pentacalia* (40). Such large *in situ* species radiations are virtually absent from the afroalpine
234 flora, probably reflecting the much smaller spatial extent and greater fragmentation of the tropical alpine
235 habitat in Africa. The high species turnover across the Andean Páramos versus the homogeneity of species
236 compositions across the isolated fragments of afroalpine habitat is consistent with the hypothesis that
237 periodic connectivity drives diversification (13). An equivalent analysis of species accumulation in the
238 Páramo is needed to further compare patterns of past extinction and resilience for tropical alpine floras in
239 general.
240

241 The history of recruitment and evolution of the afroalpine flora also differs from that of comparable oceanic
242 islands. The Macaronesian archipelagos show lower levels of endemism (20%) than tropical alpine regions
243 (> 60%), probably because they are closer to the source of their floristic diversity, northern Africa. Contrary
244 to the afroalpine flora but similar to the tropical Andean flora, Macaronesia shows striking species radiations
245 following establishment in many clades (e.g., *Aeonium* and *Echium*; 41, 42), perhaps due to a combination of
246 greater age and lower extinction rates given more stable oceanic climates (43). The islands of Hawaii are
247 more similar to the afroalpine mountains in terms of their degree of isolation, but the estimated <500 km² of
248 vegetation at high elevation in Hawaii, home to just 13 tropical alpine species, is much smaller (11). This is
249 despite a potentially greater age of the Hawaiian flora, which may originate from older, now submerged,
250 islands of the chain (44).

251 The differences between the afroalpine flora and other island- and alpine-like floras across the tropics serve
252 to highlight their unique histories and unique diversity. These floras, with their characteristic giant rosette
253 plants and dwarf shrubs, have evolved independently in similar extreme environments against common
254 backgrounds of climatic fluctuation and resulting species diversifications. Our results show that even in the
255 older African mountains, the present day tropical alpine flora has assembled remarkably recently, most likely
256 repopulating habitats impacted by extinctions of earlier colonizers. However, the sheer speed and
257 compounding impact of the current human-caused habitat destruction and climate change is unprecedented
258 in this evolutionary context. The afroalpine flora inhabits a narrow band of suitable conditions that are
259 already limited to the upper extremes of the mountains, and it is susceptible to severe and increasing
260 human-induced impacts such as overgrazing. The already decreasing extent of intact alpine habitat is
261 projected to be pushed higher into smaller areas and might disappear altogether as the climate continues to
262 warm (15, 45).

263

264 Materials and Methods

265 We collected plant material during field expeditions to all major eastern African sky islands (Ethiopia,
266 Tanzania, Kenya, Uganda). Vouchers and silica-dried leaf samples were deposited in herbaria in the
267 countries of origin and in the herbarium and DNABank at the Natural History Museum, University of Oslo (SI
268 Appendix 1, Table S1). Our taxon sampling represents 102 species that occur in the alpine zone above 3200
269 m (SI Appendix 1, Table S2) (9). We extracted DNA from silica dried leaves using Qiagen DNeasy Plant Mini
270 Kits (Qiagen, Valencia, CA) and constructed libraries using the TruSeq compatible genomic DNA library
271 preparation kits for Illumina paired-end sequencing of 550 base pair (bp) inserts with multiplexing for 96

272 samples (Illumina, San Diego, CA, USA). Sequencing was carried out on the Illumina HiSeq v2500 platform
273 (Illumina, San Diego, CA, USA) at the Norwegian Sequencing Centre (<https://www.sequencing.uio.no/>).

274 We inferred phylogenetic relationships of afroalpine species and clades based on plastid and nuclear
275 ribosomal DNA sequence markers that have been widely sampled for phylogenetic studies by incorporating
276 our new sequences into existing alignments of 1) all seed plants (17); and 2) individual seed plant clades (6,
277 18–29) (see SI Appendix 1, Table S3); most of the latter are based on dense sampling of taxa, variable
278 sequences, and shallow fossil calibrations. We used PhylUp (46) <https://github.com/mkandziora/PhylUp/>), a
279 python workflow for finding and adding sequences to the target alignments using blast+ (47) to search both
280 user-supplied data and GenBank. We first split concatenated matrices into single loci and used
281 ncbiTAXONparser (<https://github.com/mkandziora/ncbiTAXONparser>) to link taxa to species names
282 accepted by NCBI. We opted to add new sequences to the original alignments if the BLAST search returned
283 a blast e-value of <0.001. Maximum sequence length was set to 2000% to add potentially much longer
284 matching plastid sequences, which were later trimmed to alignment length. Final alignments are available
285 through the Open Data Framework (30).
286

287 We estimated ages of afroalpine species and clades based on the seed plant-wide and individual seed plant
288 clade datasets using two different molecular dating methods: penalized likelihood as implemented in treePL
289 (48) and Bayesian statistics under an uncorrelated lognormal relaxed clock model as implemented in
290 BEAST2 (49). Apart from applying current versions of the software we aimed to match the age calibrations
291 and other settings as reported in the original publications as closely as possible (see SI Appendix 1, Table
292 S8).

293 We summarized the age distribution of sampled colonization and *in situ* diversification events across the
294 afroalpine flora as a whole and compared it to expectations given different models of diversification similar to
295 the approaches in (35, 50). We analyzed the differences between consecutive node ages across
296 phylogenies (for all individual seed plant clades together and for the seed plant-wide phylogeny), thus
297 summarizing both colonization and diversification rate heterogeneity across lineages and through time. We
298 used the difference quotient function incorporating a degree of phylogenetic and age uncertainty by 1)
299 subsampling and 2) assessing separately results given the different age estimates obtained above (SI
300 Appendix 1). We compared the resulting distributions of densities of species accumulation through time,
301 calculating the R2 of the fit with those simulated assuming constant or exponential growth. Code used is
302 available via data submission through the Open Data Framework (30). Detailed methods are presented in SI
303 Appendix S1.
304

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327 **SI Appendices**

328 SI Appendix 1: Supplement Methods and Results.

329 SI Appendix 2: Figures of dated phylogenies of individual seed-plant clades.

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331

332 **Figures**

333

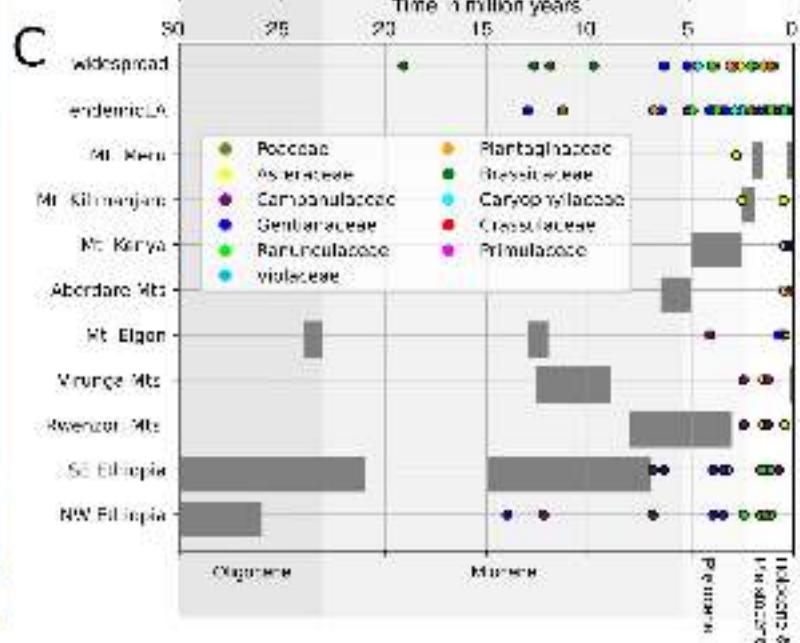
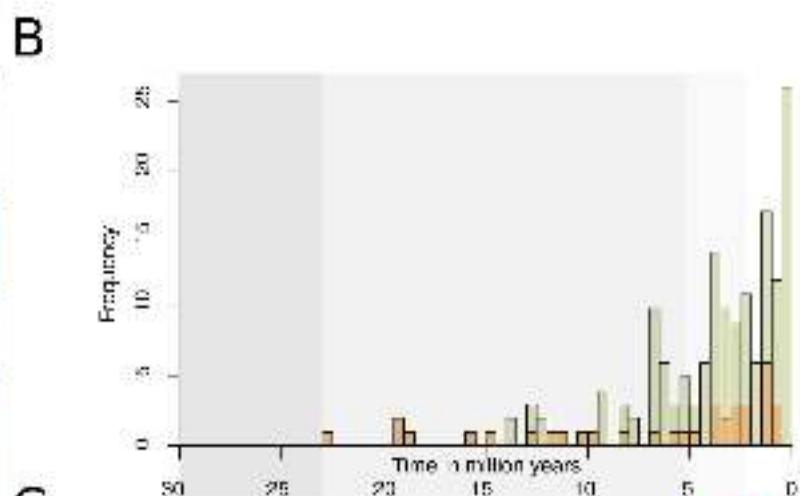
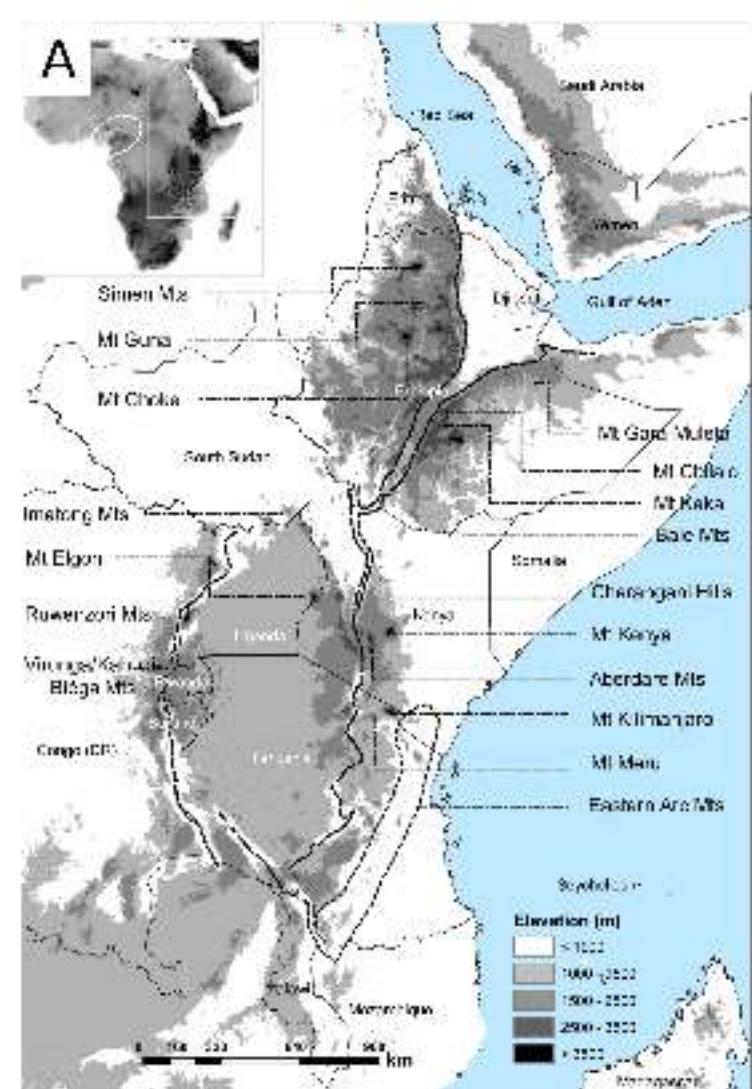
334 **Figure 1.** Geographic distribution and recruitment to the afroalpine flora (>3500 m). A) Major mountains and
335 mountain regions in eastern Africa. The Great Rift Valley is shown as stippled lines. B) Numbers of
336 afroalpine colonization (orange) and *in situ* diversification (olive) events per 0.5 Ma time bin. Colonizations,
337 here represented by stem nodes, must be interpreted as maximum age estimates for events that are more
338 recent to an unknown degree. C) Stem age estimates of afroalpine species in relation to mountain orogeny
339 (gray bars; Gehrke et al. (7)). Species are colored according to family. Local endemics (species restricted to
340 one or two mountains) are mapped to individual mountains; the remaining species are classified as ‘eastern
341 African endemics’ (‘endemicEA’) or as ‘widespread’. Age estimates in A) and B) represent both Bayesian
342 median node ages of individual seed plant clades.

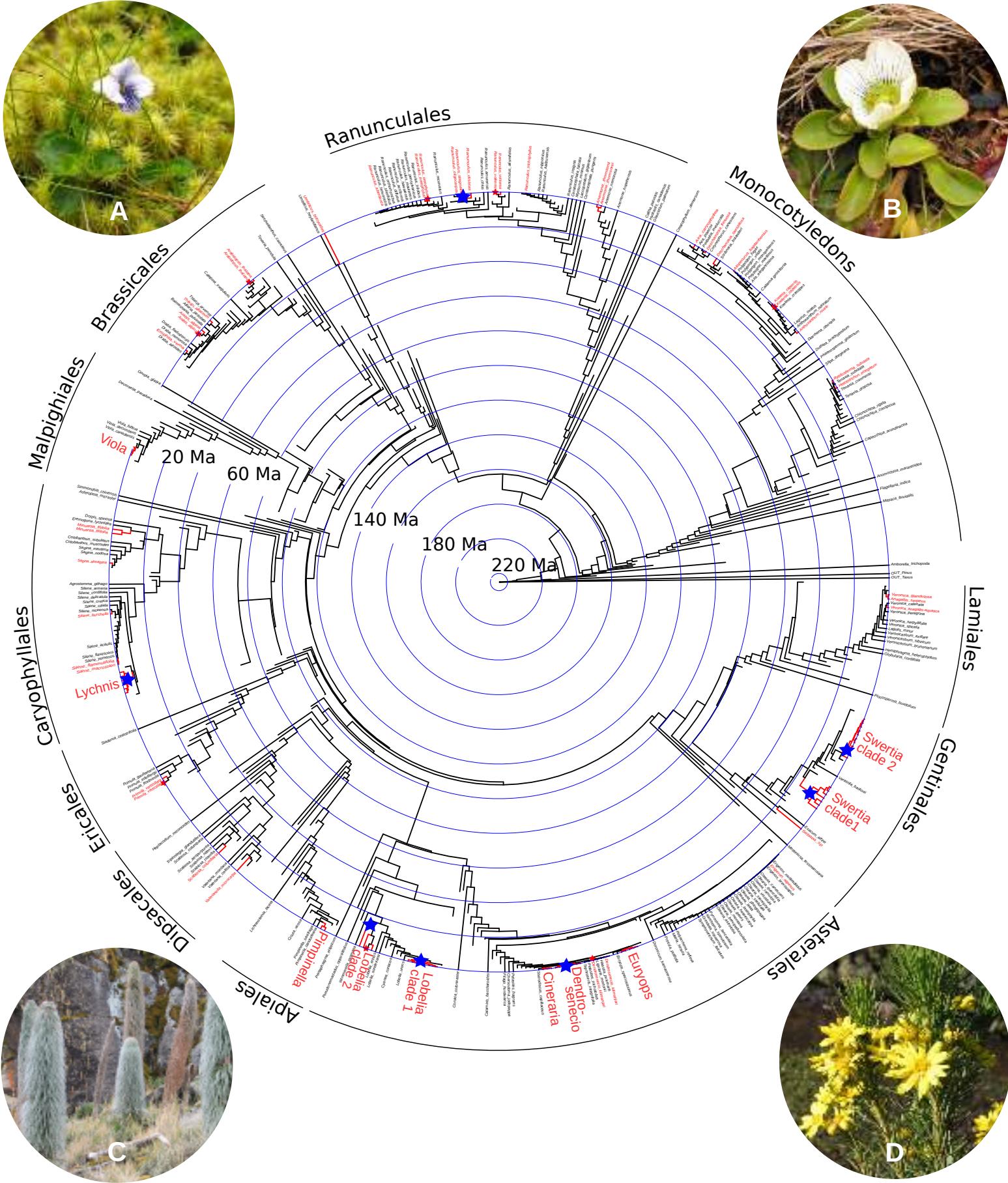
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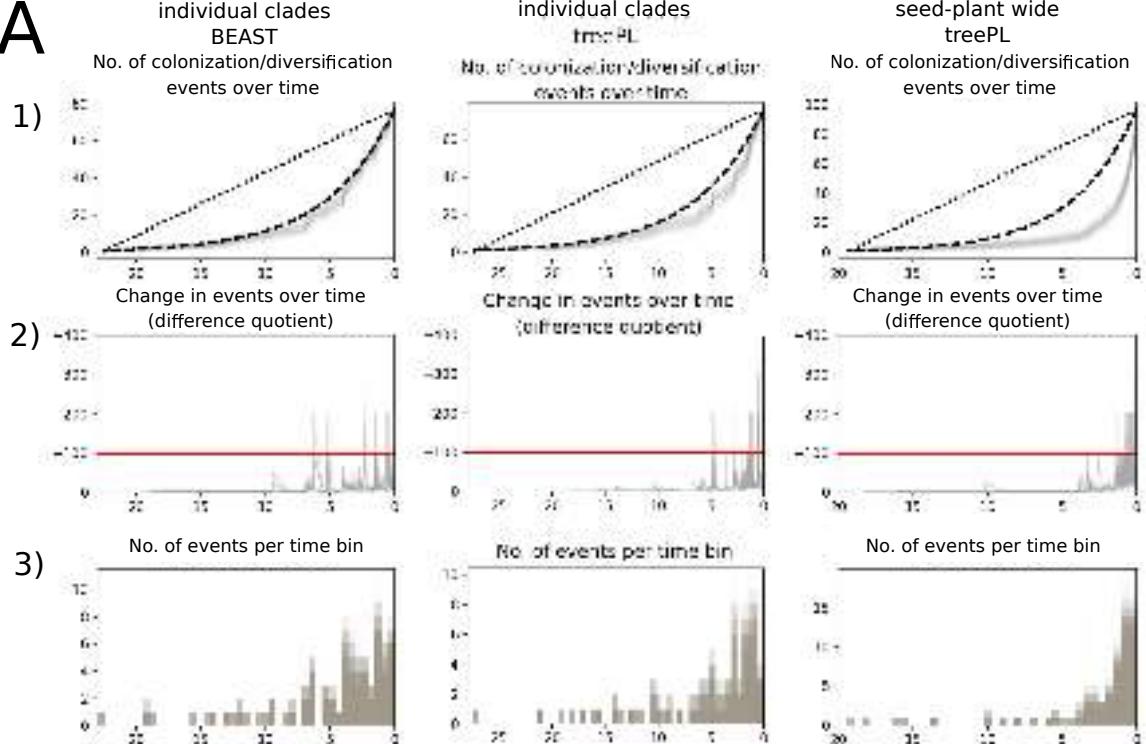
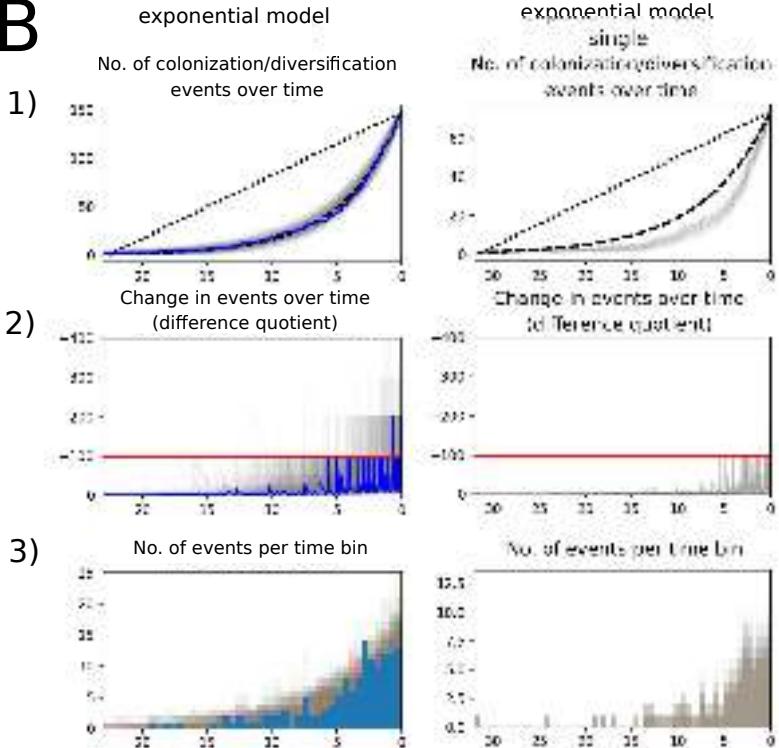
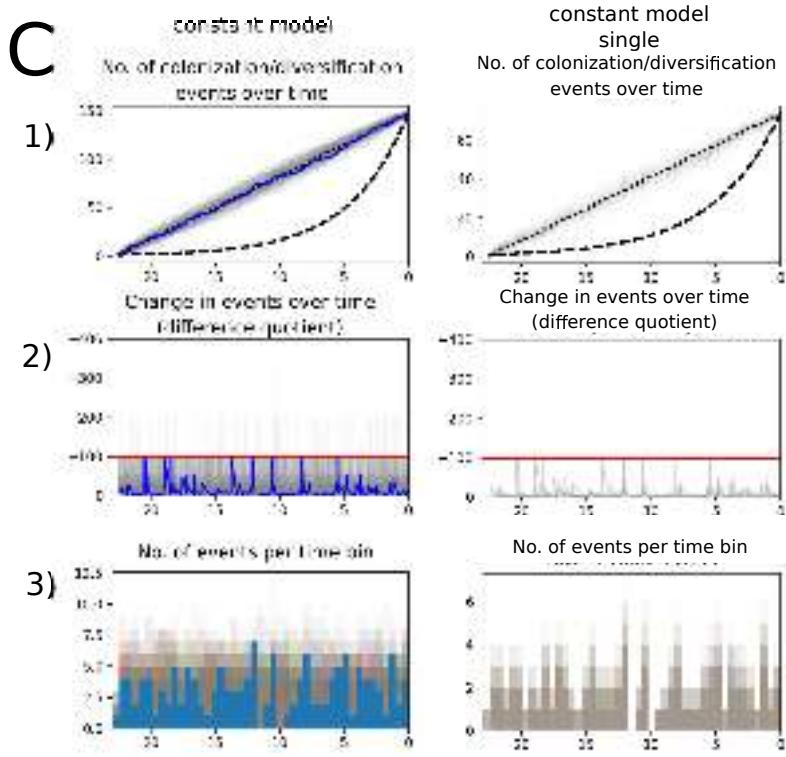
344 **Figure 2.** Seed plant-wide dated phylogeny based on our new data combined with the original matrix of
345 Janssens et al. (17) with afroalpine species indicated in red type and those of the original dataset further
346 indicated with a red colored star. Clades not including afroalpine species are pruned to crown clade nodes to
347 increase readability. Afroalpine clades supported by posterior probability ≥ 0.95 in the BEAST2 analyses of
348 the individual seed plant clades are indicated with blue stars. Circles indicate time in million years before
349 present. Photographs show examples of afroalpine species: A) *Viola eminii*, B) *Swertia crassiuscula*, C)
350 *Lobelia telekii* and D) *Euryops browneii* (photographs: B. Gehrke, available under a creative commons CC-
351 BY-NC license at <https://www.inaturalist.org/>).

352

353 **Figure 3.** Species accumulation over time in the afroalpine region based on historical lineage diversity esti-
354 mates and relative branching times obtained from the time-calibrated phylogenies. These plots differ from
355 standard lineage through time plots in that species accumulation in a region results from colonization events
356 in addition to regional diversification of multiple independent lineages. Row 1: Number of colonization/diversi-
357 fication events over time. The black dotted line marks a constant rate model and the black dashed line an
358 exponential model. Row 2: Change in number of colonization/diversification events over time calculated as a
359 difference quotient (see text). The red line shows the maximum difference of change in a constant model.
360 Row 3: number of colonization/ diversification events per 0.5-million-year intervals. Age estimates were sub-
361 sampled to 50% 200 times. Results for our three different dating methods are shown in A). Simulated results
362 are shown in B) (exponential model) and C) (constant model), with the left columns representing 200 itera-
363 tions (one highlighted in blue), and right columns a single iteration subsampled to 50%.





A**B****C**

Supplementary Information for

The enigmatic tropical alpine flora on the African sky islands is young, disturbed, and unsaturated

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This PDF file includes:

Supplementary text

Figures S1 to S8

Tables S1 to S9

Legend for Appendix S2

SI References

Other supplementary materials for this manuscript include the following:

Appendix S2

Appendix 1. Supplementary Methods and Results

Methods

Material and DNA sequencing

We sampled 102 species that occur in montane and/or alpine areas of eastern Africa (Ethiopia, Kenya, Uganda, and Tanzania), limited to those with an upper altitudinal limit above 2500 m (Table S2). Seventy of these species occur above the tree line (> 3800 m). For this project, we sampled 92 species that were collected over the years (Table S1). We collected leaves on silica gel and pressed three individual plants from each population, deposited in the following herbaria: one in the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia (ETH); one in the Natural History Museum, University of Oslo, Norway (O); and one in the country of collection: East African Herbarium, National Museum of Kenya, Nairobi, Kenya (EA); National Herbarium of Tanzania, Arusha, Tanzania (NHT); or Makerere University Herbarium, Kampala, Uganda (MUH).

In some cases, we pooled DNA of multiple individuals from a single population to obtain sufficient DNA for sequencing. We consider this to be unproblematic for species level analyses. We used FastQC (version 0.11.5; <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to determine the quality of the raw sequences. After sequencing, we removed adapters and low-quality reads using bbdduk (bbmap version 38.79; 1) with standard settings (adapter trimming: ktrim=r, k=23, mink=11, hdist=1, tpe, tbo; low quality reads: qtrim=rl, trimq=10). To assemble nuclear ribosomal and plastid sequences we used GetOrganelle (version 1.6.2e; 2). Settings were adapted to plastid and nuclear ribosomal DNA, using the recommended ones (plastid: -R 7 -k 35,85,115; nuclear ribosomal: -R 15 -k 21,45,65,85,105).

