From southern Africa and beyond: historical biogeography of the Ledebouriinae (Scilloideae, Asparagaceae)

Running title: Ledebouriinae (Scilloideae, Asparagaceae) historical biogeography

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Abstract

Aim

Within sub-Saharan Africa, plants inhabiting more seasonal and arid landscapes showcase unique distributional patterns that hint at fascinating evolutionary histories. Research on plants in these habitats have highlighted a complex interplay between climate and orogenic activities. However, despite a steady improvement in our understanding of evolution within these diverse and widespread habitats, many taxa within these regions remain understudied. The Ledebouriinae (Scilloideae, Asparagaceae) are widespread throughout sub-Saharan Africa, and can also be found in Madagascar, the Middle East, India, and Sri Lanka. Unfortunately, this widespread distribution coupled with taxonomic uncertainties have made uncovering the evolutionary history of the Ledebouriinae very difficult. Here, using the most comprehensive sampling of the lineage to date, we investigate the timing and historical biogeography of these bulbous monocots within and outside of Africa.

Location

Sub-Saharan Africa, Madagascar, Asia

Taxon

Ledebouriinae (Scilloideae, Asparagaceae)

Methods

We infer age estimates of major clades and subclades using penalized likelihood as implemented in treePL. Capitalizing on our broad geographic sampling and using BioGeoBEARS, we then

reconstruct ancestral ranges across the phylogeny to investigate the role vicariance and dispersal have played in the biogeographic history of the lineage.

Results

Our results suggest that the Ledebouriinae originated within the past ~30 myr in southeastern sub-Saharan Africa, with the major subclades arising soon thereafter. Vicariance likely led to the current distribution of *Ledebouria* in Asia, not long-distance dispersal as previously hypothesized. The two *Ledebouria* overlap in eastern Africa, but have divergent biogeographical histories, divided into mostly northern and southern clades, yet each has an independent dispersal to Madagascar. A similar north-south split is seen in *Drimiopsis*. The predominantly sub-Saharan African *Ledebouria* clade has a complex biogeographic history, with a rapid radiation estimated ~14 mya, likely driven by drastic climate change and mountain building in southern Africa.

Main conclusions

The expansion of seasonal and arid landscapes in sub-Saharan Africa coupled with mountain building likely spurred the radiation of the Ledebouriinae as well as several subclades. Fragmentation, due to Miocene-driven aridification, of a once widespread distribution led to the current distribution in Asia.

Keywords Africa, *Drimiopsis*, geophytes, *Ledebouria*, *Resnova*, Scilloideae

1 INTRODUCTION

Modern-day Africa is dominated by arid and semi-arid landscapes that contain a diversity of habitats from deserts, woodlands and savannas, to name a few (Bobe, 2006; Linder, 2014). In sub-Saharan Africa, these drier ecosystems collectively form a fairly continuous sickle-shaped corridor that connects the floras of southwestern, northeastern and western Africa, and that skirts around the wet tropics of central and western Africa (Balinsky, 1962; Bellstedt et al., 2012; Jürgens, 1997) (Figure 1). The recent onset of aridity in Africa (i.e., approximately from the Mid-Eocene onwards) is hypothesized to have been caused by the synergistic activities of rapid global cooling, tectonic events (e.g., Eastern African rift) and oceanic upwelling (i.e., Benguela current) that altered precipitation patterns across the continent (Bobe, 2006; Couvreur et al., 2021; Hagen et al., 2021; Linder, 2017; Senut et al., 2009; Sepulchre et al., 2006). These changes had immense impacts on the evolutionary trajectories of countless lineages on the African continent. For example, in the early Eocene, equatorial Africa was largely covered by tropical forests, but aridification repeatedly fragmented and reduced these habitats over time (Couvreur, 2015; Couvreur et al., 2008; Hagen et al., 2021). Evidence from plant fossils and phylogenetic studies suggest that lineages with past widespread distributions across the continent, as well as between eastern Africa and southern Asia, are now disjunct as a result of recent aridification (Ali et al., 2013; Jacobs et al., 1999; Pokorny et al., 2015; Sanmartín et al., 2010; Zhou et al., 2011). Conversely, dry and more seasonal climates have been linked to the diversification and dispersal of lineages both in and out of Africa. Today, a diversity of taxa can be found in abundance within the more arid regions of Africa as well as surrounding areas with similar climates (e.g., Arabian Peninsula) (Bruyns et al., 2014; Coe & Skinner, 1993; Jürgens, 1997; Lorenzen et al., 2012; Nylinder et al., 2016). Therefore, it is imperative we gain a broad understanding of the

historical evolution of diverse lineages across different landscapes, habitats, and biomes in order to continually refine our understanding of the past and future changes of biodiversity within Africa.

Geophytes, herbaceous plants with renewal buds located belowground on structures such as bulbs, corms, and stem tubers, are ubiquitous components of seasonal or disturbance-prone habitats, and are phylogenetically diverse (Howard et al., 2019; Pausas et al., 2018; Tribble et al., 2021). Within Africa, geophytes are major components of the Greater Cape Floristic Region and the Mediterranean Basin (Buerki et al., 2012; Procheş et al., 2006). Although geophytes are predominant elements of these two areas, the geophytic habit is widespread throughout sub-Saharan Africa, particularly within seasonal or disturbance-prone (e.g., fire) habitats (Esler et al., 1999; Kornas, 1985). Studies have reported that many African geophytes' origins coincide with the onset of increased seasonality and/or aridity within the continent (i.e., since the Eocene), with the majority of diversity evolving in response to the relatively more drastic climatic changes since the Oligocene/Miocene (Ali et al., 2012, 2013; Buerki et al., 2012; del Hoyo et al., 2009; Procheş et al., 2006). At a broad scale, therefore, geophytic lineages make excellent candidates for understanding recent evolutionary and biogeographic dynamics within seasonal and arid climates.

Scilloideae (Asparagaceae) are a bulbous geophyte lineage widespread both within and outside of Africa (Speta, 1998a). This monocotyledonous clade consist of 1,000+ taxa found throughout seasonal climates in Africa as well as Madagascar, Europe, the Middle East, and Asia, with a single lineage found in South America (i.e., *Oziroe* Raf.; (Giranje & Nandikar, 2016; Speta, 1998b). Although only a handful of studies have investigated the historical biogeography of the Scilloideae and its subclades, so far, all have pointed to sub-Saharan Africa

as the origin for the majority of the group (excluding *Oziroe* in South America), followed by a complex history within and outside of the