Dating approach and methodology

To estimate ages of afroalpine lineages, we used datasets from previously published molecular dating analyses (3–16) whenever these were available through public archives or on request from the authors, instead of building new alignments. We could thereby leverage work by experts on the respective lineages, with sampling typically carefully chosen to be representative of the entire lineage, with carefully incorporated fossil calibrations, and often with comparison of results from different analysis settings; the sum of which has passed peer-review process. Most such datasets focused on individual seed plant clades; for comparison we also used an existing dataset spanning seed plants (17) which incorporates an alternative set of fossil calibrations.

We added new sequences to the existing alignments (3–17) using PhylUp (18). PhylUp is a python workflow for finding and adding sequences to a target alignment using blast+ (19) to search for similar sequences. To further increase sampling of the closest relatives of afroalpine taxa we used PhylUp to add 10 additional taxa from GenBank for each of our own new sequences, selecting those that were most similar (but not identical) and which represented species/subspecies not already included. We first split concatenated matrices into their constituent individual marker alignments. Where information about partitioning of the alignment was not available, we used blast searches to determine locus identities and start and end positions within the concatenated alignments. Alignment information is provided in Table S3.

We used different dating approaches. For individual seed plant clades, we used a Bayesian relaxed clock model (BEAST2; 20) and also a penalized likelihood method, treePL (21; Table S8). For the seed plant wide matrix, we used penalized likelihood only (following 17) because the matrix was too large to run successfully using BEAST2. It was not always possible or desirable to implement exactly the same analysis settings as used in the original publications. In two cases (Poaceae and Dipsacales) we combined alignments and molecular dating protocols from two different publications (4–6, 13). For Gentianaceae (11), we added a maximum age constraint for the root node following (22) to restrict overall age estimates to within plausible time frames. The node constraint reported for

Cleistochloa in Poaceae (6) was omitted because the relevant node was not represented given the taxon sampling in the available alignment (13). Our BEAST2 analysis using the original Brassicaceae matrix did not converge; we achieved convergence after reducing the density of taxon sampling in the matrix.

For the treePL analyses, we first needed to reconstruct phylogenetic trees from the updated alignments using RAxML-NG (version 0.9.0) or RAxMLv8 on CIPRES (for the seed plant-wide phylogeny; 23–25). We then estimated chronograms using penalized likelihood (based on the RAxML trees) and uncorrelated lognormal relaxed clock models (estimating phylogeny and node ages at the same time). For treePL, we applied node calibrations as minimum and/or maximum age constraints, and for the BEAST2 analyses, we used the same prior distributions for node calibrations as implemented in the original publications (Table S8). For the analyses of individual seed plant clades, we used an existing script (https://github.com/tongjial/treepl_wrapper) for the treePL analysis that automatically runs the ‘prime’ step a hundred times to find the best optimization parameters and to choose the optimal cross validation (cv) parameters, before calculating an ultrametric phylogeny using the corresponding smoothing value. For the seed plant-wide analyses we followed the dating method as reported in the original analysis (17), incorporating the same set of node-defined calibration points and producing a comparable result by using their topology as a backbone constraint under RAxML. In order to compute confidence intervals for the age estimates, we calculated 50 bootstrap trees, dated them using treePL, and combined the dated trees using TreeAnnotator from the BEAST package (version 1.10).

For the BEAST2 analyses, when detailed settings were not provided in the original publications, we partitioned substitution models into pDNA and nrDNA markers, and linked trees and molecular clocks, without further assessment of model fit (Table S8). Convergence of BEAST2 analyses was assessed using Tracer version 1.7.1. Two independent BEAST2 analyses were combined using Logcombiner and TreeAnnotator (version 2.6.2) if the analyses converged and ESS values were above 200 (in a few cases only >100). Sometimes three independent but converging analyses were combined to ensure high ESS values.

To compare species ages with the age of the mountains we used the information from (26). Species were categorized as “local endemics” if they occur on a maximum of two different mountains; otherwise, either as “afrotemperate endemics” (distribution limited to DR Congo, Rwanda, Tanzania, Ethiopia, Sudan, Zimbabwe, Kenya, Uganda, Burundi, Malawi, Zambia, Yemen) or as “widespread species” (also occurring in more distant temperate regions).

Species accumulation over time

We summarized the ages of species and clades (representing colonization and *in situ* diversification events) across phylogenies for all sampled species occurring in the afroalpine flora, excluding multiple accessions of monophyletic species, and compared the resulting node age distributions through time to expectations based on hypothetical models of diversification. Age estimates were pruned to two decimal positions for this approach.

- 1) We pooled node ages across all clades analyzed, ordered them by decreasing age, and summed the accumulating number of colonization/diversification events over time, equivalent to the approach of (27). In a few cases, where there was a discrepancy between phylogenies concerning the nodes within species complexes, we omitted these nodes.
- 2) We estimated a colonization/diversification rate given age intervals between consecutive ages by calculating the difference quotient, an approach similar to (28). The difference quotient is defined as $f(b)-f(a)/(b-a)$, which in our case is $(\text{cumulative number of colonization/diversification events at age 2} - \text{cumulative number of colonization/diversification events at age 1}) / (\text{age 2} - \text{age 1})$.
- 3) We summarized the number of colonization/diversification events per time bin.

To account for phylogenetic uncertainty and uncertainty in the age estimates, we employed a

subsampling approach: We randomly selected 50% of the samples 200 times before recalculating the three estimates and overlaid the results. We compared our results to expectations assuming constant and exponential models, simulating colonization/diversification under these two models and comparing the fit of our data using the `fit_curve` method from `scipy v. 1.5.4` (29), an optimization process to find an optimal set of parameters for a defined function that best fits a given set of observations. We calculated R^2 between our dating results and data of the fitted curve. We repeated the above analysis using diversification events only to test whether the pattern we see is driven by the older colonization events.

We modeled a constant colonization/diversification rate by sampling N colonization/diversification events from a uniform distribution between 0 and the maximum age inferred by the dating method. The corresponding linear function is $N(t)=a*t+b$, where a is the slope and b is the number of new species, in our case one. The exponential model was generated by drawing samples from an exponential distribution. The corresponding (probability density) function is $N(t)=N_0*\exp^{-\lambda*t}$, where N is the number of colonization/diversification events, t is the time point and lambda is $-\ln(N_0/N_{max})/t_{max}$.

Results

Data deposition

We deposited raw reads from shotgun sequencing in an NCBI sequence read archive: <http://www.ncbi.nlm.nih.gov/bioproject/766027>. Alignments (including newly added sequences, as below), phylogenetic trees and analysis settings as well as code used for this study are available via <https://github.com/mkandziora/AfroalpineDating/>, including reference to the respective original publications on which alignments and settings were based.

Phylogenetic relationships

All newly sampled sequences were represented in at least one of the datasets. However, nine species were only included in the seed plant-wide dating, either because of mismatch between available data and existing matrices (Table S2) or, in the case of Dipsacales and Apiaceae, because of persistent problems with convergence using BEAST2. For the analyses of individual seed plant clades, we included similar sequences from GenBank in addition to our newly sampled individuals to improve the resolution of species relationships of our afroalpine samples. This added nine afroalpine species and corresponding age estimates to the results of the individual seed plant clades only.

Of the genera that are represented by more than five species in the afroalpine flora (26), six were represented by more than one species in our analyses. We found that three of these six genera colonized the afroalpine region more than once: *Lobelia* and *Swertia* both colonized twice, and *Ranunculus* colonized at least four times (of which only two colonizations resulted in diversification into two or more afroalpine species). The two *Silene* species we sampled (out of three in the flora), *S. flammulifolia* and *S. burchellii*, belonged to separate clades, each including non-afroalpine *Silene* species. The genus *Lychnis* (in our dataset nested within *Silene*), represents a further independent origin in the afroalpine region.

Nineteen species were represented by more than one individual in our analyses (Table S4). Five of them were retrieved as clearly monophyletic (>0.9 PP; according to the BEAST2 analyses, which were better supported than the RAxML analyses), and seven were retrieved as para- or polyphyletic, potentially indicating unrecognized species diversity in the afroalpine flora (these cases did not represent additional immigration events). For the remaining seven species, support was too low to confirm or reject monophyly.

Molecular dating

Based on our three dating methods, we could summarize up to three different age estimates for each of 102 afroalpine species, with and without confidence intervals (Figure S3-S5, Table S2). For the analyses of individual seed plant clades, we used treePL (rate-smoothing a single ML tree to obtain point estimates for node ages) and BEAST2 (estimating the phylogeny and age estimates at the same time, including confidence intervals from posterior probability distributions). For the seed plant-wide analysis we used only treePL (using an ML tree with confidence intervals estimated from bootstrapping). As the two datasets represent different species (as represented in published data) with our newly added sequences, the two datasets delivered two somewhat different sets of results (91 and 93 age estimates from not fully overlapping species sampling).

More than 60% of the BEAST2/treePL median age estimates fell within the seed plant-wide confidence intervals, and >40% of the seed plant-wide median age estimates fell within the individual seed plant clade confidence intervals. Because fossil-calibrated molecular dating results should in principle be interpreted as minimum ages (and other means of rate calibration are subject to various sources of error), deviation between results based on different calibrations can be expected and needs to be assessed. The BEAST2 and treePL analyses resulted in only a few old (> 10 Ma) species, but the two methods identified different species (Table S6). The age estimates were very different for two species: *Umbilicus botryoides* (individual lineage analysis 0.98 Ma, seed plant-wide analysis 19.41 Ma) and *Sebaea* sp. (3.26 Ma and 18.25 Ma); the much younger ages inferred by the better sampled individual lineage analyses may be caused by using more informative, and thus more reliable, age constraints for these lineages. In the case of *Umbilicus*, it should be noted that the Crassulaceae is difficult to date as no fossils are available; the age estimate from the seed plant-wide dating, which is informed by (albeit more distant) fossil calibrations, might be more appropriate.

The inferred ages and resolution in our phylogenies of individual seed plant clades were very similar to those in the original publications (Table S9), except for *Veronica*, for which we estimated the crown age to 10.82 Ma (7.11-15.69 highest posterior density [HPD]) instead of 16.13 Ma (12.46-20.59 HPD, BEAST2 age estimates). We used the same node calibrations as in the original publication; the different results might be due to the use of both ITS and *trnLF* in the original publication whereas our analysis only included ITS, because our sampling for the *trnLF* alignment did not include the nodes needed for calibration.

Notably, our estimates for species stem ages from the treePL and BEAST2 analyses were young compared to the ages of the tropical African mountains, also when considering the maximum confidence intervals (Figures S2 and S3). The median age over all BEAST2 analyses was 2.44 Ma (0.7 - 4.85; median of all HPDs), the treePL median age was 2.57 Ma, and the seed plant-wide median age was 0.84 Ma (0.12 - 7.81; median of all HPDs). All three dating approaches estimated that the majority of species (73 of 102) were younger than 5 Ma. Eighty-four of the species were younger than 10 Ma according to their median age estimates (Figure S3, BEAST2 analyses, Table S7).

We found no correlation between age and altitudinal distribution (Pearson correlation: $R = 0.11$, $p = 0.3$) and no association between age and biogeographic subregion (Figure S6 and S7). Species older than 10 Ma were typically either widespread or only found in Ethiopia and mostly found in alpine habitats. The clades that diversified were often composed of species that occur both above and below the treeline. From our results, it was rarely possible to discern whether clades originated in alpine or montane habitats (Figure S7).

As might be expected from the young stem node ages, the crown nodes of the six afroalpine clades (i.e., *in situ* diversifications) that we recovered were also younger than most of potential alpine or montane habitats (Figure S1).

Accumulation of species over time did not change its main results when not including colonizations events (Figure S8).

Table S1. Voucher information. Abbreviations: HC – herbarium code.

family	genus	species name (according to ncbi)	author	collector	collection date	unique id	country	locality	eleva- tion	latitude	longitud- e	HC	Genban- k accessio- n	sample_id
Apiaceae	Pimpinell a	<i>Pimpinella oreophila</i> var <i>oreophila</i>	AFROAL Hook. f. (Hochst.)	P II team H.Wolff Sch.Bip	2009-01- 29 O-DP-36064	Kenya	Mt Elgon: S of Mt Koitobos	3629	1.10067	34.6215	O	SAMN21 599645	KN0314- 2_Pimpinella_oreo- phila_var_oreop-	
Apiaceae	Pimpinell a	<i>Pimpinella pimpinelloides</i>	AFROAL A.Rich. Sch.Bip	P II team	2007-10- 30 O-DP-30849	Ethiopia	Simen Mts: Silki	3760	13.3333	3	38.23333	O	SAMN21 599646	ET0415- 2_Pimpinella_pi- mpinelloides
Asteracea e	Cineraria	<i>Cineraria abyssinica</i>	AFROAL A.Rich. Sch.Bip	P II team	2007-10- 13 O-DP-33982	Ethiopia	Bale Mts: Habera Bale Mts:	3484	7.01867	39.72067	O	SAMN21 599647	ET1413- 2_Cineraria_aby- ssinica	
Asteracea e	Cineraria	<i>Cineraria abyssinica</i>	AFROAL A.Rich.	P II team	2007-11- 12 O-DP-31694	Ethiopia	Sanetti Plateau	4143	6.85502	39.87802	O	SAMN21 599648	ET0652- 2_Cineraria_delt- oidea	
Asteracea e	Cineraria	<i>Cineraria sebaldii</i>	AFROAL Cufod.	P II team	2007-10- 24 O-DP-44436	Ethiopia	Simen Mts: Dirni Gate	3716	13.2879	8	38.11882	O	SAMN21 599649	ET0225- 2_Cineraria_seb- aldii
Asteracea e	Dendrose necio	<i>Dendrosenecio adnivalis</i> var <i>adnivalis</i>	(Stapf) E.B.Kno x (R.E.Fr. & T.C.E.F r.)	AFROAL P II team	2008-08- 10 O-DP-40704	Uganda	Rwenzori Mts: Upper Bigo Valley	3561	0.38602	29.92632	O	SAMN21 599650	UG2305- 3_Dendroseneci- o_adnivalis_var_- adnivalis	
Asteracea e	Dendrose necio	<i>Dendrosenecio battiscombei</i>	E.B.Kno x (R.E.Fr. & T.C.E.F r.)	AFROAL P II team	2009-02- 11 O-DP-27466	Kenya	Aberdare Mts: Mt Kinangop area	3069	0.54265	36.71993	O	SAMN21 599651	KN0482- 1_Dendroseneci- o_battiscombei	
Asteracea e	Dendrose necio	<i>Dendrosenecio brassiciformis</i>	r.) AFROAL Mabb.	P II team	2009-02- 12 O-DP-42217	Kenya	Aberdare Mts: Mt Satima area	3865	0.31065	36.63192	O	SAMN21 599652	KN0516- 4_Dendroseneci- o_brassiciformis	
Asteracea e	Dendrose necio	<i>Dendrosenecio elgonensis</i> ssp Fr.)	(T.C.E. AFROAL P II team	2009-01- O-DP-34825	Kenya	Mt Elgon: S of Mt	3915	1.10567	34.60183	O	SAMN21 599653	KN0025- 4_Dendroseneci		
					20									

Koitobos												ensis	
Asteracea e	<i>Euryops</i>	<i>Euryops pinifolius</i>	A.Rich.	AFROAL P II team	2008-10- 08 O-DP-33607	Ethiopia	Mt Choke	3960	10.642	37.83567	O	SAMN21 599662	ET1330- 2_Euryops_pinifo lius
Asteracea e	<i>Euryops</i>	<i>Euryops prostratus</i>	B.Nord.	AFROAL P II team	2007-11- 21 O-DP-32614	Ethiopia	Bale Mts: Batu	4116	6.85003	39.85317	O	SAMN21 599663	ET0889- 2_Euryops_prost ratus
Brassicac eae	<i>Arabidop sis</i>	<i>Arabidopsis thaliana</i>	(L.) Heynh.	AFROAL P II team	2007-10- 24 O-DP-29948	Ethiopia	Simen Mts: Dirni Gate Mt Meru: Saddle Hut	3716	13.2879 8	38.11882	O	SAMN21 599664	ET0177- 3_Arabidopsis_th aliana
Brassicac eae	<i>Arabis</i>	<i>Arabis alpina</i>	L.	AFROAL P II team	2008-11- 27 O-DP-38474	Tanzania area		3594	-3.217	36.769	O	SAMN21 599665	TZ0375- 3_Arabis_alpina
Brassicac eae	<i>Erophila</i>	<i>Erophila verna</i> var <i>macrosperma</i>	Sebald	AFROAL P II team	2007-11- 13 O-DP-31710	Ethiopia	Bale Mts: Sanetti	4000	NA	NA	O	SAMN21 599666	ET0667- 2_Erophila_vern a_var_Macrosper ma
Brassicac eae	<i>Thlaspi</i>	<i>Thlaspi alliaceum</i> (<i>Mummenhoffia</i> <i>alliacea</i>)	L. R.E.Fr. & (E.Wim m.)	AFROAL P II team	2007-11- 13 O-DP-31715	Ethiopia	Bale Mts: Sanetti Mt Elgon: Near camp site at end of car road	4050	NA	NA	O	SAMN21 599667	ET0671- 2_Thlaspi_alliace um
Campanul aceae	<i>Lobelia</i>	<i>Lobelia aberdarica</i>	T.C.E.F r. (E.Wim m.)	AFROAL P II team	2009-01- 29 O-DP-27246	Kenya		3557	1.09317	34.62367	O	SAMN21 599668	KN0394- 1_Lobelia_aberd arica
Campanul aceae	<i>Lobelia</i>	<i>Lobelia acrochila</i>	E.B.Kno x	AFROAL P II team	2008-10- 17 O-DP-34377	Ethiopia	Bale Mts: Dinsho Mt Kilimanjaro : Shira Plateau near Mt	3281	7.05815	39.7657	O	SAMN21 599669	ET1503- 3_Lobelia_acroc hilus
Campanul aceae	<i>Lobelia</i>	<i>Lobelia deckenii</i> ssp <i>deckenii</i>	Hemsl.	AFROAL P II team	2008-11- 03 O-DP-37017	Tanzania	- Simba	3636	3.03425	37.243	O	SAMN21 599670	TZ0025- 2_Lobelia_deck enii_ssp_deckeni
Campanul aceae	<i>Lobelia</i>	<i>Lobelia erlangeriana</i>	Engl.	AFROAL P II team	2008-10- 18 O-DP-34155	Ethiopia	Bale Mts: Betw. Goba and Sanetti, 4	2918	6.77312	39.72578	O	SAMN21 599671	ET1510- 14_Lobelia_erlan geriana

								km from				
Campanulaceae	<i>Lobelia</i>	<i>Lobelia lindblomii</i>	Mildbr.	AFROAL P II team	2009-02-12 O-DP-27650	Kenya	Goba Aberdare Mts: Mt Satima area	-	3806 0.30533	36.62483	O	SAMN21 4_Lobelia_lindblo mii
Campanulaceae	<i>Lobelia</i>	<i>Lobelia mildbraedii</i>	Engl.	AFROAL P II team	2009-02-17 O-DP-28572	Kenya	Aberdare Mts: Near Wanderi Gate	-	2571 0.32017	36.7685	O	SAMN21 2_Lobelia_mildbr aedii
Campanulaceae	<i>Lobelia</i>	<i>Lobelia rhynchopetalum</i>	Hemsl.	AFROAL P II team B.	2007-10-21 O-DP-29729	Ethiopia	Simen Mts: Saha	13.2827	3711 3	38.11077	O	SAMN21 ET0122- 2_Lobelia_rhync hopetalum
Campanulaceae	<i>Lobelia</i>	<i>Lobelia schimperi</i>	Hochst. ex dhin & G. A.Rich.	Gebreme Tassew	2013-03-26 O-DP-54720	Ethiopia	ETH: Simen Mts: Sherafit Virunga Mts: Mt Muhavura, along trail to summit	13.2779	2780 5	38.07445	O	SAMN21 BG52- 1_Lobelia_schim peri
Campanulaceae	<i>Lobelia</i>	<i>Lobelia stuhlmannii</i>	Stuhlmann	AFROAL P II team	2008-07-26 O-DP-43042	Uganda	Aberdare Mts: Mt Kinangop area, Gura River Virunga Mts: Mt Muhavura, summit	-	3600 1.38272	29.67798	O	SAMN21 UG2088- 1_Lobelia_stuhl mannii
Campanulaceae	<i>Lobelia</i>	<i>Lobelia telekii</i>	Schwei nf.	AFROAL P II team	2009-02-12 O-DP-27438	Kenya	River Virunga Mts: Mt Shasheme ne and Goba, 11 km from	NA NA NA	NA	NA	O	SAMN21 KN0475- 3_Lobelia_telekii
Campanulaceae	<i>Lobelia</i>	<i>Lobelia wollastonii</i>	Baker f.	AFROAL P II team	2008-07-28 O-DP-40212	Uganda	Shasheme Arsi: Betw. Shasheme ne and Goba, 11 km from	-	4139 1.38277	29.67783	O	SAMN21 UG2173- 3_Lobelia_wollas tonii
Caryophylaceae	<i>Lychnis</i>	<i>Lychnis abyssinica</i> (=Silene abyssinica)	(Hochst . Liden	AFROAL P II team	2008-09-22 O-DP-33240, 22 O-DP-33225	Ethiopia	Shasheme ne	ET1300_18_3_L	2318 7.25575	39.1564	O	SAMN21 ychnis_abyssinic a
Caryophylaceae	<i>Lychnis</i>	<i>Lychnis</i>	(Hochst	AFROAL	2007-10- O-DP-44203	Ethiopia	Simen Mts:	3574 13.2666	38.10782	O	SAMN21 ET0098-	

Iaceae	<i>Lychnis abyssinica</i> (=Silene abyssinica)	.) Liden	P II team	20		Close to Gich Camp Site		599680	X_Lychnis_abyssinica_LGS
Caryophylaceae	<i>Lychnis crassifolia</i> (=Silene kenyana)	(T.C.E. Fr.)	AFROAL	2009-02-		Aberdare Mts: Mt Kinangop area			KN0494-
Caryophylaceae	<i>Lychnis kigesiensis</i> subsp. <i>ragazziana</i> (=Silene kigesiensis)	M.Popp	P II team	11 O-DP-27504	Kenya	3086 -0.5425 36.7175 O	SAMN21 599681	2_Lychnis_crassifolia	
Caryophylaceae	<i>Lychnis kigesiensis</i> subsp. <i>ragazziana</i>	(Ousted)	AFROAL	2008-10-06	O-DP-41981 Uganda	Echuya Forest: Kanaba Swamp Betw. Keffa and Masha, Chenga Village Aberdare Mts: Mt	2300 1.25533 29.80933 O	SAMN21 599682	UG2600-1_Lychnis_kigesensis_subsp_ragazziana
Caryophylaceae	<i>Lychnis kiwuensis</i> (=Silene kiwuensis)	(T.C.E. Fr.)	AFROAL	2008-10-01	O-DP-33528 Ethiopia	Simen Mts: Gich Camp Site	2257 8.27212 35.7909 O	SAMN21 599683	ET1320-3_Lychnis_kiwue nsis
Caryophylaceae	<i>Lychnis rotundifolia</i> (=Silene afromontana)	(Oliv.) M.Popp	AFROAL P II team	2009-02-12	O-DP-27521 Kenya	Satima area	3686 0.32183 36.64067 O	SAMN21 599684	KN0497-4_Lychnis_rotun difolia
Caryophylaceae	<i>Minuartia filifolia</i>	(Forssk.) Mattf.	AFROAL P II team	2007-10-25	O-DP-30277 Ethiopia	Simen Mts: Dirni Gate	3652 13.2697 2 38.10588 O	SAMN21 599685	ET0266-1_Minuartia_filifolia
Caryophylaceae	<i>Minuartia filifolia</i>	(Forssk.) Mattf.	AFROAL P II team	2007-10-24	O-DP-29995 Ethiopia	Mt Kenya: Shipton's Cave	3716 13.2879 8 38.11882 O	SAMN21 599686	ET0189-3_Minuartia_filifolis
Caryophylaceae	<i>Sagina afroalpina</i>	Hedberg	AFROAL P II team	2009-07-09	O-DP-29202 Kenya	Aberdare Mts: Mt Satima area	4193 0.13358 - 37.2765 O	SAMN21 599687	KN0944-2_Sagina_afroal pina
Caryophylaceae	<i>Silene burchellii</i>	Otth ex DC.	AFROAL P II team	2009-02-14	O-DP-27930 Kenya	Simen Mts: Dirni Gate	3526 0.33417 - 36.64067 O	SAMN21 599688	KN0594-1_Silene_burchel lii_var_burchellii
Caryophylaceae	<i>Silene flammulifolia</i>	Steud. ex	AFROAL P II team	2007-10-24	O-DP-30053 Ethiopia	Dirni Gate	3716 13.2879 38.11882 O 8	SAMN21 599689	ET0205-2_Silene_flammu

				A.Rich. Steud.									
Caryophyl laceae	<i>Silene</i>	<i>Silene</i> <i>macrosolen</i>	ex A.Rich.	AFROAL P II team	2007-10- 27 O-DP-30359	Ethiopia	Simen Mts: Bwahit Mt	4035	13.2513 5	38.20225 O	SAMN21 599690	4_Silene_macros olen	lifolia ET0289-
Dipsacale s	<i>Scabiosa</i>	<i>Scabiosa</i> <i>columbaria</i>	L.	AFROAL P II team	2008-11- 14 O-DP-37816	Tanzania	Kilimanjaro : Horombo Bale Mts:	3650	3.14215 -	37.44065 O	SAMN21 599691	2_Scabiosa_colu mbaria	TZ0218-
Dipsacale s	<i>Valerianel</i> <i>la</i>	<i>Valerianella</i> <i>microcarpa</i>	Loisel.	AFROAL P II team	2007-11- 15 O-DP-31946	Ethiopia	Garba Guracha Virunga	4101	6.87028 -	39.8678 O	SAMN21 599692	2_Valerianella_m icrocarpa	ET0734-
Gentianac eae	<i>Sebae</i>	<i>Sebea sp</i>		AFROAL P II team	2008-07- 27 O-DP-40093	Uganda	Mts: Mt Muhavura	3800	NA NA	O	SAMN21 599693	3_Sebea_sp	UG2145-
Gentianac eae	<i>Swertia</i>	<i>Swertia</i> <i>abyssinica</i>	Hochst.	AFROAL P II team	2007-11- 23 O-DP-33146	Ethiopia	Bale Mts: Angaso	3875	6.8931 -	39.89735 O	SAMN21 599694	3_Swertia_abyss inica	ET1031-
Gentianac eae	<i>Swertia</i>	<i>Swertia adolfi-</i> <i>friderici</i>	Mildbr. & Gilg	AFROAL P II team	2009-06- 24 O-DP-39272	Tanzania	Mbeya Mts Virunga Mts: Mt Mgahinga, near	2731	8.83633 -	33.37083 O	SAMN21 599695	5_Swertia_adolfi- friderici	TZ0710-
Gentianac eae	<i>Swertia</i>	<i>Swertia brownii</i> J.Shah	AFROAL P II team	2008-07- 23 O-DP-39501	Uganda	Mgahinga Camp Site	2340	1.35275 -	29.6201 O	SAMN21 599696	1_Swertia_brown ii	UG2008-	
Gentianac eae	<i>Swertia</i>	<i>Swertia</i> <i>crassiuscula</i> ssp <i>crassiuscula</i>	Gilg	AFROAL P II team	2009-01- 29 O-DP-36039	Kenya	Mt Elgon: S of Mt Koitobos	3629	1.10067 -	34.6215 O	SAMN21 599697	2_Swertia_crassi uscula_ssp_cras siuscula	KN0309-
Gentianac eae	<i>Swertia</i>	<i>Swertia</i> <i>crassiuscula</i> ssp <i>robusta</i>	Sileshi	AFROAL P II team	2008-10- 18 O-DP-34488, 18 O-DP-34489	Ethiopia	Bale Mts: Sanetti Aberdare Mts: along the car road towards	3700	6.76667 -	39.75 O	SAMN21 599698	45_Swertia_cras siuscula_ssp_rob usta	ET1515-
Gentianac eae	<i>Swertia</i>	<i>Swertia</i> <i>crassiuscula</i> var <i>leucantha</i>	(T.C.E. Fr.) Sileshi	AFROAL P II team	2009-02- 16 O-DP-28318	Kenya	Satima	3697	0.33883 -	36.6515 O	SAMN21 599699	1_Swertia_crassi uscula_var_leuca ntha	KN0681-

Gentianac eae	Swertia	<i>Swertia engleri</i> var <i>engleri</i>	Gilg	AFROAL P II team	2007-10- 23 O-DP-43467	Ethiopia	Simen Mts: Saha	3718	13.2852 5 38.11838 O	SAMN21 599700	ET0136- i_Swertia_engler i_var_engleri
Gentianac eae	Swertia	<i>Swertia engleri</i> var <i>woodii</i>	(J.Shah) Sileshi	AFROAL P II team	2008-10- 14 O-DP-34071	Ethiopia	Bale Mts: Habera Virunga Mts: Mt	3482	7.00733 39.70983 O	SAMN21 599701	ET1454- 4_Swertia_engler i_var_woodii
Gentianac eae	Swertia	<i>Swertia</i> <i>kilimandscharica</i>	Engl.	AFROAL P II team	2008-07- 29 O-DP-40220	Uganda	Muhavura, along trail to 1st Hut	2900	NA NA O	SAMN21 599702	UG2175- 1_Swertia_kilima ndscharica
Gentianac eae	Swertia	<i>Swertia</i> <i>macrosepala</i>	<i>ssp</i> <i>macrosepala</i>	Gilg	AFROAL P II team	2008-07- 22 O-DP-39494	Uganda	Virunga Mts: Mt	-	SAMN21 599703	UG2006- 4_Swertia_macro sepala_ssp_mac
Gentianac eae	Swertia						Mgahinga	3470	1.38427 29.64018 O		ET0443- 1_Swertia_pumil a
Gentianac eae	Swertia	<i>Swertia pumila</i>	Hochst.	AFROAL P II team	2007-10- 31 O-DP-30948	Ethiopia	Simen Mts: Silki Sidamo: Wendo Abela	3912	13.3490 7 38.2625 O	SAMN21 599704	1_Swertia_pumil a
Gentianac eae	Swertia	<i>Swertia</i> <i>quartiana</i>	A.Rich. (Hochst)	AFROAL P II team	2008-09- 24 O-DP-33260	Ethiopia	Giorgis Church	1990	6.9158 38.50017 O	SAMN21 599705	ET1304- 5_Swertia_quarti niana
Gentianac eae	Swertia	<i>Swertia</i> <i>schimperi</i>	Griseb.	AFROAL P II team	2007-10- 25 O-DP-44309	Ethiopia	Simen Mts: Near Gich Camp Site	NA	NA NA O	SAMN21 599706	ET0187- x_Swertia_schim peri
Gentianac eae	Swertia	<i>Swertia</i> <i>subnivalis</i>	T.C.E.F r.	AFROAL P II team	2009-01- 19 O-DP-34720	Kenya	Mt Elgon: Mt	4224	1.1239 34.60198 O	SAMN21 599707	KN0004- 3_Swertia_subni valis
Gentianac eae	Swertia	<i>Swertia</i> <i>tetandra</i>	Hochst.	AFROAL P II team	2008-09- 29 O-DP-33195	Ethiopia	Koitobos Addis Ababa:	NA	NA NA O	SAMN21 599708	ET1294- 3_Swertia_tetand ra
Gentianac eae	Swertia						Kality				KN0085- 4_Swertia_uniflor a
Gentianac eae	Swertia	<i>Swertia</i> <i>uniflora</i>	Mildbr. & Gilg	AFROAL P II team	2007-01- 23 O-DP-35048	Kenya	Mt O-DP- 39276-O-	3953	1.124 34.59033 O	SAMN21 599709	TZ0711- 4_Swertia_uniflor a
Gentianac eae	Swertia	<i>Swertia</i> <i>usambarensis</i>	Engl.	AFROAL P II team	2009-06- DP-39278, 24 O-DP-48440	Tanzania	Mbeya Mts	2616	8.83683 33.3725 O	SAMN21 599710	4568_Swertia_us ambarensis

Gentianaceae	<i>Swertia volkensii</i>	<i>Swertia volkensii</i>	Gilg	AFROAL P II team	2007-11- 13 O-DP-31750, 13 O-DP-31751	Ethiopia	Bale Mts: Sanetti	4050 NA	NA	O	SAMN21 599711	ET0680-x23_Swertia_volkensii	
Poaceae	<i>Aira caryophyllea</i>	<i>Aira caryophyllea</i>	L.	AFROAL P II team	2007-10- 24 O-DP-44263	Ethiopia	Simen Mts: Dirni Gate	13.2879 3716	8 38.11882	O	SAMN21 599712	ET0167-T_Aira_caryophyllea	
Poaceae	<i>Alopecurus baptrrhenus</i>	<i>Alopecurus baptrrhenus</i>	S.M.Phillips	AFROAL P II team	2007-11- 01 O-DP-31197	Ethiopia	Simen Mts: Silki Mt	13.3284 3681	7 38.24297	O	SAMN21 599713	ET0495-5_Alopecurus_baptarrhenius	
Poaceae	<i>Anthoxanthum nivale</i>	<i>Anthoxanthum nivale</i>	K.Schum. (L.)	AFROAL P II team	2008-11- 03 O-DP-37045	Tanzania	Kilimanjaro : Shira Plateau	-	3406 2.98662	37.22237	O	SAMN21 599714	TZ0031-2_Anthoxanthum_nivale
Poaceae	<i>Deschampsia cespitosa</i>	<i>Deschampsia cespitosa</i>	P.Beauv.	AFROAL P II team	2007-11- 18 O-DP-32350	Ethiopia	Bale Mts: Sanetti, Konten	4019 6.85542	39.89647	O	SAMN21 599715	ET0831-3_Deschampsia_cespitosa	
Poaceae	<i>Avenella flexuosa</i>	<i>Avenella flexuosa</i>	(L.) Drejer (Hochst. ex A.Rich.)	AFROAL P II team	2008-11- 18 O-DP-38285	Tanzania	Mt Kilimanjaro : Horombo	-	3710 -3.1375	37.43683	O	SAMN21 599716	TZ0328-3_Deschampsia_flexuosa_var_afrmontana
Poaceae	<i>Helictotrichon elongatum</i>	<i>Helictotrichon elongatum</i>	C.E.Hubb.	AFROAL P II team	2007-10- 24 O-DP-30063	Ethiopia	Simen Mts: Dirni Gate	13.2879 3716	8 38.11882	O	SAMN21 599717	ET0209-3_Helictotrichon_elongatum	
Poaceae	<i>Koeleria capensis</i>	<i>Koeleria capensis</i>	Nees	AFROAL P II team	2007-10- 23 O-DP-29614	Ethiopia	Simen Mts: Saha	13.2852 3718	5 38.11838	O	SAMN21 599718	TZ0097-1_Koeleria_capeensis	
Poaceae	<i>Rytidosperma subulata</i>	<i>Rytidosperma subulata</i>	(A.Rich. Cope)	AFROAL P II team	2007-11- 20 O-DP-32749	Ethiopia	Bale Mts: Angaso	3986 6.88218	39.8883	O	SAMN21 599719	ET0925-2_Rytidosperma_subulata	
Plantaginaceae	<i>Veronica anagallis-aquatica</i>	<i>Veronica anagallis-aquatica</i>	L.	AFROAL P II team	2007-11- 03 O-DP-31570	Ethiopia	Simen Mts: Silki	13.3270 3682	5 38.24247	O	SAMN21 599731	ET0691-1_Veronica_anagallis-aquatica	
Plantaginaceae	<i>Veronica arvensis</i>	<i>Veronica arvensis</i>	L.	AFROAL P II team	2007-10- 24 O-DP-30129	Ethiopia	Simen Mts: Dirni Gate	13.2879 3716	8 38.11882	O	SAMN21 599732	ET0299-14_Veronica_arvensis	
Plantaginaceae	<i>Veronica glandulosa</i>	<i>Veronica glandulosa</i>	Hochst. ex	AFROAL P II team	2007-10- 23 O-DP-29615	Ethiopia	Simen Mts: Saha	13.2852 3718	38.11838	O	SAMN21 599733	ET0097-2_Veronica_glandulosa	

Benth.												dulosa
		<i>Anagallis serpens subsp. meyeri-johannis</i>										ET0090-3_Anagallis_serpens_subsp_meyeri-johannis
Primulaceae	<i>Anagallis</i>	<i>Primula</i>	<i>Primula verticillata</i>	(Engl.) Taylor	AFROAL P II team	2007-10-21 O-DP-29581	Ethiopia	Simen Mts: Saha	3711	13.2827	3 38.11077 O	SAMN21599720
Primulaceae	<i>Anemone</i>	<i>Anemone thomsonii var friesiorum</i>	Forssk.	AFROAL P II team	2007-10-28 O-DP-30520	Ethiopia	Simen Mts: Near Chenek Camp Site	NA	NA	NA	O	SAMN21599721
Ranunculaceae	<i>Anemone</i>	<i>Anemone thomsonii var thomsonii</i>	Ulbr.	AFROAL P II team	2007-11-02 O-DP-31586	Ethiopia	Simen Mts: Silki	3643	13.3285	38.24092 O	SAMN21599722	
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus aberdaricus</i>	Oliv.	AFROAL P II team	2008-11-30 O-DP-38990	Tanzania	Mt Meru: Saddle Hut area	3589	3.21783	-	36.754 O	SAMN21599723
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus distrias</i>	Ulbr. Steud.	AFROAL P II team	2007-11-02 O-DP-31436	Ethiopia	Simen Mts: Silki	3643	13.3285	38.24092 O	SAMN21599724	
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus oligocarpos</i>	ex A.Rich. Hochst.	AFROAL P II team	2007-10-21 O-DP-29766	Ethiopia	Simen Mts: Saha	3711	13.2827	3 38.11077 O	SAMN21599725	
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus oreophytus</i>	ex A.Rich. Hochst.	AFROAL P II team	2007-11-01 O-DP-30977	Ethiopia	Simen Mts: Silki	3681	13.3284	7 38.24297 O	SAMN21599726	
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus stagnalis</i>	Delile Hochst.	AFROAL P II team	2008-11-30 O-DP-38965	Tanzania	Mt Meru: Saddle Hut area	3166	3.22075	36.78395 O	SAMN21599727	
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus trichophyllum</i>	ex A.Rich.	AFROAL P II team	2009-01-31 O-DP-27222	Kenya	Simen Mts: Mt Elgon: S of Mt Koitobos	3873	1.107	34.60317 O	SAMN21599728	
Ranunculaceae	<i>Ranunculus</i>	<i>Ranunculus volkensii</i>	Chaix Engl.	AFROAL P II team	2007-11-02 O-DP-31595	Ethiopia	Simen Mts: Silki	3643	13.3285	38.24092 O	SAMN21599729	
Ranunculaceae					2008-11-04 O-DP-37229	Tanzania	Mt Kilimanjaro : Shira	3536	-3.0056	37.24155 O	SAMN21599730	

Violaceae	<i>Viola</i>	<i>Viola abyssinica</i>	Steud. ex Oliv.	AFROAL P II team	2008-07- 27	O-DP-39897	Uganda	Plateau Virunga Mts: Mt Muhavura, along trail to summit Virunga Mts: Mt Muhavura, along trail to summit Rwenzori	3700	-1.3782	29.67333	O	SAMN21 599734	UG2096- ca	2_Viola_abyssinica
Violaceae	<i>Viola</i>	<i>Viola eminii</i>	R.E.Fr.	AFROAL P II team	2008-07- 25	O-DP-39721	Uganda	Mts: Lower Bigo Valley	3550	1.37628	29.67153	O	SAMN21 599735	UG2035- 1_Viola_eminii	
Violaceae	<i>Viola</i>	<i>Viola nannae</i>	R.E.Fr. Hochst.	AFROAL P II team	2008-08- 11	O-DP-40505	Uganda	Simen	3425	0.38502	29.9273	O	SAMN21 599736	UG2247- 2_Viola_nannae	
Crassula ceae	<i>Umbilicu</i> <i>s</i>	<i>Umbilicus</i> <i>botryoides</i>	ex A.Rich.	AFROAL P II team	2007-10- 25	O-DP-30133	Ethiopia	Mts: Gich Camp Site	13.269	38.1058	72	8 O	SAMN2 1599737	ET0230- 2_Umbilicus_bot ryoides	

Table S2. Distribution and age of species. Distribution and elevation of sampled species according to the Flora of East Africa and the Flora of Eritrea. In some cases, information are adapted to more recent information(indicated). *Lychnis* information are also based on Ousted (1985) A taxonomic revision of the genus *Uebelinia* Hochst.(Caryophyllaceae). Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie, pp.421-459. *Cineraria* information based on Cron, G.V., Balkwill, K. and Knox, E.B., 2006. A revision of the genus *Cineraria* (Asteraceae, Senecioneae). Kew Bulletin, pp.449-535.. *Viola* information are solely based on information provided by “Conservatoire et Jardin botanique de Genève” (<http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=175422>). Abbreviations: Ci: confidence interval; sA: strict Afroalpine (>3800m, according to Gehrke et al. (3)); osA: occurs in the strict Afroalpine and below; obsA: occurs only below the strict Afroalpine (< 3800m)

family	genus	species name (according to ncbi)	individual clades				seed plant wide – treePL								distribution coding	sample_id		
			BEAST2				treePL		elevation in m				Ci		altitud			
			media n	Ci max	Ci min	age	media n	ma x	Ci min	ma x	Ci min	ma x	e coding	Distribution				
Apiaceae	<i>Pimpinella</i>	<i>Pimpinella</i> <i>oreophila</i>							230	420				Ethiopia, Kenya, Rep. South Sudan, Tanzania, Uganda, Cameroon, Gulf of Guinea Islands	endemicEA	KN0314- 2_Pimpinella_ore ophila_var_oreop hila		
Apiaceae	<i>Pimpinella</i>	<i>Pimpinella</i> <i>pimpinelloides</i>					1.83	5.82	0.16	0	0	osA		Native to Ethiopia (Simen)	Simen	ET0415- 2_Pimpinella_pi mpinelloides		
Asteraceae	<i>Cineraria</i>	<i>Cineraria</i> <i>abyssinica</i>	2.58	7.71	0.19	1.25	0.37	1.52	0.01	0	0	osA		Ethiopia, Saudi Arabia, Yemen Ethiopia, Kenya, Uganda, Tanzania, Rep. South Sudan, DR Congo, Rwanda, Malawi, Zambia, Zimbabwe, South Africa	endemicEA	ET1413- 2_Cineraria_abys sinica		
Asteraceae	<i>Cineraria</i>	<i>Cineraria</i> <i>deltoides</i>	2.58	7.71	0.19	1.25	0.5	0.71	0.02	0	0	osA	110	430	Zimbabwe, South Africa	widespread	ET0652- 2_Cineraria_delt oidea	
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio</i> <i>adnivalis</i>	0.42	2.5	0	0.59	0.48	9.75	0.01	325	450	osA	0	0	Uganda, DR Congo; Rwenzori Mts	endemic to Mt	UG2305- 3_Dendrosenecio	

Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio battiscombei</i>	0.41	2.51	0	0.59	0.55	4.68	0.01	295	400	0	Rwenzori	_adnivalis_var_a
												0	Kenya; endemic to	dnivalis
												0	Mt Kenya and	KN0482-
												0	Aberdare Mts	1_Dendrosenecio_battiscombei
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio brassiciformis</i>	0.42	2.5	0	0.59	0.46	5.52	0	295	395	0	Kenya; endemic to	KN0516-
												0	Aberdare Mts	4_Dendrosenecio_bassiciformis
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio cheranganiensis</i>	1	3.61	0.02	0.99				260	350	0	Kenya; endemic to	Dendrosenecio_c
												0	Cherangani Hills	heranganiensis
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio elgonensis</i>	0.41	2.51	0	0.54	0.22	2.36	0.01	275	422	0	Kenya, Uganda;	KN0025-
												5	endemic to Mt Elgon	4_Dendrosenecio_elgonensis_ssp_elgonensis
												0	MtElgon Uganda, Rwanda,	UG2207-
												0	DR Congo; endemic	5_Dendrosenecio
												0	to Mt Rwenzori,	_eric-
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio erici-rosenii</i>	1.46	4.85	0.12	1.98	0.33	8.23	0.03	275	447	0	Muhi, Mt Kahuzi, Mt	RwenzoriMts
												5	Nyiragongo	rosenii_ssp_eric-
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio johnstonii</i>	0.49	2.95	0	0.67				275	335	0	Tanzania; endemic	MtKilimanjar
												0	to Mt Kilimanjaro	Dendrosenecio_johnstonii
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio keniensis</i>	0.44	2.7	0	0.59	0.22	3.2	0.01	330	427	0	Kenya; endemic to	KN0792-
												5	Mt Kenya	3_Dendrosenecio_keniensis
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio keniodendron</i>	0.44	2.7	0	0.54	0.46	2.54	0	365	435	0	Kenya; endemic to	KN0781-
												0	Mt Kenya and	1_Dendrosenecio_keniodendron
												0	Aberdare Mts	TZ0343-
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio kilimanjari</i>	0.49	2.95	0	0.67	0.23	2.56	0	300	427	0	Tanzania; endemic	MtKilimanjar
												5	to Mt Kilimanjaro	_kilimanjari_ssp_kilimanjari
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio meruensis</i>	2.79	8.75	0.13	1.34				285	335	0	Tanzania; endemic	Dendrosenecio_
												0	to Mt Meru	meruensis
												0	MtMeru	

Asteraceae	<i>Erigeron</i>	<i>Erigeron alpinus</i>	3.86	8.82	0.55	1.36	0.03	1.76	0	375	395	0	0	Ethiopia and Kenya. Also Europe to N.	ET0928- 2_Erigeron_alpin us	
Asteraceae	<i>Euryops</i>	<i>Euryops brownei</i>	0.54	3.06	0	0.6	0.52	4.25	0	155	375	0	0	Iran	KN0599- 2_Euryops_brow nei	
Asteraceae	<i>Euryops</i>	<i>Euryops dacrydioides</i>	2.5	7.07	0.07	2.2	0.3	6.2	0	330	470	0	0	Tanzania; endemic to Mt Kilimanjaro	TZ0237- 2_Euryops_dacry dioides	
Asteraceae	<i>Euryops</i>	<i>Euryops elgonensis</i>	0.54	3.06	0	0.6	0.52	4.47	0	300	420	0	0	Kenya, Uganda; endemic to Mt Elgon	KN0016- 2_Euryops_elgon ensis	
Asteraceae	<i>Euryops</i>	<i>Euryops pinifolius</i>	1.61	5.73	0.02	1.44	0.43	4.25	0	320	370	0	0	Ethiopia; Simen Mts, Mt Choke	ET1330- 2_Euryops_pinifo lius	
Asteraceae	<i>Euryops</i>	<i>Euryops prostratus</i>	1.61	5.73	0.02	1.75	0.3	4.5	0	390	440	0	0	Ethiopia, endemic to Bale Mts	ET0889- 2_Euryops_prost ratus	
Brassicaceae	<i>Arabidopsis</i>	<i>Arabidopsis thaliana</i>	11.91	15.7	9	8.21	3.66	2.11	6.14	0.01	145	440	0	0	Europe and much of Asia.	ET0177- 3_Arabidopsis_th aliana
Brassicaceae	<i>Arabis</i>	<i>Arabis alpina</i>	19.09	25.1	4	13.0	3.81	8.75	0.31	0	200	495	0	0	widespread within the Arctic Circle.	TZ0375- 3_Arabis_alpina
Brassicaceae	<i>Erophila</i>	<i>Erophila verna</i>	9.77	14.4	5	5.26	2.74	1.02	7.93	0.12	390	450	0	0	Ethiopia. Simen	ET0667-

ae		<i>mildbraedii</i>				3	0	0	Rwanda, Tanzania, Uganda, DR Congo Burundi, Malawi,	2_Lobelia_mildbr aedii			
Campanulaceae	<i>Lobelia1</i>	<i>Lobelia mildbraedii2</i>	3.45	6.68	1.23	8.87	180	300	Rwanda, Tanzania, Uganda, DR Congo	Lobelia_mildbrae dii			
Campanulaceae	<i>Lobelia1</i>	<i>Lobelia rhynchopetalum</i>	3.45	6.68	1.23	10.3	0.17	8.59	0.06	endemicEA ET0122- 2_Lobelia_rhync hopetalum			
Campanulaceae	<i>Lobelia1</i>	<i>Lobelia stuhlmannii</i>	1.25	2.94	0.3	3.67	0.5	7.81	0.24	UG2088- RwenzoriMts 1_Lobelia_stuhlm annii			
Campanulaceae	<i>Lobelia1</i>	<i>Lobelia telekii</i>	5.18	8.59	2.66	14.0	0.79	7	0.12	295	455	Mt.Elon, Aberdare Mts, Mt Kenya Uganda, Rwanda, DR Congo; endemic to Mt Rwenzori, Virunga Mts Kenya, Uganda; endemic to Mt.Elon, Aberdare Mts, Mt Kenya Uganda, Rwanda, DR Congo; endemic to Mt Rwenzori, Virunga Mts	KN0475- endemicEA 3_Lobelia_telekii
Campanulaceae	<i>Lobelia1</i>	<i>Lobelia wollastonii</i>	2.44	5.09	0.75	6.05	0.5	7.81	0.24	335	425	RwenzoriMts 3_Lobelia_wollas tonii	UG2173-
Campanulaceae	<i>Lobelia2</i>	<i>Lobelia erlangeriana</i>	6.88	12.4	4	2.82	8.12	35.1	1.99	320	410	Ethiopia; Bale Mts, Arsi Kenya, Uganda; endemic to Ethiopia; Bale Mts, Arsi Kenya, Uganda; endemic to	ET1510- 14_Lobelia_erlan geriana
Campanulaceae	<i>Lobelia2</i>	<i>Lobelia lindblomii</i>	4.09	8.6	1.08	4.76	3.1	1	1.17	310	425	Mt.Elon, Aberdare Mts	KN0527- 4_Lobelia_lindblo mii
Campanulaceae	<i>Lobelia2</i>	<i>Lobelia schimperi</i>	20.0	10.5	12.23	5	43.2	6.03	8	240	380	Ethiopia; endemic to Simen Mts	BG52- 1_Lobelia_schim peri
Caryophyllaceae	<i>Lychnis</i>	<i>Lychnis abyssinica</i> (=Silene abyssinica)	1.98	3.25	1.03	0.98	1.37	14.3	0.47	135	400	Ethiopia, Kenya, Rwanda, Uganda, DR Congo, Burundi, Eritrea, Nigeria,	ET1300_18_3_L ychnis_abyssinic a
							1		0	0			

Caryophyllaceae	<i>Lychnis abyssinica</i> 2 (= <i>Silene abyssinica</i>)	4.67	6.81	2.85	2.57	2.4	12.9	1 1.15	0	0	osA	Cameroon	Ethiopia, Kenya, Rwanda, Uganda, DR Congo, Burundi, Eritrea, Nigeria, Cameroon	widespread	ET0098-X_Lychnis_abyssinica_LGS
Caryophyllaceae	<i>Lychnis crassifolia</i> (= <i>Silene kenyana</i>)	2.45	4.14	1.26	1.53	2.11	10.2	0.68	0	0	osA	Kenya, Tanzania	endemicEA	KN0494-2_Lychnis_crassifolia	
Caryophyllaceae	<i>Lychnis kigesiensis</i> (= <i>Silene kigesiensis</i>)	1.98	3.25	1.03	0.98	1.37	11.3	0.47	0	0	obsA	Uganda, Rwanda, Ethiopia	endemicEA	UG2600-1_Lychnis_kigesensis_subsp_ragazziana	
Caryophyllaceae	<i>Lychnis kiwuensis</i> (= <i>Silene kiwuensis</i>)	2.45	4.14	1.26	1.53	2.11	10.2	0.68	0	0	obsA	Uganda, Ruanda, Burundi, DR Congo, Ethiopia	endemicEA	ET1320-3_Lychnis_kiwue nsis	
Caryophyllaceae	<i>Lychnis rotundifolia</i> (= <i>Silene afromontana</i>)	3.9	5.81	2.36	2.88	1.98	16.6	8 0.68	0	0	osA	Aberdare Mts, Mt. Kenya, Mt Kilimanjaro Ethiopia; Gughe highland, Amaro Mts	endemicEA	KN0497-4_Lychnis_rotundifolia	
Caryophyllaceae	<i>Lychnis scottii</i> (= <i>Silene scottii</i>)	3.22	4.91	1.87	1.96				270	330		Ethiopia, Tanzania, Eritrea, Rep. South Sudan, Yemen, Saudi Arabia,	Bale	Uebelina_scotii	
Caryophyllaceae	<i>Minuartia filifolia</i>	5.05	7.94	2.88	3	10.19	13.9	3 4.03	0	0	osA	Somalia	endemicEA	ET0266-1_Minuartia_filifolia	
Caryophyllaceae	<i>Sagina afroalpina</i>	2.87	4.8	1.29	2.78	1.44	27.1	16 1.15	315	460	osA	Ethiopia, Kenya,	endemicEA	KN0944-	

ae										0	0	Uganda, DR Congo Ethiopia, Kenya, Uganda, Tanzania, Eritrea, Rep. South Sudan, Somaliland, South Africa, Angola, Rwanda, DR Congo, Tropical Arabia	2_Sagina_afroalpina		
Caryophyllaceae	<i>Silene</i>	<i>Silene burchellii</i>	2.94	5.68	1.09	9.62	0.66	21.1	6	0.34	0	0	osA	widespread	KN0594-1_Silene_burchellii_var_burchellii
Caryophyllaceae	<i>Silene</i>	<i>Silene flammulifolia</i>	0.51	1.2	0.08	3.72	0.26	3.8	0.11	0	0	obsA	Ethiopia, Somalia, Yemen	ET0205-2_Silene_flammulifolia	
Caryophyllaceae	<i>Silene</i>	<i>Silene macrosolen</i>					0.26	3.8	0.11	0	0	obsA	Ethiopia, Kenya, Tanzania, Rep. South Sudan	ET0289-4_Silene_macrosolen	
Crassulaceae	<i>Umbilicus</i>	<i>Umbilicus botryoides</i>	0.98	1.97	0.31	2.76	19.41	65.7	8	4.92	0	0	osA	Ethiopia, Kenya, Tanzania, Uganda, DR Congo, Djibouti, Eritrea, Rwanda, Somalia, Sudan, Cameroon	ET0230-2_Umbilicus_botryoides
Dipsacales	<i>Scabiosa</i>	<i>Scabiosa columbaria</i>					0.99	5.48	12.8	1.08	0	0	obsA	Ethiopia, Kenya, Uganda, DR Congo, Tanzania, also Cameroon, South Africa and Europe	TZ0218-2_Scabiosa_columbaria
Dipsacales	<i>Valerianella</i>	<i>Valerianella microcarpa</i>					12.2	8	22.5	10.23	220	410	(Mediterranean region)	Ethiopia, Kenya, North Africa, Europe	ET0734-2_Valerianella_microcarpa
Gentianaceae	<i>Sebaea</i>	<i>Sebaea</i> sp	3.26	5.76	1.28	2.35	18.25	9	4.99				obsA	UG2145-3_Sebea_sp	
Gentianaceae	<i>Swertia</i> 1	<i>Swertia</i>	5.17	8.43	2.24	4.8	0.58	3.11	0.1	150	335	obsA	Ethiopia, Kenya,	widespread	ET1031-

Gentianaceae	<i>Swertia1</i>	<i>Swertia usambarensis</i>	6.33	9.65	3.39	5.8	0.45	5.89	0.07	135	310	Cameroon, Nigeria Ethiopia, Kenya, Uganda, Tanzania, Burundi, Rwanda, DR Congo, Zimbabwe, Mozambique, Malawi	widespread	TZ0711- 4568_Swertia_us ambarensis
Gentianaceae	<i>Swertia2</i>	<i>Swertia adolfi-friderici</i>	3.32	7.09	1.9	2.57	2.25	8	0.27	230	300	Uganda, Rwanda, DR Congo, Burundi Ethiopia, Kenya, Uganda, Tanzania, Burundi, Rwanda,	endemicEA	TZ0710- 5_Swertia_adolfi-friderici
Gentianaceae	<i>Swertia2</i>	<i>Swertia kilimandscharica</i>	0.71	2.75	0	2.7	1.14	4	0.58	210	400	Burundi, Rwanda, DR Congo, Malawi	endemicEA	UG2175- 1_Swertia_kilim andscharica
Gentianaceae	<i>Swertia2</i>	<i>Swertia macrosepala</i>	0.22	1.08	0	0.35	3.33	9.79	0.27	335	420	Tanzania, Uganda, Rwanda, DR Congo, Ethiopia	endemicEA	UG2006- 4_Swertia_macro sepala_ssp_maca osepala
Gentianaceae	<i>Swertia2</i>	<i>Swertia schimperi</i>	4.09	7.09	1.9	2.57	1.14	4	0.58	140	390	Ethiopia, Uganda, Tanzania, Malawi, South Sudan	endemicEA	ET0187- x_Swertia_schim peri
Gentianaceae	<i>Swertia2</i>	<i>Swertia subnivalis</i>	0.22	1.08	0	0.35	3.33	4	0.33	360	420	Kenya; endemic to Mt. Kenya.	MtKenya	KN0004- 3_Swertia_subni valis
Gentianaceae	<i>Swertia2</i>	<i>Swertia uniflora</i>	0.71	2.75	0	1.55	6.11	7	0.27	375	430	Kenya, Uganda; endemic to Mt Elgon	MtElgon	KN0085- 4_Swertia_uniflor a
Gentianaceae	<i>Swertia2</i>	<i>Swertia volkensii</i>	1.67	3.57	0.39	1.26	5.19	6	0.27	285	450	Ethiopia, Kenya, Tanzania	endemicEA	ET0680- x23_Swertia_volk ensii
Poaceae	<i>Aira</i>	<i>Aira caryophyllea</i>	2.89	5.53	0.7	1.73	2.11	11.3	0.56	210	450	Ethiopia, Kenya, Uganda, Tanzania, Rep. South Sudan.	widespread	ET0167- T_Aira_caryophyl lea

Poaceae	<i>Alopecurus</i>	<i>Alopecurus baptarrhenius</i>		1.4	2.72	0.37	1.22	0.4	4.29	0.03	0	0	osA	Also in South Africa, Northern Europe and Asia. Introduced to many other parts of the world	ET0495- 5_Alopecurus_ba ptarrhenius		
Poaceae	<i>Anthoxanthum</i>	<i>Anthoxanthum nivale</i>		2.1	4.37	0.42	1.6	1.38	8.95	0.28	0	0	osA	Ethiopia; Bale Mts (also in Shewa Region) Kenya, Uganda, DR Congo, Rwanda, Tanzania	Bale, Simen endemicEA	TZ0031- 2_Anthoxanthum _nivale TZ0328- 3_Deschampsia_	
Poaceae	<i>Avenella</i>	<i>Avenella flexuosa</i>		11.29	16.9	2	6.04	11.4	0.88	7.36	0.37	0	0	osA	Ethiopia, Uganda, Kenya, Tanzania, DR Congo Ethiopia, Kenya, Uganda, DR Congo, Tanzania. Also in temperate regions of both hemispheres and mountains of the Cameroun	endemicEA	flexuosa_var_afr omontana
Poaceae	<i>Deschampsia</i>	<i>Deschampsia cespitosa</i>		1.29	2.46	0.35	0.95	3.76	9.07	0.12	0	0	osA	290 430 Ethiopia, Kenya, Uganda, DR Congo, Rwanda, Tanzania, Rep. South Sudan, Cameroon, Malawi, Zimbabwe, Madagascar	endemicEA	ET0831- 3_Deschampsia_	
Poaceae	<i>Helictotrichon</i>	<i>Helictotrichon elongatum</i>		1.43	2.61	0.28	0.33	1.32	2.83	0.05	0	0	osA	180 450 Ethiopia, Kenya, Uganda, Tanzania, Rep. South Sudan.	widespread	ET0209- 3_Helictotrichon_	
Poaceae	<i>Koeleria</i>	<i>Koeleria capensis</i>		1.15	2.48	0.15	0.9	1.49	7.02	0.02	180	530	osA	0 0 Also in Cameroon,	widespread	widespread TZ0097- 1_Koeleria_cape nsis	

Poaceae	<i>Rytidospermum</i> a	<i>Rytidosperma</i> <i>subulata</i>	1.43 2.61 0.28 0.75	0.77 2.97 0.07	300 0	430 0	osA	Southern Africa Ethiopia; Simen Mts, Bale Mts. Also in Northern Yemen.	endemicEA	ET0925- 2_ <i>Rytidosperma_</i> <i>subulata</i>	
Plantaginacea e	<i>Veronica</i>	<i>Veronica</i> <i>abyssinica</i>	3.11 5.03 1.54 1.37		120 0	390 0	osA	Zambia, DR Congo, Zimbabwe	widespread	<i>Veronica</i> <i>abyssinica</i>	
Plantaginacea e	<i>Veronica</i>	<i>Veronica</i> <i>anagallis-</i> <i>aquatica</i>	1.47 2.62 0.7 0.4	1.11 3.62 0.12	480	300 0	obsA	Ethiopia, Kenya, Uganda, Tanzania, also in Rwanda, Zambia, Zimbabwe	widespread	ET0691- 1_ <i>Veronica_anag</i> <i>allis-aquatica</i>	
Plantaginacea e	<i>Veronica</i>	<i>Veronica</i> <i>glandulosa</i>	6.79 10.0 1 4.35 2.65	0.74 7.87 0.2	0	200 0	410 0	osA	Ethiopia, Kenya, Uganda, DR Congo, Rwanda, Tanzania	endemicEA	ET0097- 2_ <i>Veronica_glan</i> <i>dulosa</i>
Primulaceae	<i>Anagallis</i>	<i>Anagallis</i> <i>serpens</i>		0.74 0.2	7.87	250 0	450 0	osA	Rep. South Sudan, Zimbabwe		
Primulaceae	<i>Primula</i>	<i>Primula</i> <i>verticillata</i>	0.91 2.02 0.22 1.21	4.84 8.63 0.11	0	200 0	420 0	osA	Ethiopia, Somalia, Saudi Arabia, Yemen	endemicEA	ET0330- 2_ <i>Primula_vertici</i> <i>llata</i>
Ranunculacea e	<i>Anemone</i>	<i>Anemone</i> <i>thomsonii</i>		10.4 3.48	5	270 0	390 0	osA	Tanzania, Uganda, DR Congo	endemicEA	KN0605- 2_ <i>Anemone_tho</i> <i>msonii_var_friesi</i> <i>orum</i>

e		<i>simensis</i>					0	0	Mts, Bale Mts, Arisi Ethiopia, Kenya, Uganda, Tanzania, DR Congo		simiensis KN0386-		
Ranunculaceae		<i>Ranunculus</i>					225	475			2_Ranunculus_stagnalis		
e	<i>Ranunculus2</i>	<i>stagnalis</i>	0.44	1.43	0	0.79	0.63	8.91	0.03	0	0 osA	endemicEA	
Ranunculaceae		<i>Ranunculus</i>											
e	<i>Ranunculus2</i>	<i>stagnalis2</i>	1.09	2.02	0.39	3.1	0.37			osA		Ranunculus_stagnalis2	
Ranunculaceae		<i>Ranunculus</i>					305	350				Ranunculus	
e	<i>Ranunculus2</i>	<i>tembensis</i>	1.09	2.02	0.39	3.1	0.42	4.37	0.01	0	0 obsA	Simen, Bale	tembensis
Violaceae	<i>Viola</i>	<i>Viola abyssinica</i>					120	340				UG2096-2_Viola_abyssinica	
							0.61	5.28	0.08	0	0 obsA	widespread	
Violaceae	<i>Viola</i>	<i>Viola eminii</i>					200	405				UG2035-1_Viola_eminii	
							0.32	4.96	0.04	0	0 osA	endemicEA	
Violaceae	<i>Viola</i>	<i>Viola nannae</i>					4.9	0.0	255	360		MtKenya, Aberdares	UG2247-2_Viola_nannae
							0.32	6	4	0	0 obsA	Aberdare	

Table S3. Alignment information.

Alignment dataset	Locus	No. of seq. in original dataset	No of seq. added from new material	No of seq. added from GenBank	Total no. of seq.	Total seq. length	Missing new samples
Seed plant wide	<i>rbcL</i>	22399	92	0	27982	682	0
	<i>matK</i> (incl. <i>trnK</i>)	27891	92	0	22489	4215	0
	concatenated				36197	4897	
Asteraceae	<i>trnLF</i>	29	17	61	107	890	0
	<i>rbcL</i>	29	17	15	55	1429	0
	<i>ndhF</i>	29	17	20	64	1718	0
	concatenated				90	4031	0
Brassicaceae	<i>trnLF</i>	55	4	37	96	1064	0
	<i>rbcL</i>	26	4	29	59	1153	0
	<i>ndhF</i>	95	4	22	121	2068	0
	<i>nad4</i>	97	0	0	97	1481	4
	<i>matK</i>	58	4	29	91	1045	0
	ITS	211	4	34	249	819	0
	<i>chl</i>	26	0	0	26	999	4
	<i>adh</i>	14	0	0	14	1986	4
	concatenated ¹				54	7270	
Campanulaceae	<i>trnLF</i>	76	11	31	119	1347	0
	<i>rbcL</i>	67	11	27	106	1400	0
	<i>ndhF</i>	31	11	29	72	1234	0
	concatenated				118	3776	
Caryophyllaceae	ITS	63	10	58	132	766	0
Crassulaceae	<i>trnLF</i>	90	1	9	100	1168	0
	<i>rps16</i>	58	1	9	68	1052	0
	<i>matK</i>	89	1	9	99	1232	0
	ITS	322	1	9	332	1005	0
	concatenated				324	4440	
Dipsacales	ITS	120	2	13	122	743	0
	<i>matK</i>	126	2	20	128	1226	0

¹no *chl*, *adh* used for concatenation, samples with less than 4 loci were removed, alignment edited.

	<i>psbAH</i>	109	2	4	111	459	0
	<i>trnL</i>	123	2	16	125	1028	0
	concatenated				149	3448	0
Gentianaceae	<i>trnLF</i>	162	19	58	239	667	0
	ITS	153	18	39	210	732	1
	concatenated				181	1384	
Poaceae (dataset A)	<i>rbcL</i>	250	8	28	286	1486	0
	<i>ndhF</i>	250	8	30	288	2451	0
	<i>matK</i>	250	8	27	285	3321	0
	concatenated				282	7442	
Plantaginaceae	ITS – Plantago	38	2	23	63	741	0
Primulaceae	<i>trnLF</i>	13	2	9	24	1070	0
	<i>rps16</i>	11	2	9	22	879	0
	<i>rpl16</i>	13	2	8	22	1099	0
	<i>rbcL</i>	13	2	10	25	1396	0
	<i>ndhF</i>	9	2	5	16	1938	0
	<i>matK</i>	13	1	8	23	1573	1
	concatenated				20	7911	
Ranunculaceae	<i>trnL-matK</i>	237	7	42	286	2013	2
	<i>psbJ-petA</i>	225	7	24	256	762	2
	ITS	238	6	39	283	656	3
	concatenated				249	3425	

Table S4. Species represented by more than one individual in the phylogenies of individual seed plant clades generated using BEAST2 and retrieved as monophyletic or non-monophyletic. PP = posterior probability.

Species retrieved as monophyletic	
> 0.9 PP	<i>Arabidopsis thaliana</i> , <i>Arabis alpina</i> , <i>Minuartia filifolia</i> , <i>Lobelia deckenii</i> (excl. <i>L. d. bequaertii</i>), <i>Silene burchellii</i>
< 0.9 PP	<i>Lobelia telekii</i> , <i>Lobelia acrochila</i> , <i>Cineraria abyssinica</i>
Non-monophyletic	
> 0.9 PP	Potentially paraphyletic species (nested species in parenthesis): <i>Veronica glandulosa</i> (<i>V. abyssinica</i>); <i>Lobelia aberdarica</i> (<i>L. bambuseti</i>); <i>Lobelia stuhlmannii</i> (<i>L. wollastonii</i>)
	Potentially polyphyletic species: <i>Ranunculus stagnalis</i> ; <i>Lychnis abyssinica</i> ; <i>Lobelia mildbraedii</i> , <i>Lobelia deckenii</i> (<i>Lobelia deckenii</i> spp. <i>bequaertii</i> clade with <i>L. stuhlmannii</i> , <i>L. wollastonii</i>); <i>Swertia crassiuscula</i> (Ethiopian sample together with <i>S. engleri</i> , distantly related to other <i>S. crassiuscula</i>)
< 0.9 PP	<i>Ranunculus trichophyllum</i> ; <i>Veronica anagallis-aquatica</i> ; <i>Lobelia rhynchopetalum</i> (<i>L. acrochilua</i>); <i>Ranunculus volvensii</i> (<i>R. ficariifolius</i> , <i>R. cheiophyllum</i>)

Table S5. BEAST2 age estimates of afroalpine clades containing min. two sampled species. Asterix indicates unsupported stem nodes.

family	clade	number of species	crown age	stem age
Asteraceae	<i>Dendrosenecio</i> *	11	7.89 (2.07 - 16.77)	22.62 (15.18 - 30.73)
Asteraceae	<i>Cineraria</i> *	2	2.58 (7.71 - 0.19)	3.54 (0.55 - 8.28)
Asteraceae	<i>Euryops</i> *	6	6.34 (1.93 - 13.03)	14.61 (7.4 - 22.87)
Campanulacea e	Giant <i>Lobelia</i> *	14	9.49 (5.01 - 15.17)	15.79 (9.63 - 22.98)
Campanulacea e	Herbal <i>Lobelia</i>	4	12.23 (6.03 - 20.05)	18.7 (10.37 - 28.99)
Caryophyllacea e	<i>Lychnis</i>	7	6.62 (4.33 - 9.4)	8.35 (9.4 - 5.56)
Gentianacea e	Swertia clade 1	9	13.99 (9.82 - 18.63)	19.02 (14.19 - 24.44)
Gentianacea e	Swertia clade 2	6	4.09 (1.9 - 7.09)	10.49 (5.08 -

				17.19)
Plantaginaceae	Veronica	7	3.93 (2.2 - 6.14)	6.79 (4.35 - 10.01)
Ranunculaceae	<i>Ranunculus</i> clade 1	3	4 (2.06 - 6.13)	5.53 (3.83 - 7.58)
Ranunculaceae	<i>Ranunculus</i> clade 2	4	2.2 (1.13 - 3.47)	3.49 (2.31 - 4.8

Table S6. Lineages estimated to be older than 10 Ma in at least one of the analyses (Table S2). Differentiated into being part of an afroalpine clade or not. Sorted according to families, and then sorted according to the dating method and then alphabetically. Bold indicates taxa having across all available dating methods age estimates > 10Ma.

family	genus	taxon	number of species	individual seed plant clades – BEAST2	individual seed plant clades – treePL	individual seed plant wide – treePL
Asteraceae	<i>Dendrosenecio</i>	<i>Dendrosenecio</i>	11	22.62		0.77
Asteraceae	<i>Euryops</i>	<i>Euryops</i>	6	14.61		0.93
Campanulaceae	<i>Lobelia2</i>	<i>Lobelia 2</i>	4	18.7		13.68
Campanulacea	<i>Lobelia1</i>	<i>Lobelia 1</i>	12	15.79		1.42
Caryophyllacea	<i>Lychnis</i>	<i>Lychnis</i>	7	8.35		3.35
Gentianaceae	<i>Swertia1</i>	<i>Swertia 1</i>	11	19.02		2.94
Gentianaceae	<i>Swertia2</i>	<i>Swertia 2</i>	7	10.49		8.82
Plantaginaceae	<i>Veronica</i>	<i>Veronica</i>	2	6.79		
Brassicaceae	<i>Arabis</i>	<i>Arabis alpina</i>	1	19.09	6.14	3.81
Brassicaceae	<i>Thlaspi</i>	<i>Thlaspi alliaceum</i>	1	12.7	4.41	1.57
Brassicaceae	<i>Arabidopsis</i>	<i>Arabidopsis thaliana</i>	1	11.91	3.66	2.11
Caryophyllacea	<i>Minuartia</i>	<i>Minuartia filifolia</i>	1	5.05	13.93	10.19
Crassulaceae	<i>Umbilicus</i>	<i>Umbilicus botryoides</i>	1	0.98	2.76	19.41
Dipsacales	<i>Valerianella</i>	<i>Valerianella microcarpa</i>	1		12.28	10.23
Gentianaceae	<i>Sebaea</i>	<i>Sebaea sp</i>	1	3.26	2.35	18.25
Poaceae	<i>Avenella</i>	<i>Avenella flexuosa</i>	1	11.29	11.49	0.88

Table S7. Statistics for the different dating methods (Table S2). Abbreviations: ci – confidence intervals.

	individual seed plant clades – BEAST2			individual seed plant clades – treePL		seed plant-wide – treePL		
	stem age	ci max	ci min	stem age	stem age	ci min	ci max	
min	0.15	0.82	0	0.33	0.03	0	0.20	
max	19.09	25.14	13.05	14.02	19.41	7.87	65.78	
median	2.44	4.85	0.70	2.57	0.84	0.12	7.81	
mean	3.37	6.00	1.68	3.81	2.12	0.53	10.28	
number of species		91		93		93		
<=5	73	47	82	79	84	90	31	
< 10	84	81	90	84	88	91	60	
>= 10	7	10	1	9	5	0	31	
>=15	1	7	0	0	2	0	14	

Table S8. Molecular dating settings for individual seed plant clades.

lineage		constraint	BEAST2						treePL				
			linked			starting tree	substitution rate model			prior settings		min	max
			on rates	substituti	linked trees		linked clocks	n	rate				
Asteraceae	mrca	incl. All samples	no	yes	yes	yes	GTR+G+I	uniform	73	101	73	101	
	Barnadesi							Exponenti					
	oideae	incl. <i>Barnadesia, Dasyphyllum</i>						al	1.5	72.1	72.1	100	
	Famathin	excl. <i>Acicarpha, Barnadesia, Boopis, Dasyphyllum,</i>						Exponenti					
	antus	<i>Famatianthus, Schlechtendalia</i>						al	1.5	47.5	47.5	65	
		excl. <i>Tropaeolum minus, Moringa oleifera, Carica</i>											
		<i>papaya, Batis maritima, Reseda lutea, Capparis</i>											
Brassicaceae	ingroup	<i>flexuosa, Cleome viscosa</i>	cp and nr										
Campanulaceae	mrca	all	split	yes	yes	no	GTR+G+I	normal	89.5	1	87.9	91.1	
		incl. <i>Brighamia insignis, Clermontia kakeana,</i>											
		<i>Cyanea angustifolia, Cyanea koolauensis,</i>											
		<i>Delissea undulata, Lobelia hypoleuca, Lobelia</i>											
	Hawaii	<i>yuccoides, Trematolobelia macrostachys</i>						uniform	0	29.8	set	29.8	
	Campanul	incl. <i>Campanula asperuloides, Campanula latifolia,</i>									infinit		
	oideae	<i>Campanula trachelium, Legousia hybrida</i>											
		excl. <i>Corriola andina, Corriola litoralis,</i>											
		<i>Paronychia argentea, Paronychia lindheimeri,</i>											
		<i>Gymnocarpos rotundifolius, Gymnocarpos</i>											
		<i>decander, Herniaria glabra, Philippiella patagonica,</i>											
		<i>Drymaria cordata, Pycnophyllum bryoides, Cerdia,</i>											
		<i>Polycarpon tetraphyllum, Illecebrum verticillatum,</i>											
	Alsinoide	<i>Cardionema ramosissimum, Loeflingia hispanica,</i>											
	ae-	<i>Dicheranthus plocamoides, Pteranthus</i>	Only										
Caryophyllacea	Caryophyl	<i>dichotomus, Scopulophila parryi, Sphaerocoma</i>	Only 1	1	Only 1					0.0/1.			
e	loideae	<i>aucherii, Spergularia rubra, Telephium imperati</i>	locus	locus	locus	no	GTR+G+I	lognormal	34 0	34	42.5		
									105.		93.4		
Crassulaceae	mrca	incl. all	yes	yes	yes	no	GTR+G+I	normal	94	7.6	3	120.59	
	Crassulac	excl. <i>Aphanopetalum, Glischrocaryon,</i>						normal	58.2	19.5	26.2	88.39	
	eae	<i>Gonocarpus, Haloragis, Laurembergia,</i>							7		3		
		<i>Meionectes, Myriophyllum, Penthorum,</i>											

Proserpinaca, Tetracarpaea, Trihaloragis													
												not	
	Macarone	sia	incl. <i>Aeonium</i> , <i>Greenovia</i> , <i>Monanthes</i>					GTR+G+I	uniform	0	21 set	21	
Dipsacales ¹	mrca	all			no	yes	yes	yes	uniform	44	62	62	
Gentianaceae	ingroup	all			no	yes	yes	yes	GTR+G+I	52.0	7	40.4	68.8
		<i>Emmenop</i>							Normal	1.0/1.			
	terys	incl. <i>Emmenopteris</i> , <i>Fadogia</i> , <i>Vangueria</i>							lognormal	45	0	45	65
	sectGenti												
	ana	incl. <i>G.cruciata</i> , <i>G. decumbens</i>							lognormal	1	5	5	25
									1.0/1.				
	Lisianthus	incl. <i>Lisianthus</i>							lognormal	40	0	40	60
	crownPoa								infinit				
Poaceae	ceae	excl. <i>Anomochloa</i>			yes	yes	yes	yes	GTR+G+I	90	y	90	no set
	BEP+PA	excl. <i>Anomochloa</i> , <i>Leptaspis</i> , <i>Pharus</i> , <i>Puelia</i> ,							1.0/1.				
	CCMAD	<i>Streptochaeta</i>							lognormal	55	0	55	75
									1.0/1.				
	<i>Distichlis</i>	incl. <i>Bouteloua</i> , <i>Distichlis</i>							lognormal	14	0	14	34
Plantaginaceae	Aragoa	incl. <i>Aragoa</i>			yes	yes	yes	no	GTR+G+I	0	3.3	0	3.3
	PlatArag	incl. <i>Plantago</i> and <i>Aragoa</i>							uniform	1	19.4	19.4	24
									Exponenti	39.9	21.0		
Primulaceae	mrca	all			yes	yes	yes	no	GTR+G+I	9	11.49	9	58.9
	ingroup	excl. <i>Androsace</i> , <i>Anagallis</i> , <i>Soldanella</i>							normal	15.9	2.1/0.		
Ranunculaceae	Ranuncul				Only 1	Only 1	Only 1			7	63	18.9	39
	aceae	excl. <i>Anemone quinquefolia</i> , <i>Isopyrum</i>			locus	locus	locus	no	GTR+G+I	46.6	1	44.6	48.2
		excl. <i>Anemone quinquefolia</i> , <i>Arcteranthis</i> ,							normal				
		<i>Beckwithia andersonii</i> , <i>Callianthemoides</i>											
		<i>semiverticillatus</i> , <i>Coptidium lapponicum</i> , <i>Coptidium</i>											
		<i>pallasii</i> , <i>Cyrtorhyncha ranunculina</i> , <i>Ficaria</i>											
		<i>fascicularis</i> , <i>Ficaria verna</i> , <i>Halerpestes</i> ,											
		<i>Hamadryas Isopyrum</i> , <i>Oxygraphis polypetala</i> ,											
	Myosurus	<i>Peltocalathos baurii</i>							Exponenti	1	23	23	not set
	gen_dist	incl. <i>Ranunculus carpaticola</i> , <i>Ranunculus notabilis</i>							normal	0	0.914	0	2.56

island	incl. <i>R.caprarum</i> , <i>R. peduncularis</i>	/0.25	not	uniform	0.1	2 set	2
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¹based on Bell&Donoghue 2012

Table S9. Comparison of our age estimates to previous Bayesian estimates. Abbreviations: CI – confidence intervals.

lineage	node	median	Individual clades – BEAST2		compared to publication (references see main text)		
			min 95% CI	max 95% CI	median	min 95% CI	max 95% CI
Asteraceae	Asteraceae crown	84.5	76.08	94.08	80.5	75.41	86.99
	Senecioneae crown	28.66	26.21	35.87	24.59	17.76	31.45
	Root - Moringaceae/Brassicaceae split						
Brassicaceae	Brassicaceae ae split	89.46	87.47	91.4	72	47.9	90.5
	Brassicaceae crown	47.19	35.82	58.35	37.6	24.2	49.4
Campanulaceae	N2	47.58	32.84	64.37	45.5	30.9	59.2
	N3	42.78	30.07	57.59	39.7	27.4	53.1
	N4	26.04	16.03	37.38	24.5	15.1	36.6
	N5	19.53	12.44	27.65	20.8	12.4	30.5
	N7	33.55	24.06	43.89	36.7	25.1	49.5
	N8	31.3	19.96	39.75	32.8	22	45.4
	N9	29.48	22.22	41.61	29.6	18.9	41.3
	<i>Faccinia</i> stem	10.37	6.77	14.38	9.41	5.02	13.93
	<i>Faccinia</i> crown	3.93	2.16	6.58	3.3	1.67	5.18
Crassulaceae	<i>Stellaria</i> - <i>Faccinia</i> root	27.01	21.36	32.22 25.46			
	excl. <i>Crassula</i> and <i>Calanchoe</i>	101.38	87	115.78	107.54	93.9	121.43
		60.16	46.32	74.82	65.92	53.39	79.53
Gentianaceae					Pirie et al. 2015 does not report ages incl. root constraint		
Poaceae	Poaceae crown	96.18	90	109.39			
	BEP+PACCMAP	61.79	55.15	72.16	57	51	75
Plantaginaceae	Danthonooideae	27.47	20.27	36.3	30	21	38
	stem <i>Veronica</i>	14.69	9.81	20.99	19.91	15.47	25.09
	crown <i>Veronica</i>	10.82	7.11	15.69	16.13	12.46	20.59
Primulaceae	<i>Primula</i> crown	11.15	7.78	15.58		results not shown	
Ranunculaceae	<i>Ranunculus</i> stem	20	16.13	23.45	21.25	14.13	28.43
	<i>Ranunculus</i> crown	17.74	14.01	21.19 18.11			

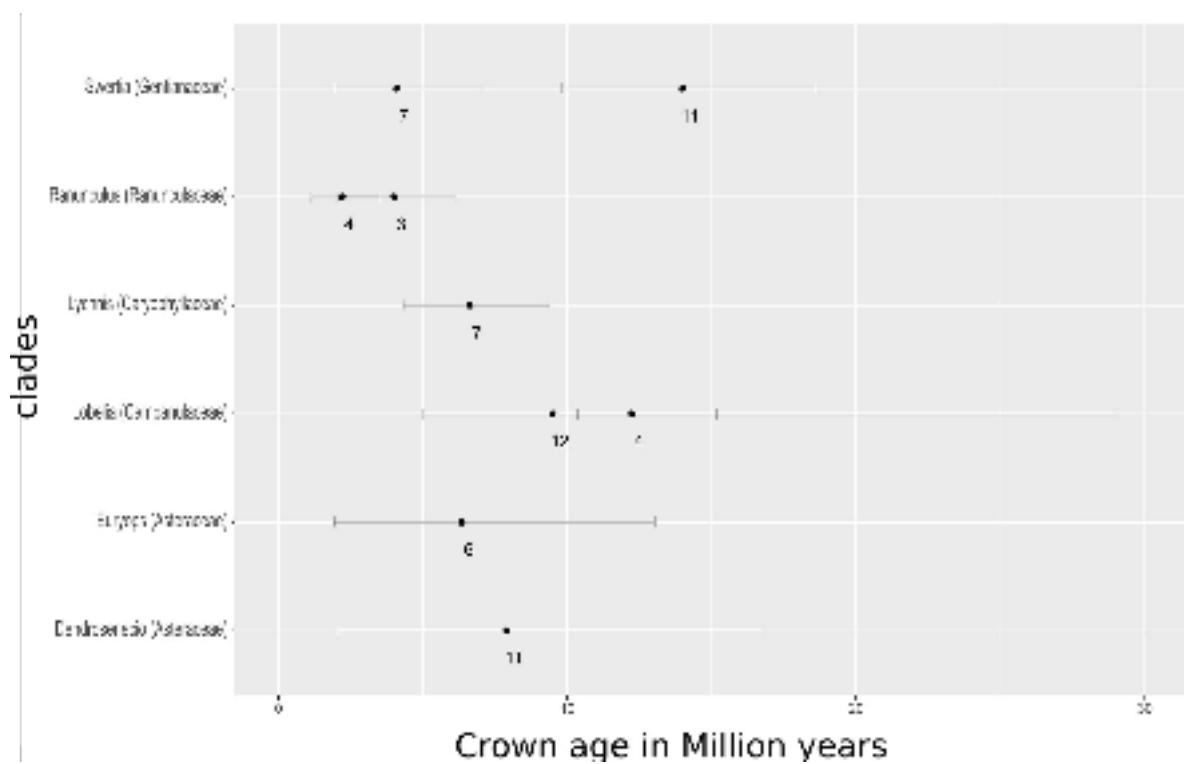


Figure S1. BEAST2 crown ages from the seed plant wide dating analysis indicating the start of lineage radiations. Numbers depict number of species sampled in clades.

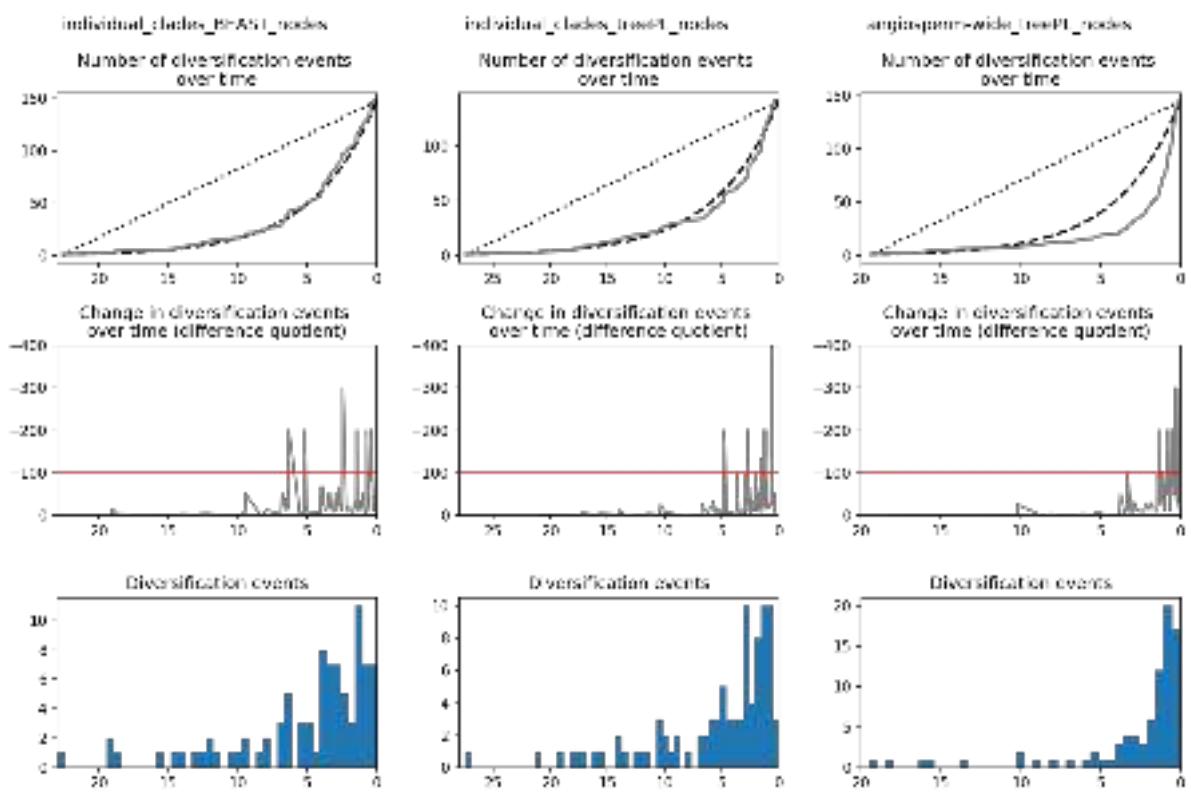


Figure S2. Species accumulation over time in the afroalpine region based on historical lineage diversity estimates and relative branching times obtained from the time-calibrated phylogenies. These plots differ from standard lineage through time plots in that species accumulation in a region results from colonization events in addition to regional diversification. Row 1: Number of colonization/diversification events over time. The black dotted line marks a constant rate model and the black dashed line an exponential model. Row 2: Change in number of colonization/diversification events over time calculated as a difference quotient (see text). The red line shows the maximum difference of change in a constant model. Row 3: number of colonization/ diversification events per 0.5-million-year intervals.

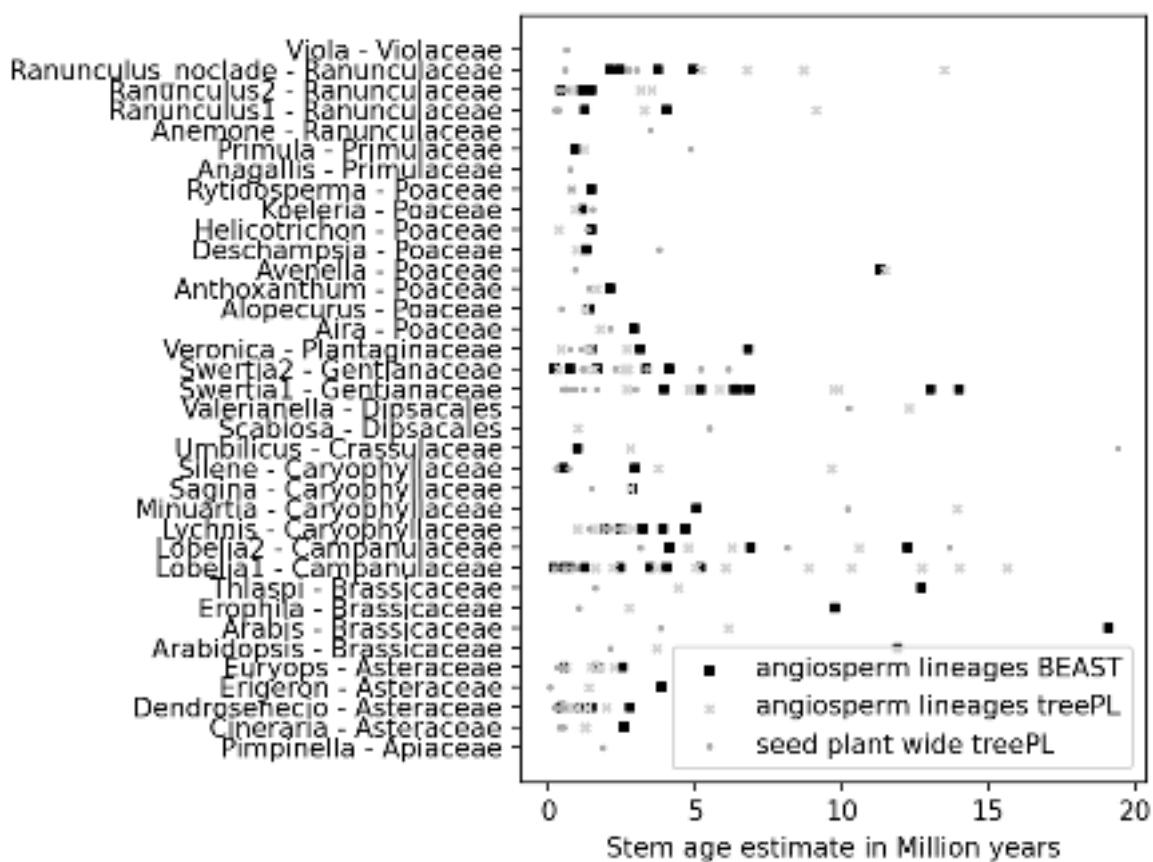


Figure S3. Median age estimates of afroalpine species based on the three different dating approaches.

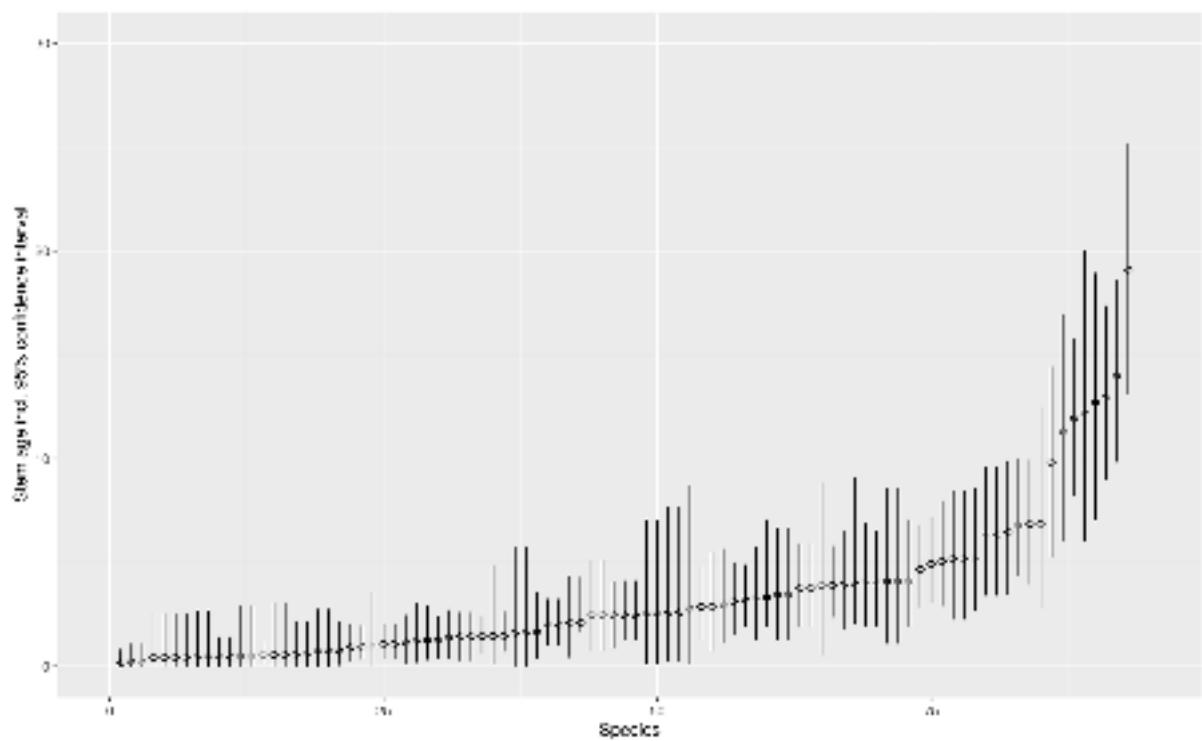


Figure S4. Individual seed plant clades stem age estimates (BEAST2) of all afroalpine species, ordered by median age.

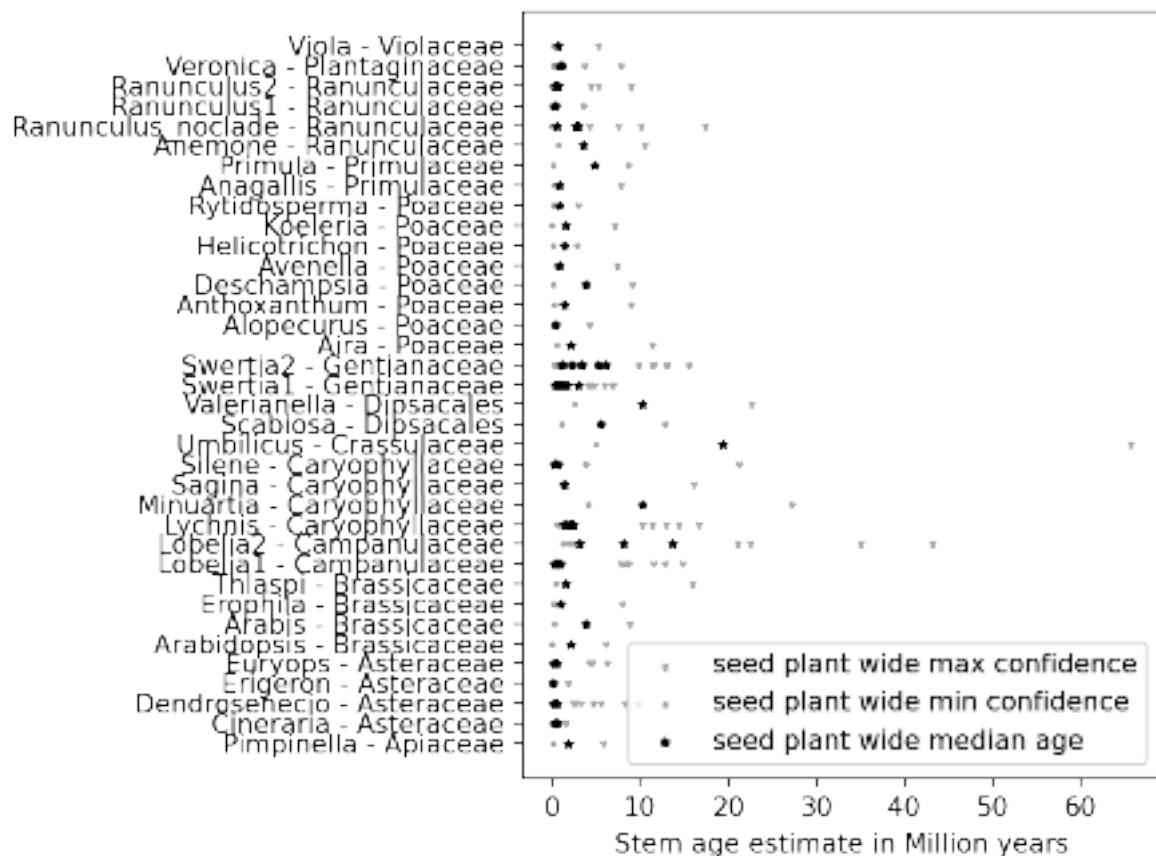


Figure S5. treePL stem ages from of the seed plant wide dating analysis, showing median and 95% confidence interval.

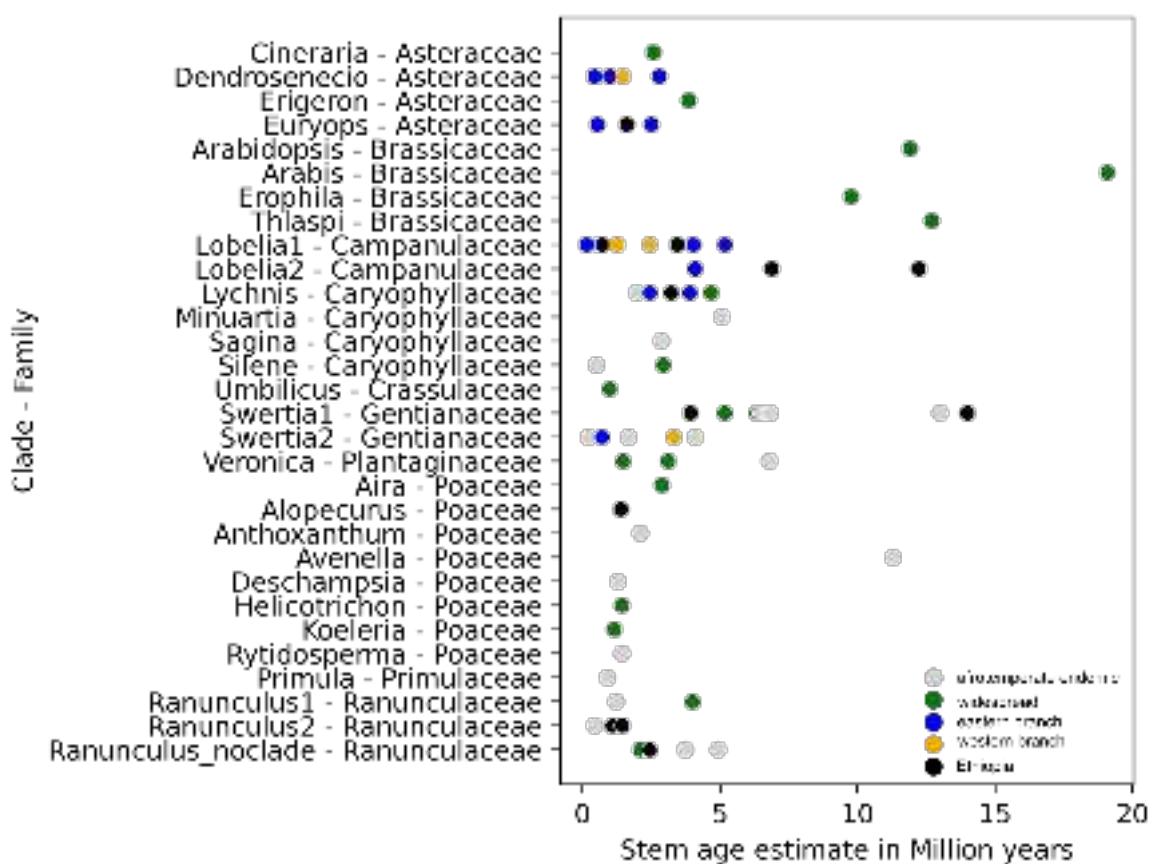


Figure S6. BEAST2 stem ages from the seed plant wide dating analysis and geographic distribution of species. Distribution of species has been assigned to subregions, species found in more than one subregion but being restricted to eastern Africa are coded as ‘afrotropical endemic’ and if they occur beyond these limits as “widespread”. See text for details on distribution coding.

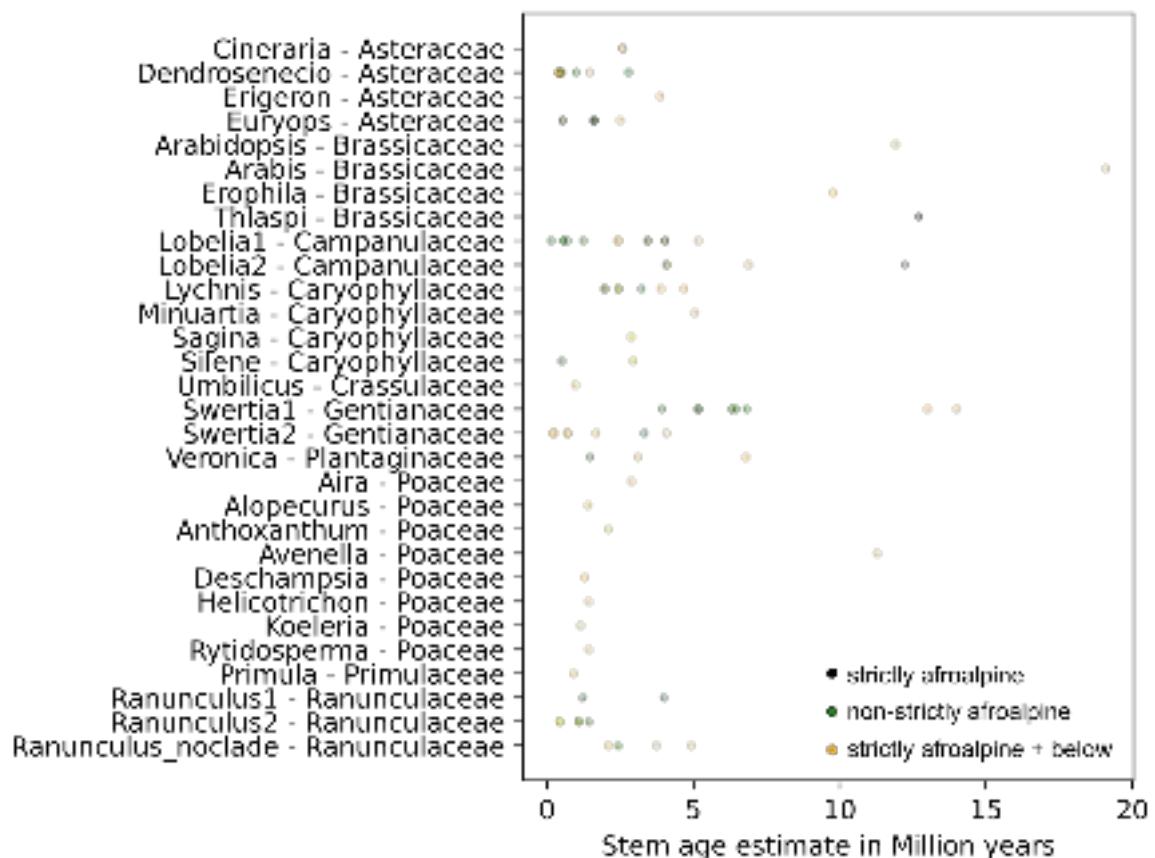


Figure S7. BEAST2 stem ages from the seed plant wide dating analysis. The species are categorized as 'strictly afroalpine (lower altitudinal limit above 3800 m)', 'strictly afroalpine + below' and 'non-strictly afroalpine' (upper altitudinal limit below 3800 m), median age estimates are 7.16 ($n=2$), 2.45 Ma ($n=32$) and 2.21 Ma ($n=57$) respectively.

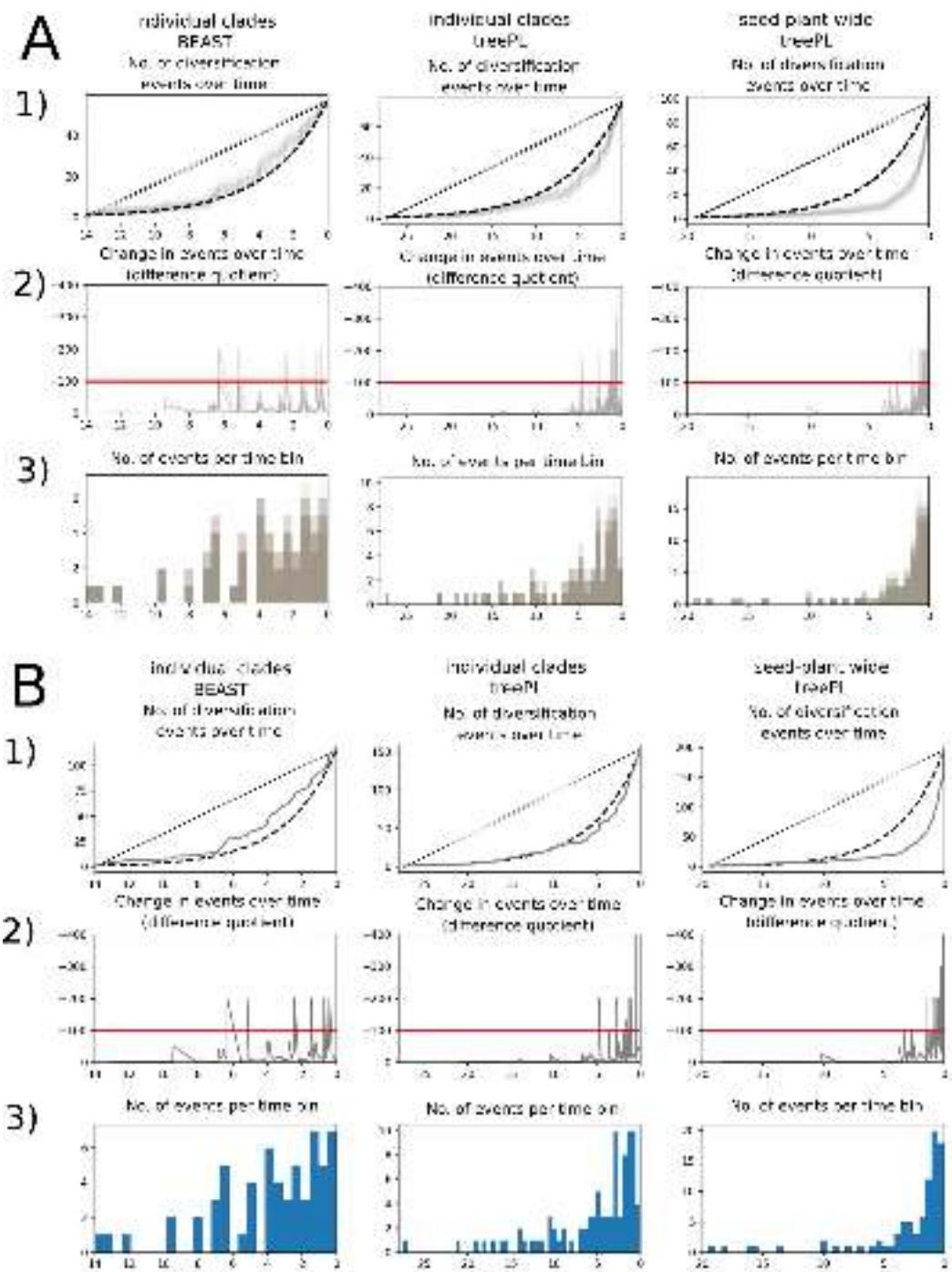


Figure S8. Species diversification (excluding colonization events) over time in the afroalpine region based on historical lineage diversity estimates and relative branching times obtained from the time-calibrated phylogenies. These plots differ from standard lineage through time plots in that species accumulation in a region results from colonization events in addition to regional diversification. Row 1: Number of diversification events over time. The black dotted line marks a constant rate model and the black dashed line an exponential model. Row 2: Change in number of diversification events over time calculated as a difference quotient (see text). The red line shows the maximum difference of change in a constant model. Row 3: number of diversification events per 0.5-million-year intervals. Row 3: number of colonization/ diversification events per 0.5-million-year intervals. A. Results for the three different dating methods are shown in A) and B). For A) age estimates were subsampled to 50% 200 times; B) shows the actual data.

Legends for further SI Appendices:

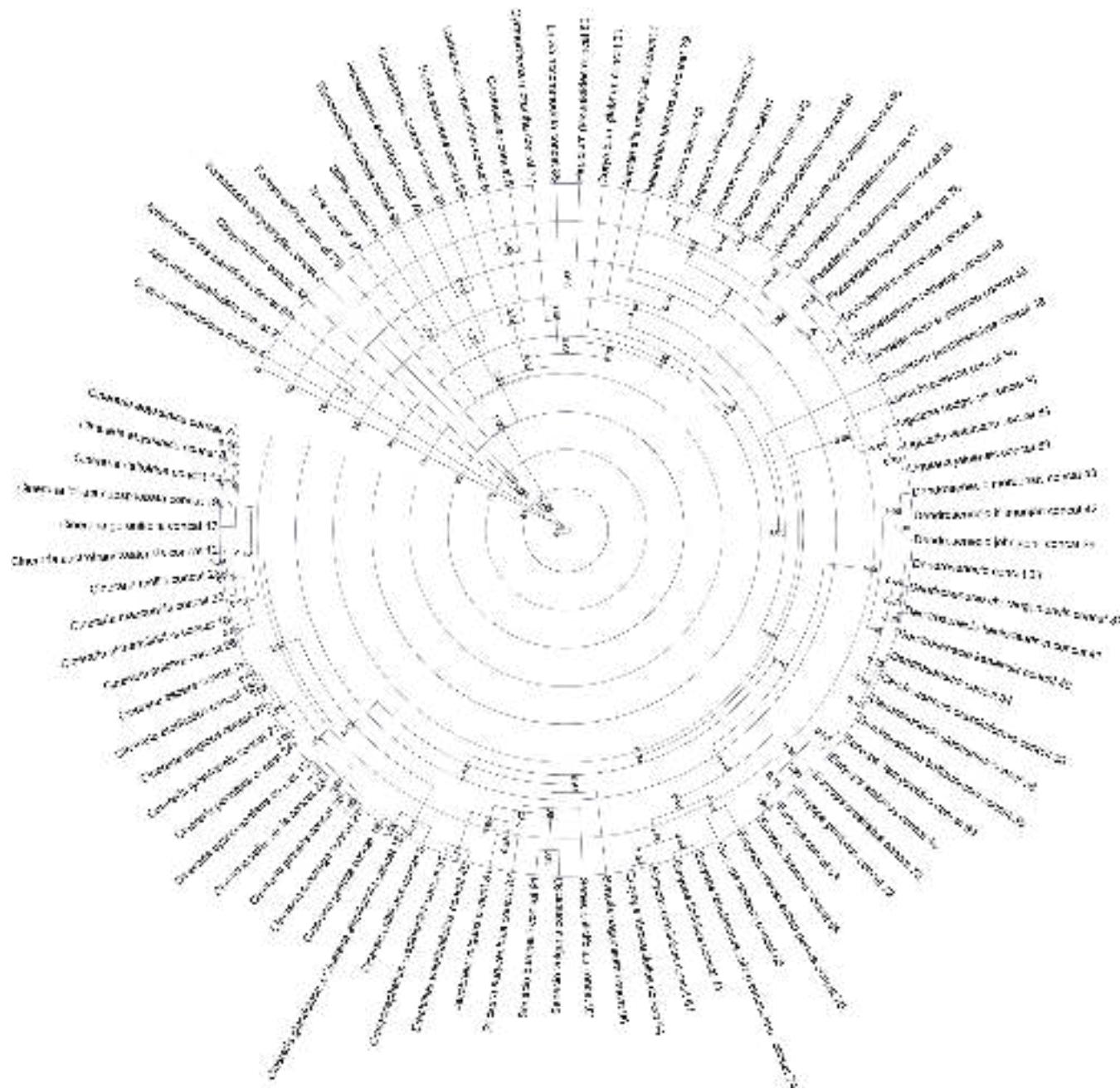
SI Appendix S2: Figures of dated phylogenies of individual seed-plant clades.

SI References

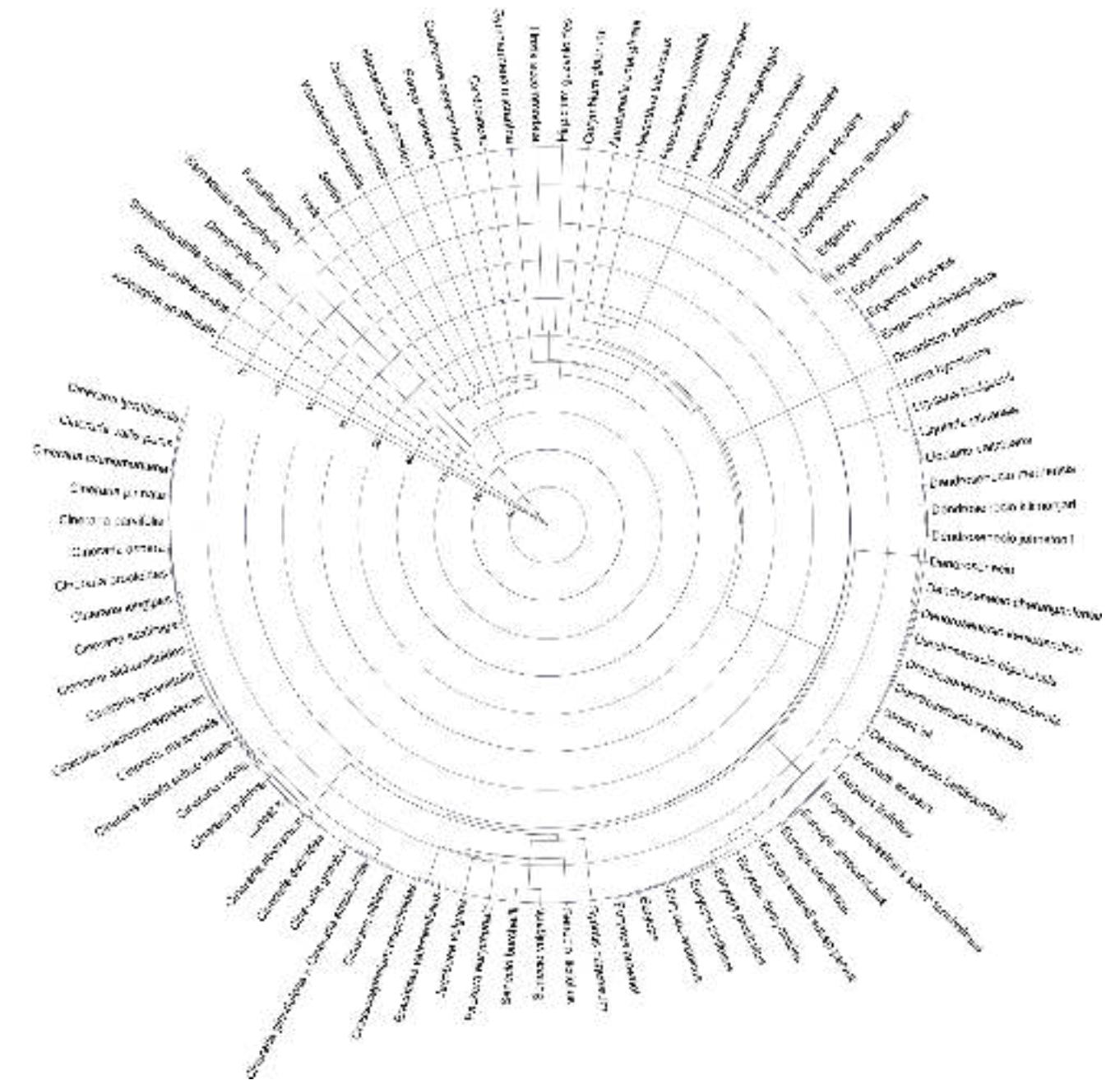
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Asteraceae

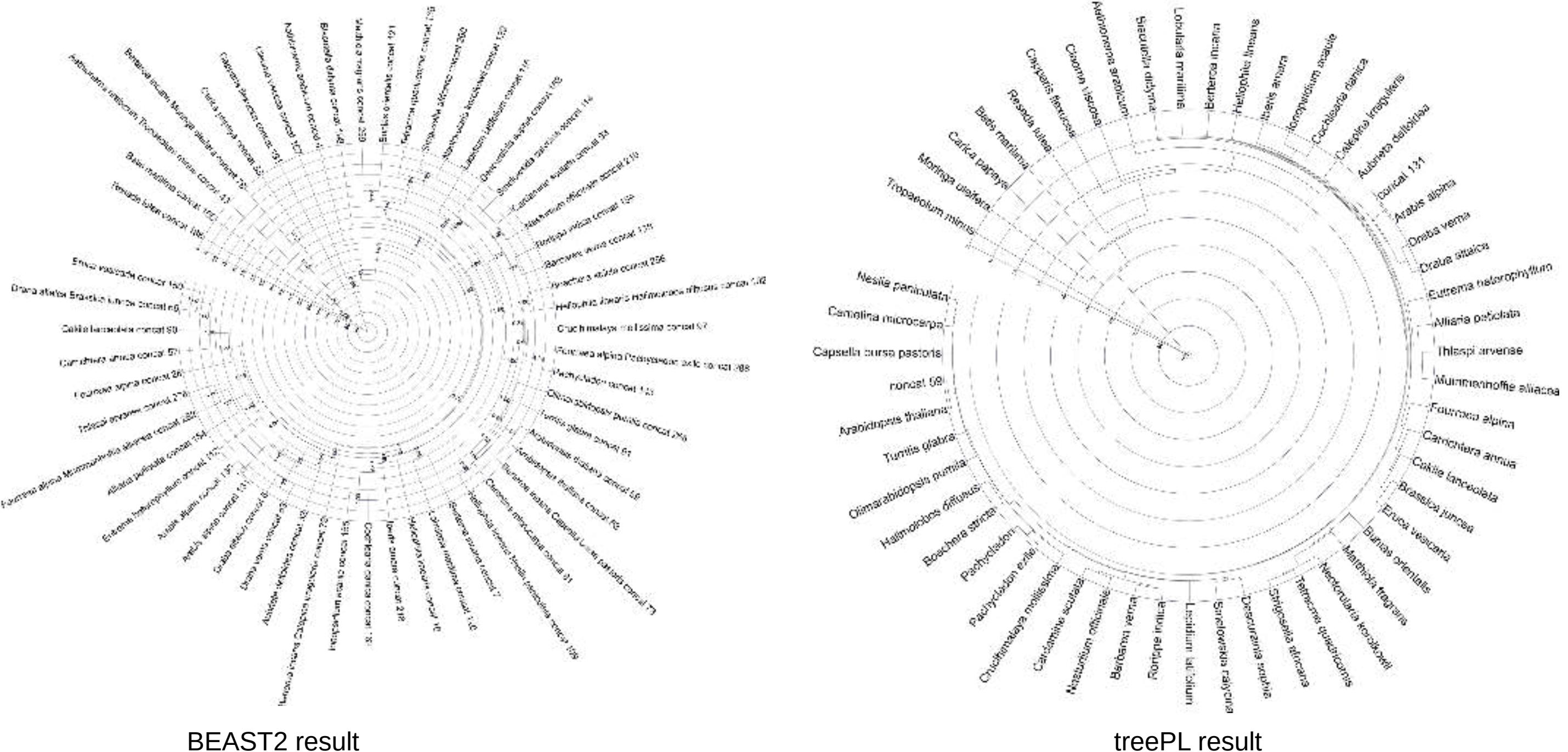


BEAST2 result



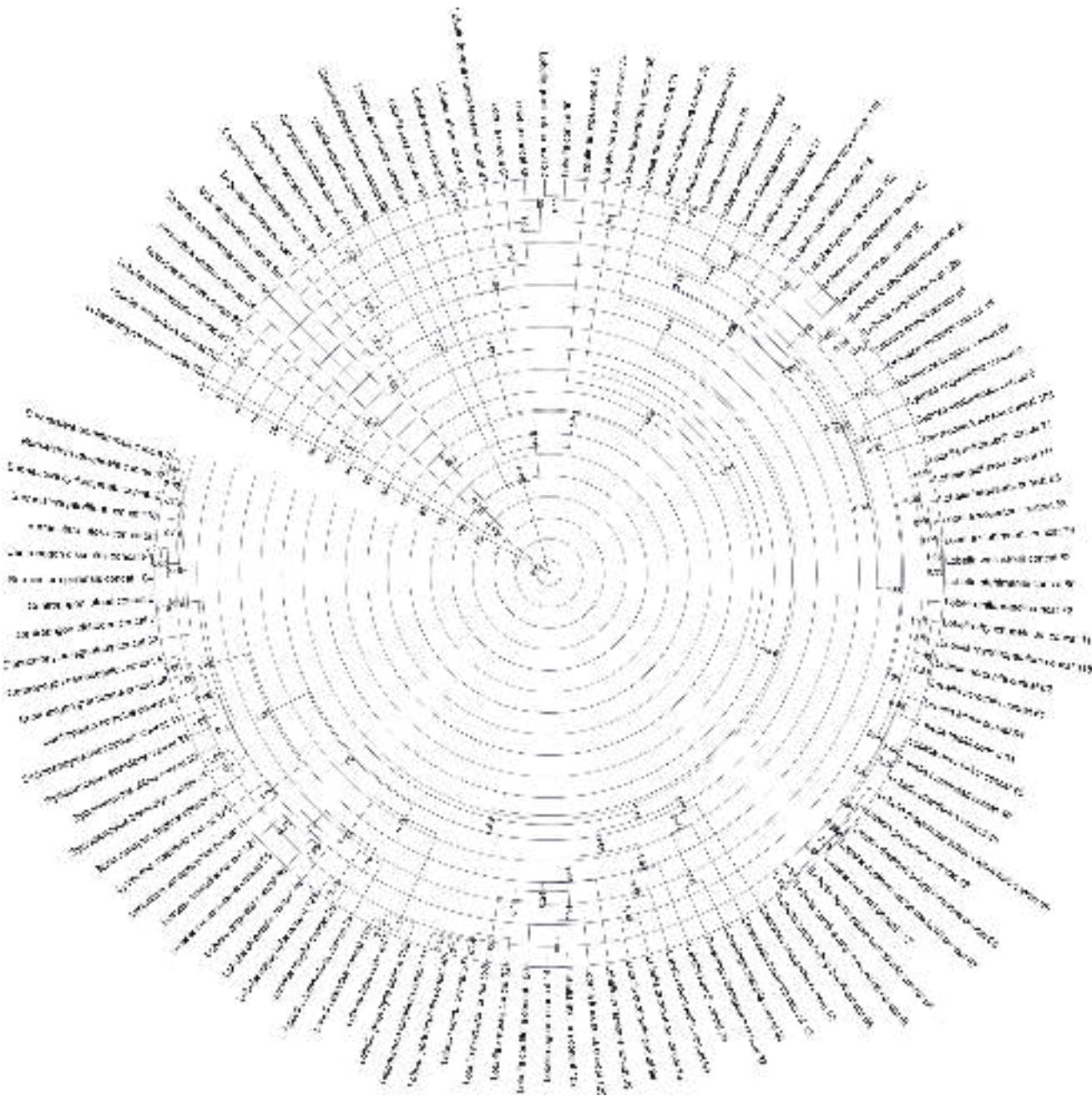
treePL result

Brassicaceae

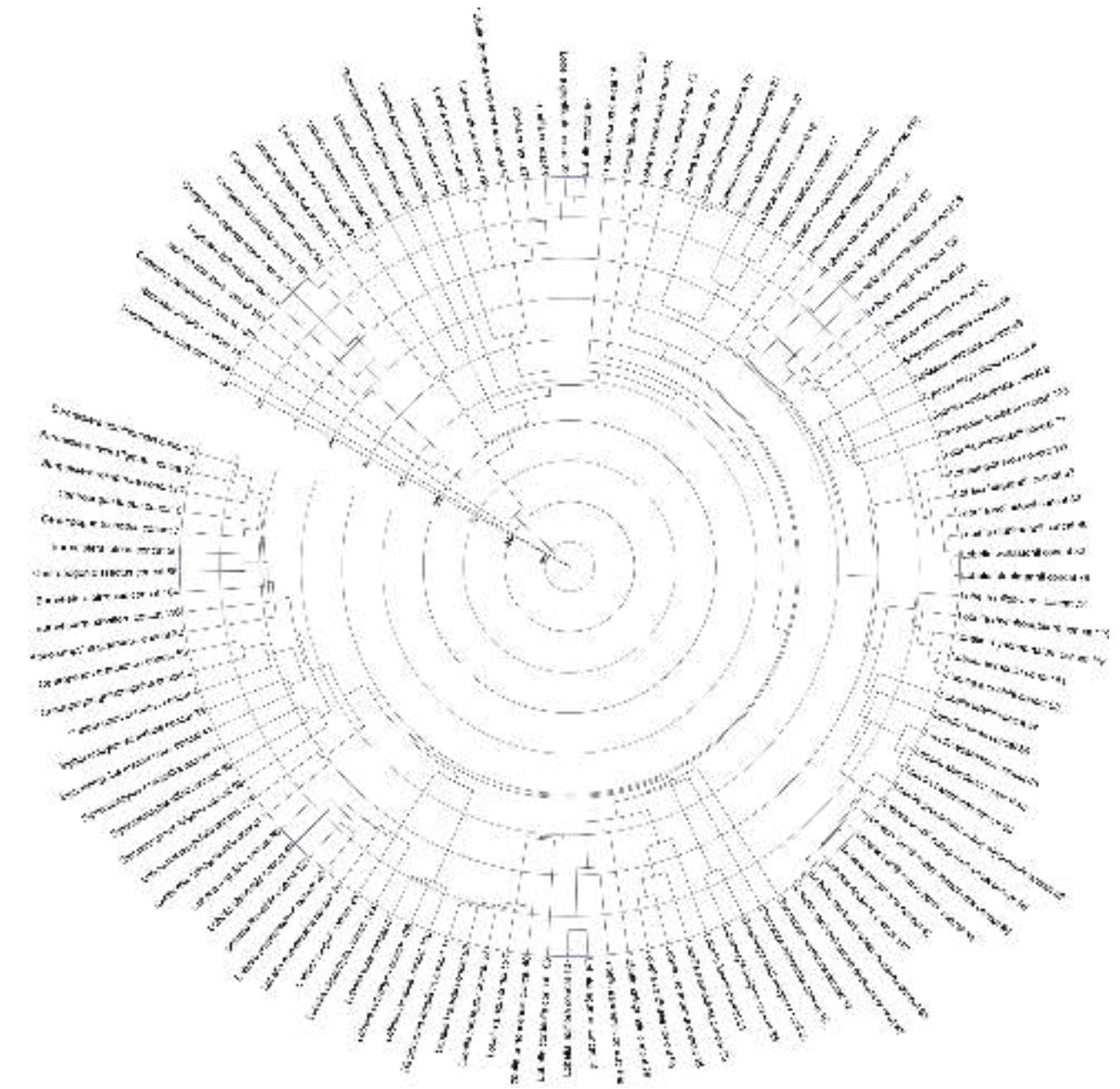


Appendix 2 of The enigmatic tropical alpine flora on the African sky islands is young, disturbed, and unsaturated by M. Kandziora, B. Gehrke, M. Popp, A. Gizaw, C. Brochmann, M.D. Pirie

Campanulaceae

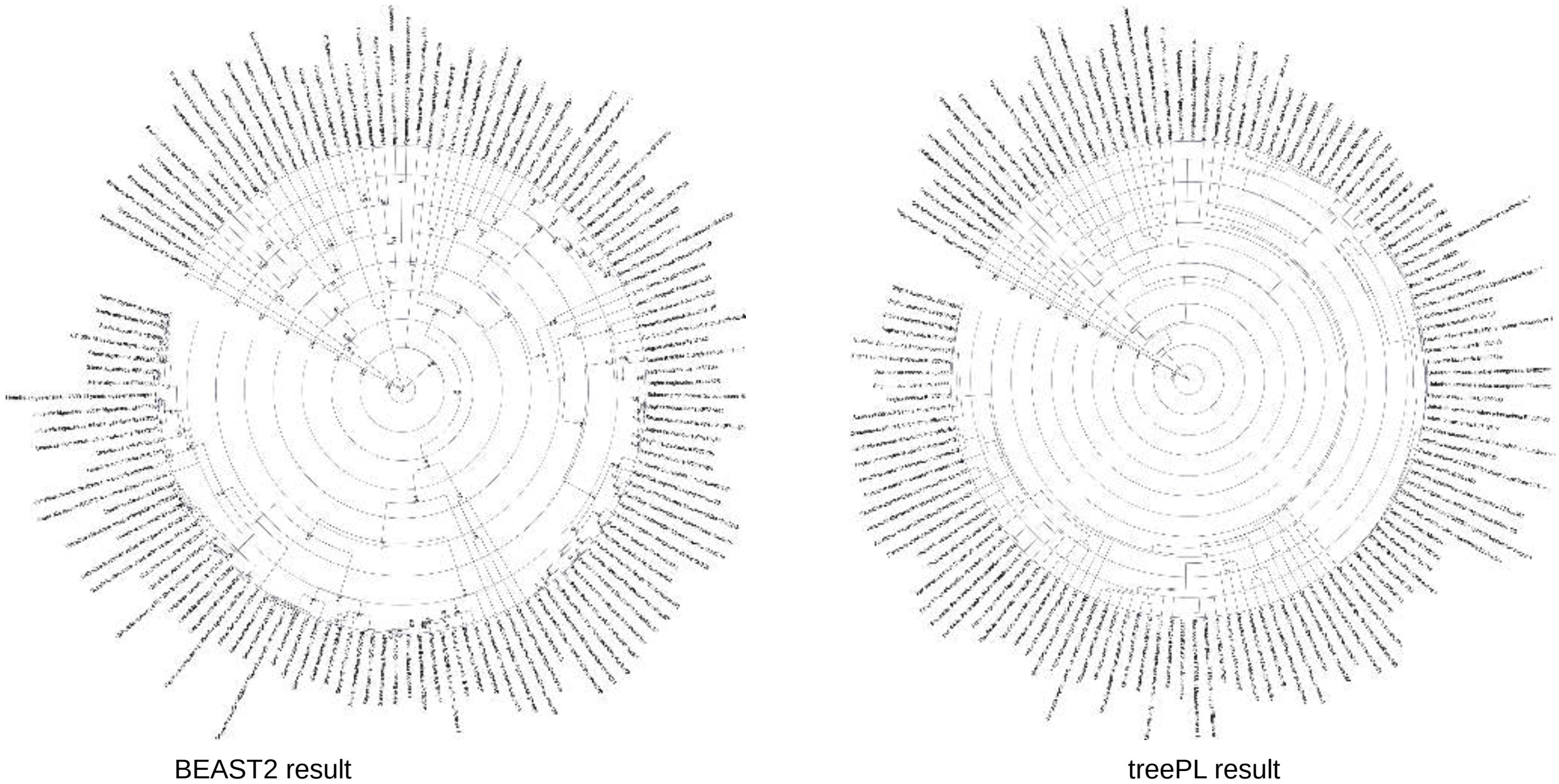


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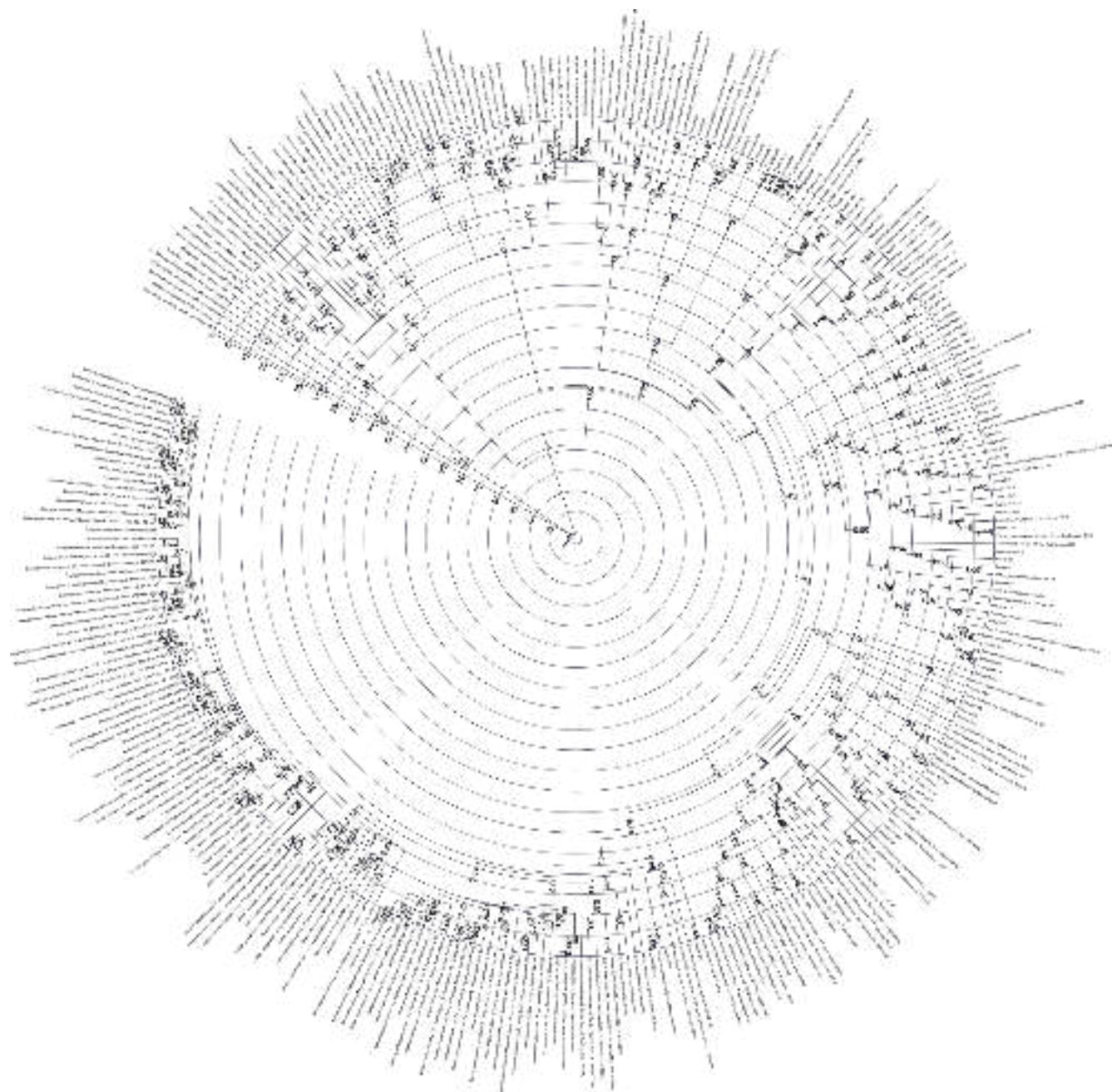


treePL result

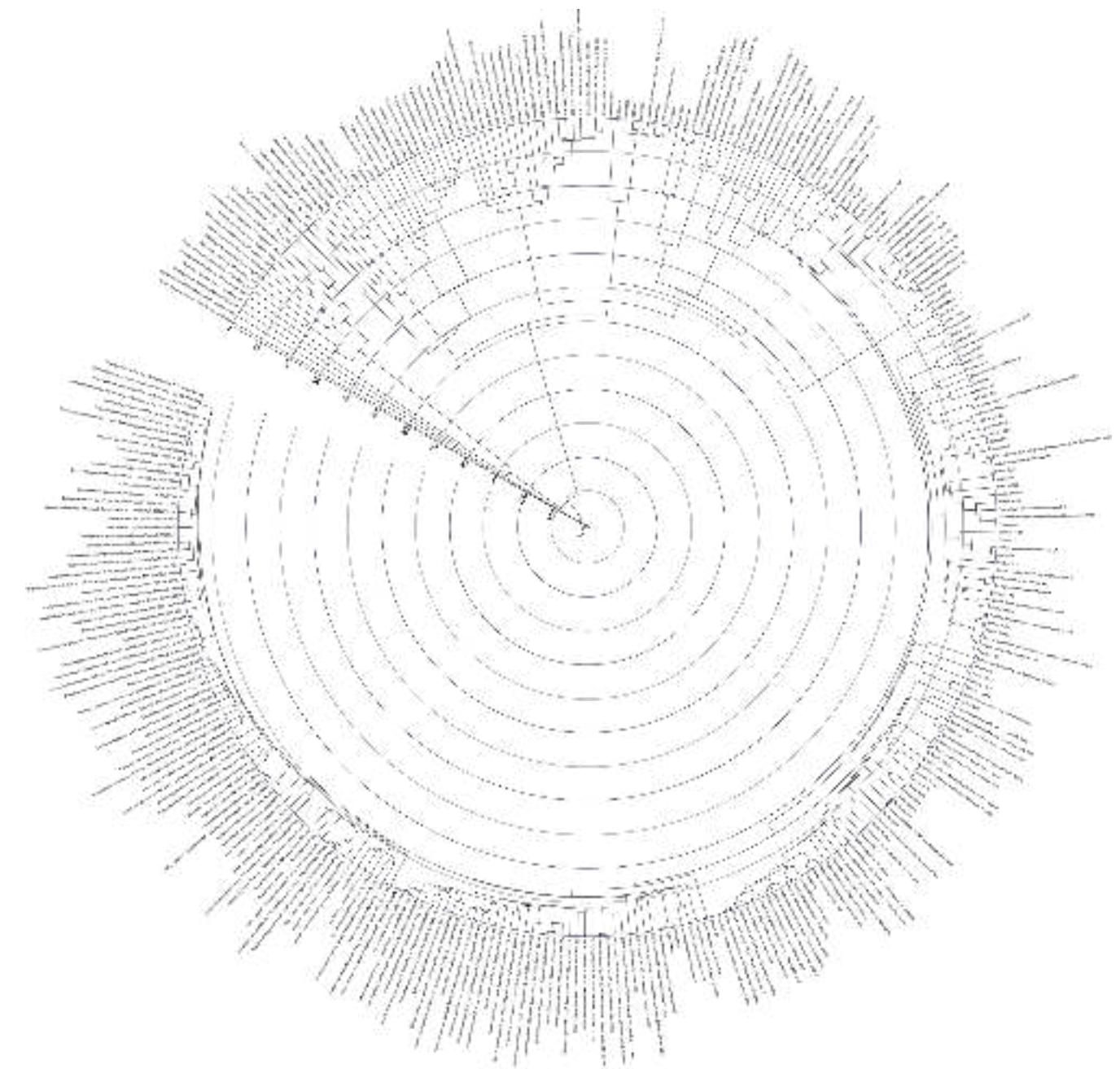
Caryophyllaceae



Crassulaceae



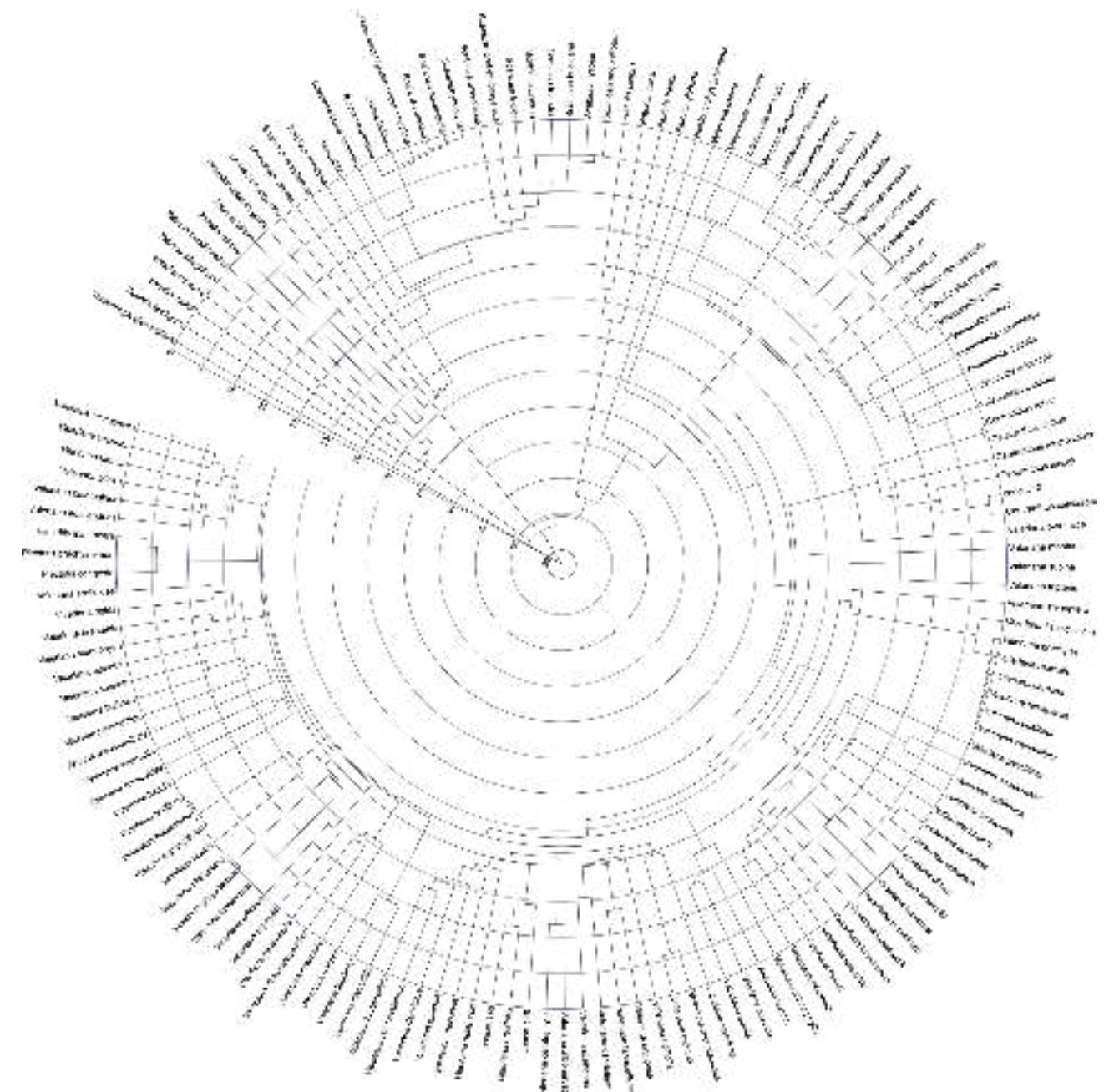
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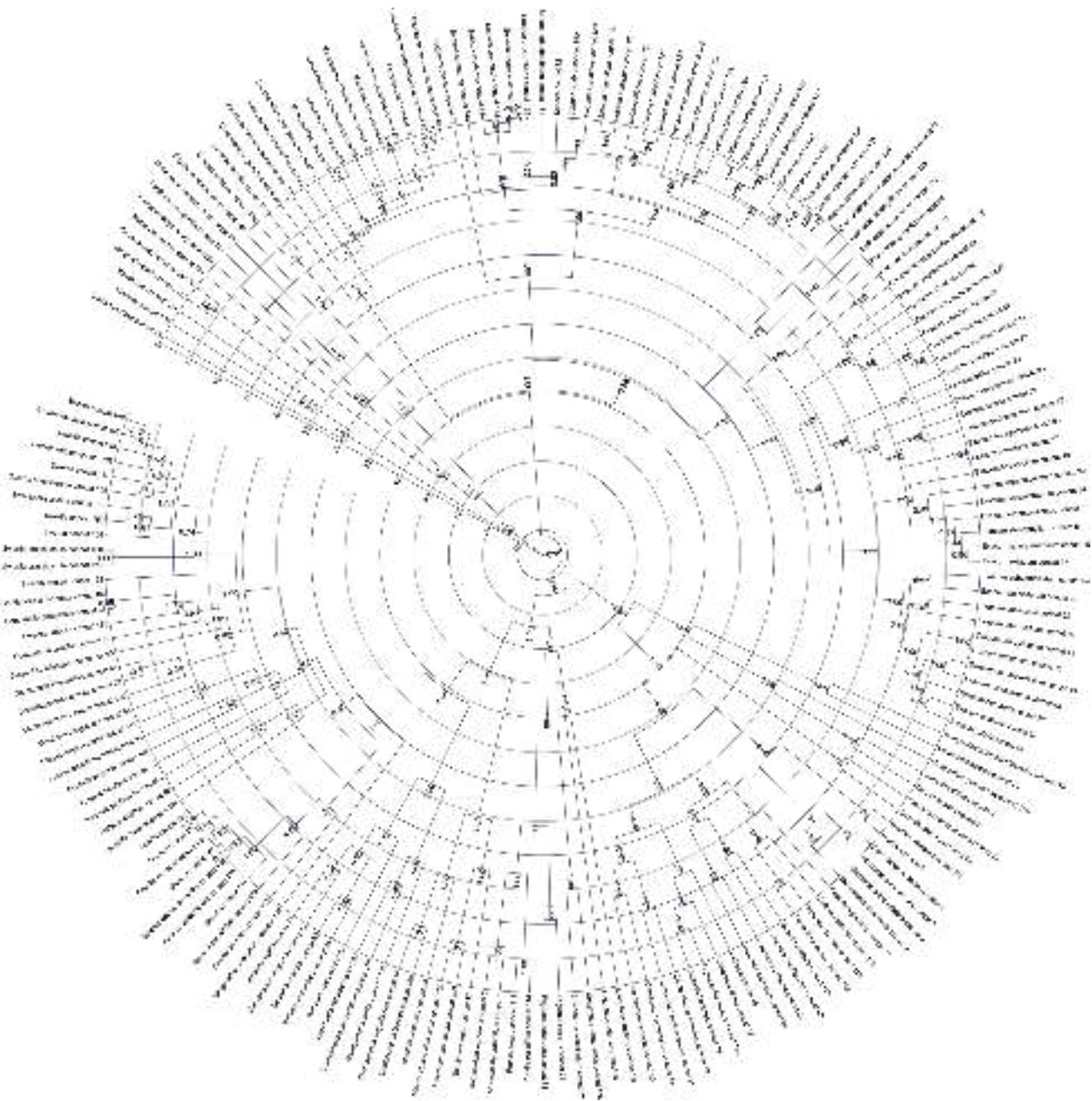
Dipsacales

BEAST2 result

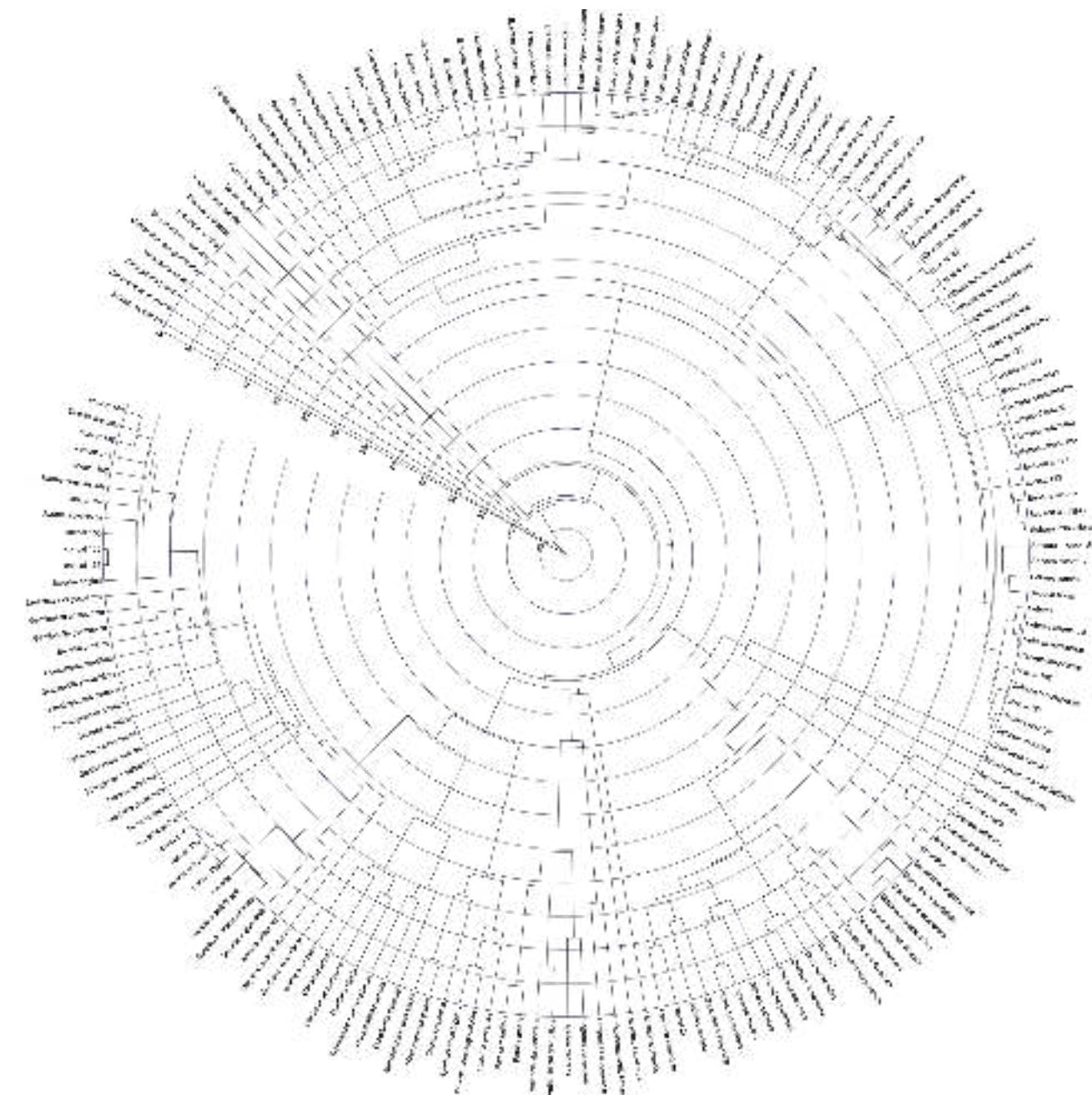


treePL result

Gentinaceae

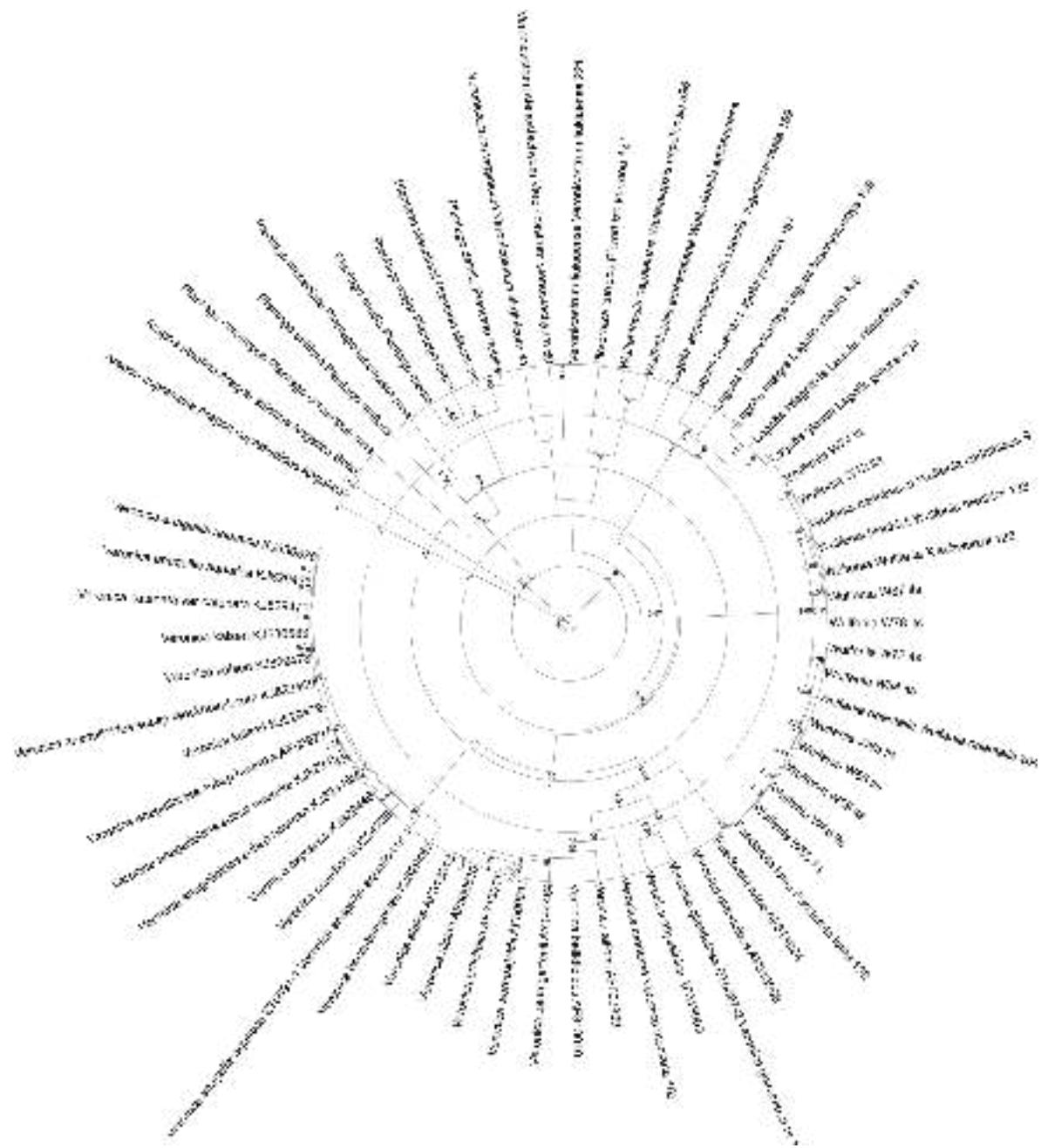


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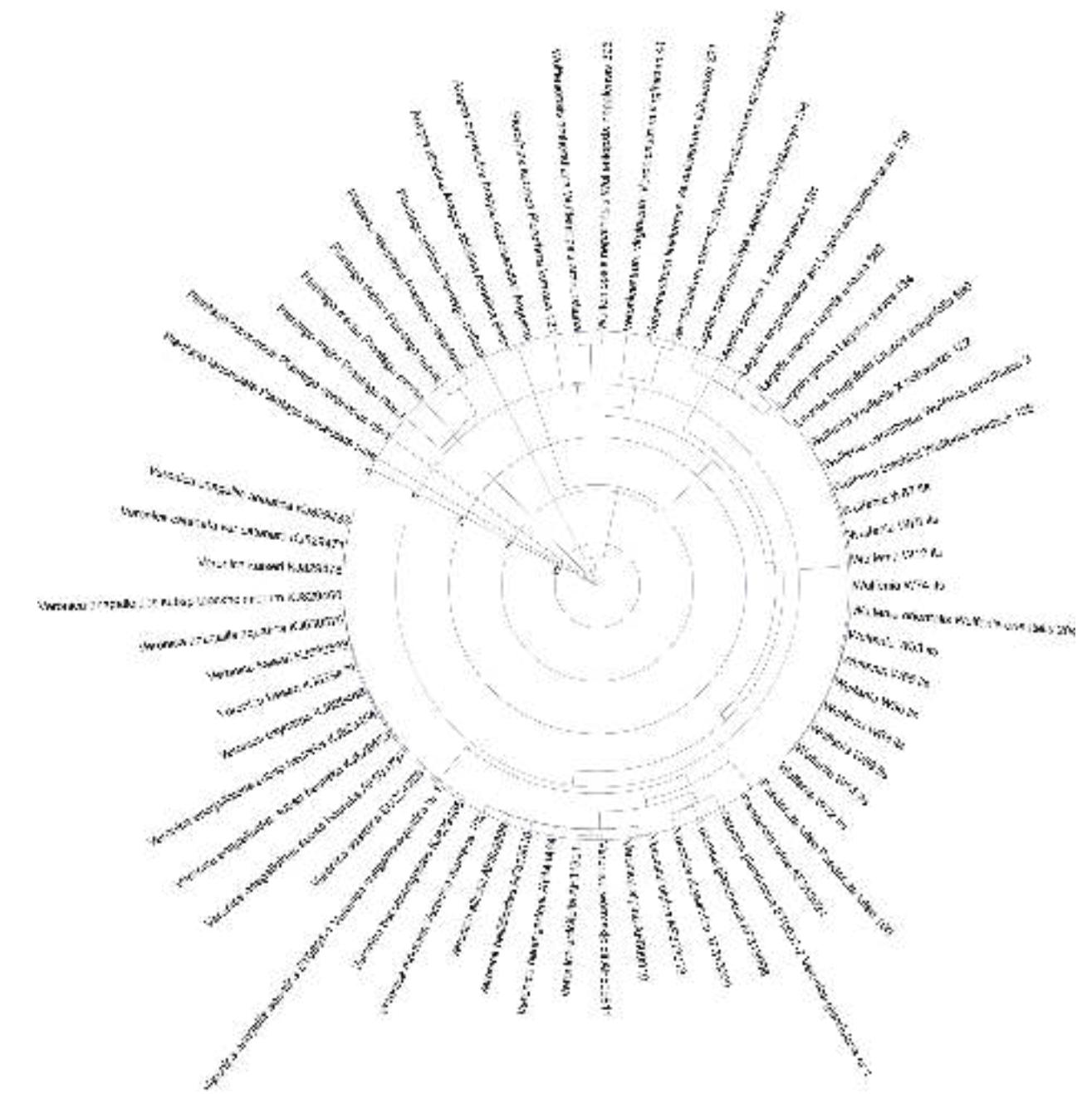


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Plantaginaceae

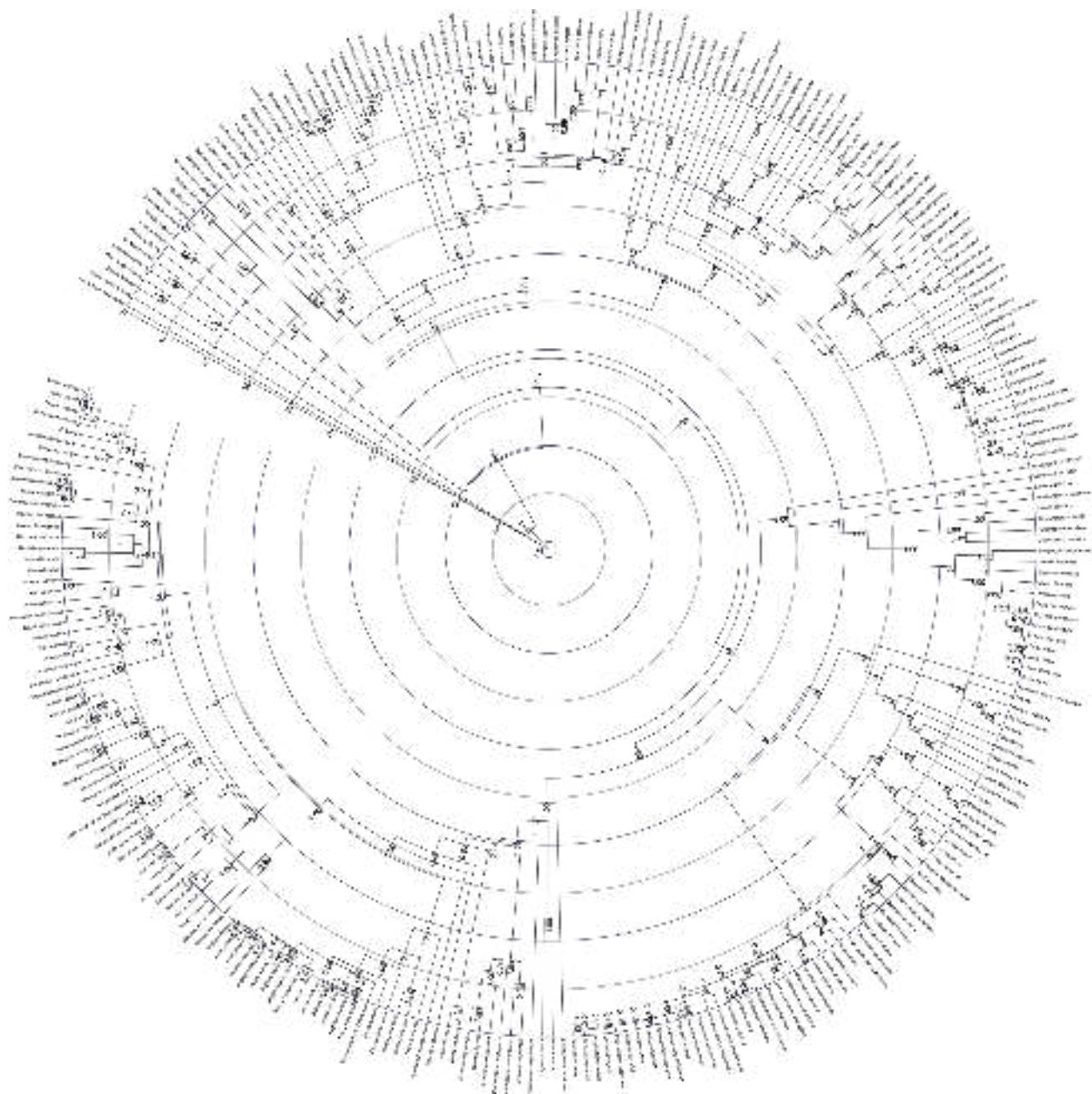


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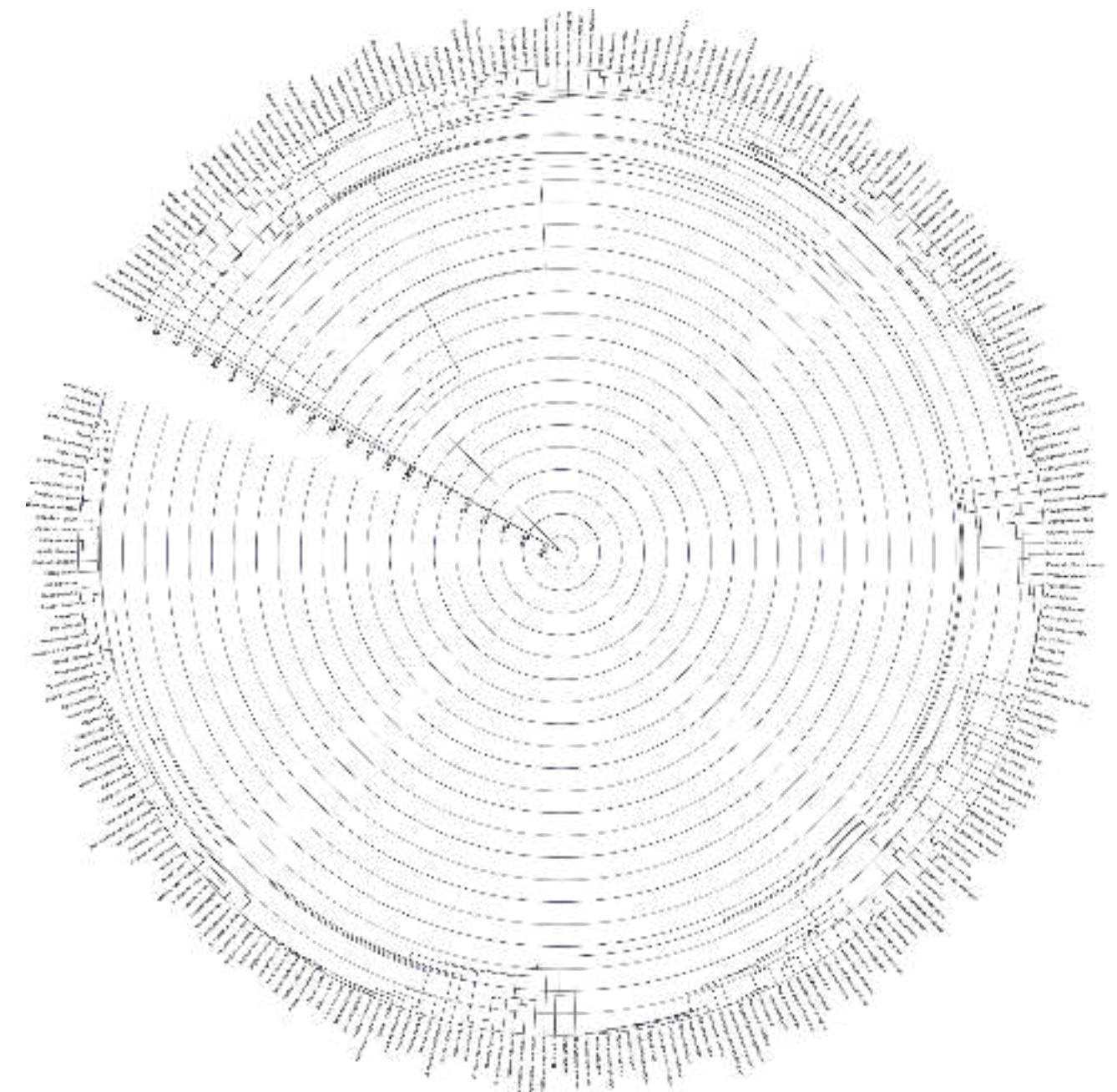


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Poaceae

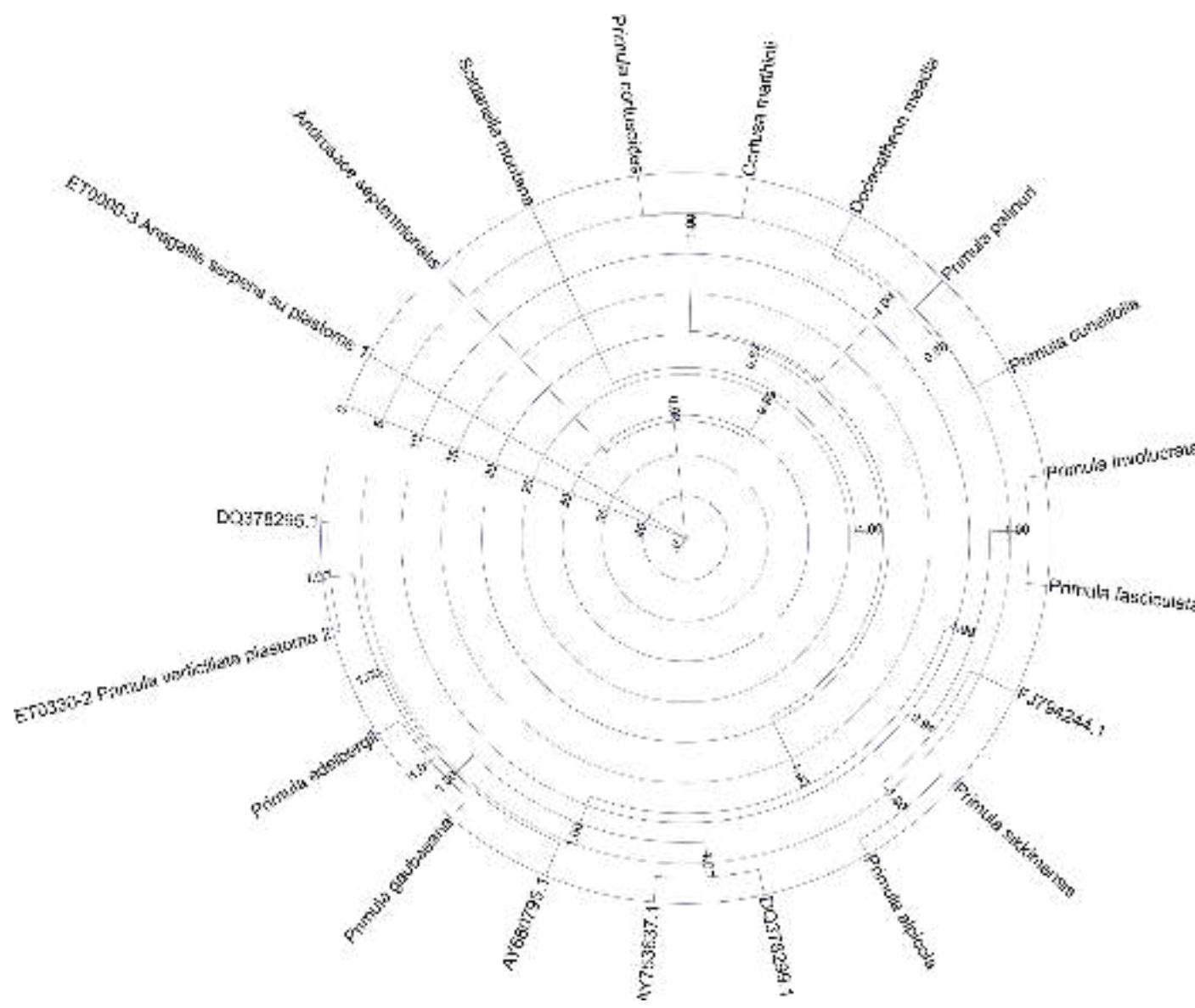


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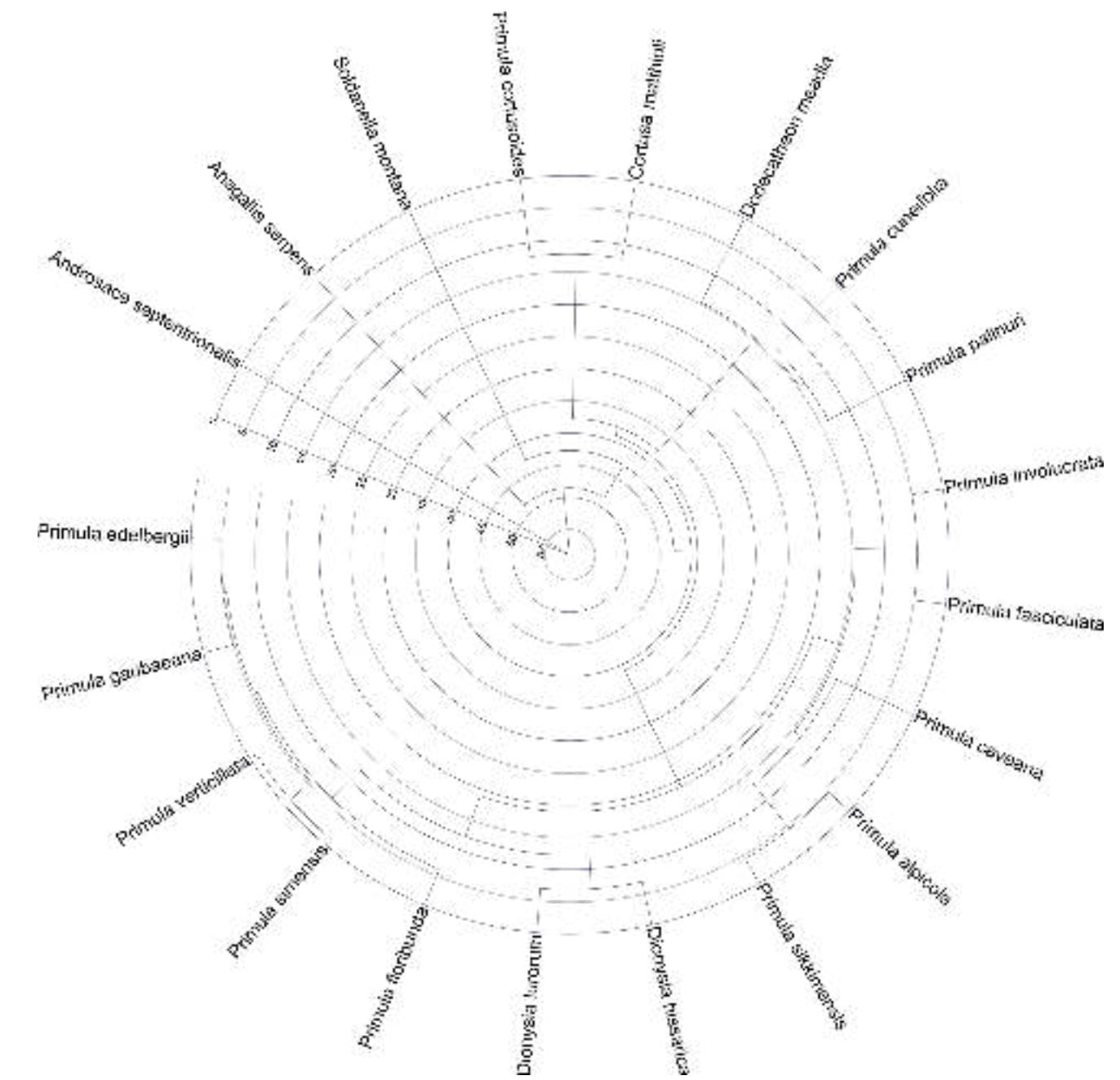


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Primulaceae

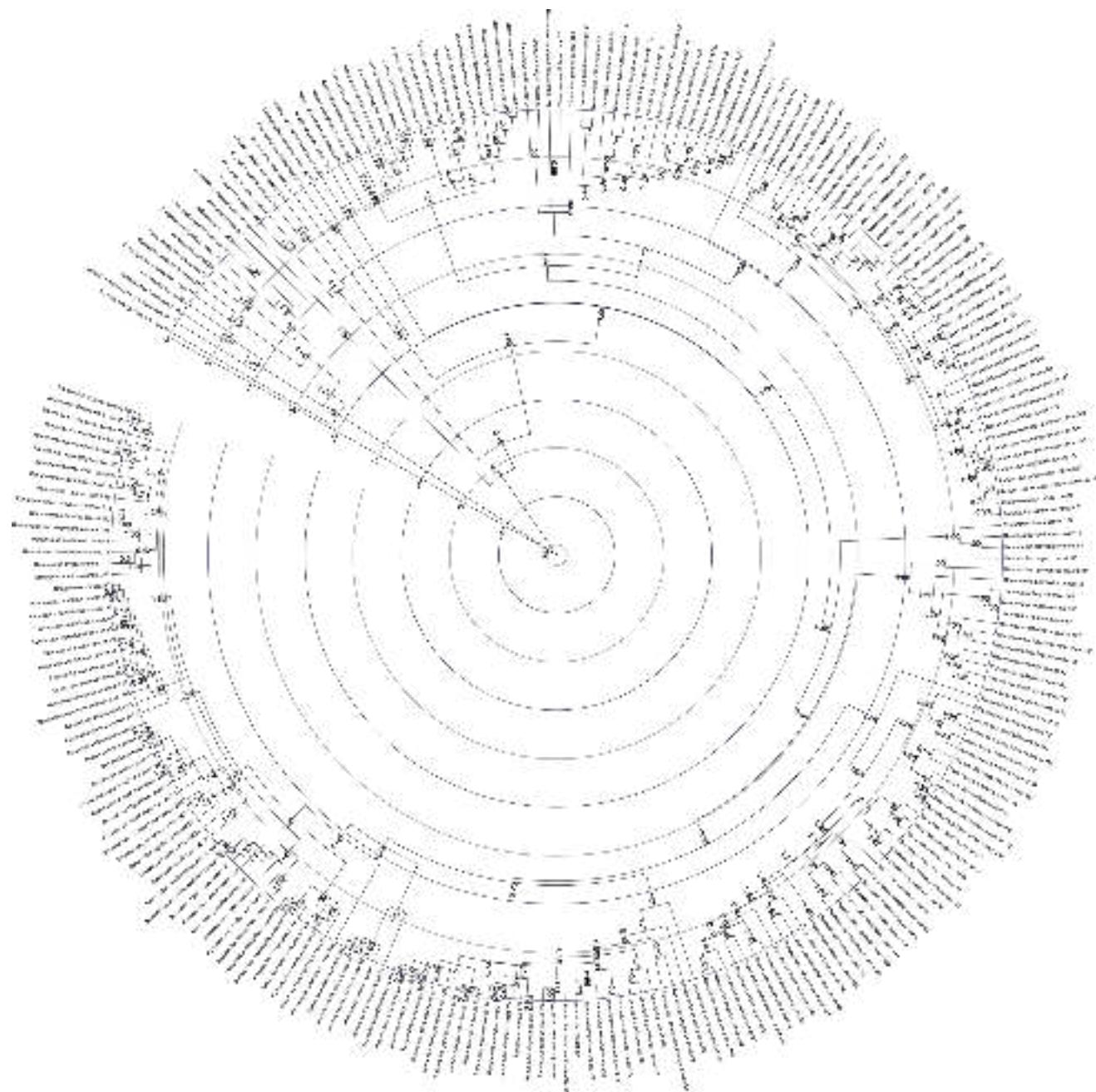


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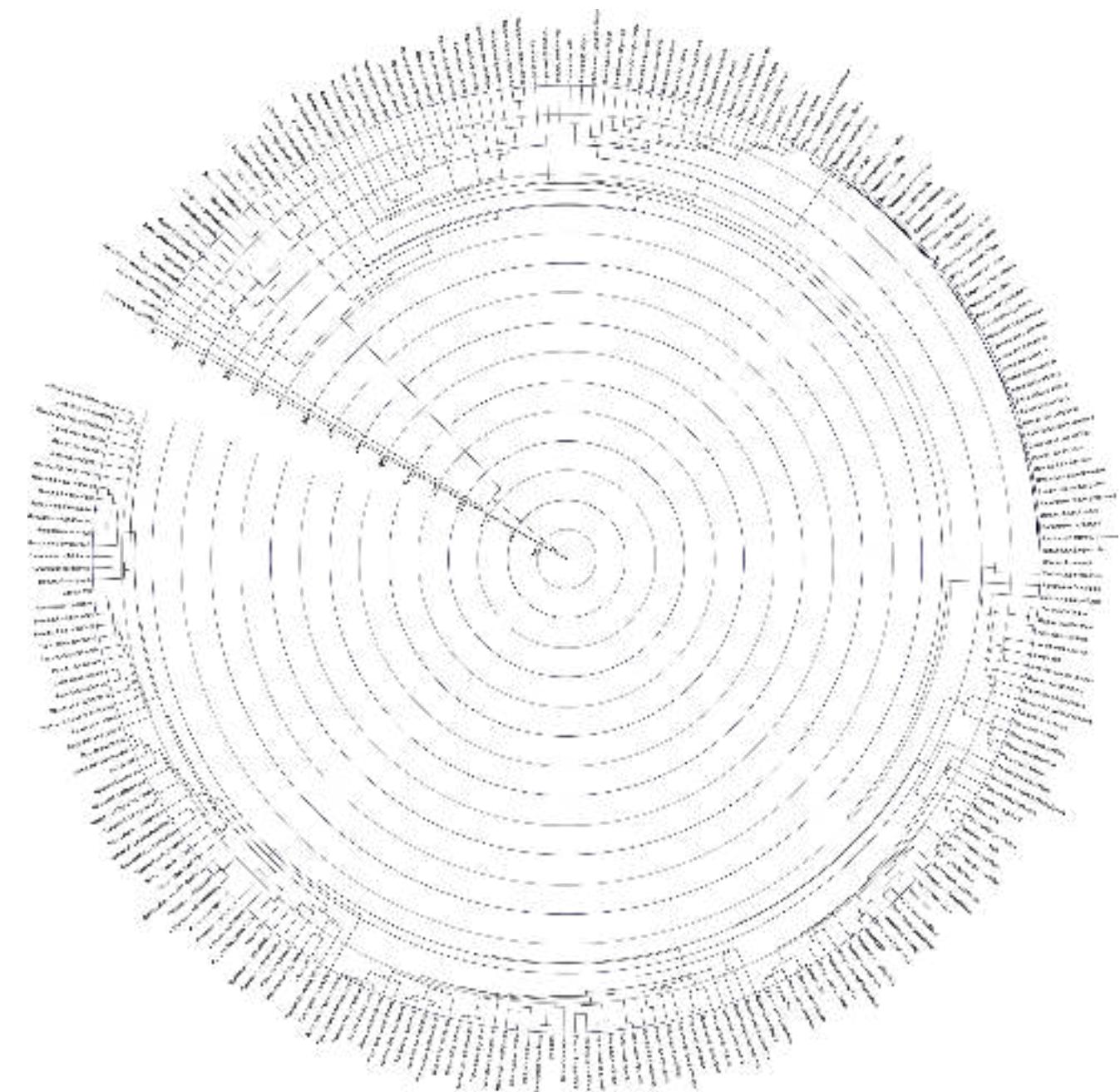


treePL result

Ranunculaceae



BEAST2 result



treePL result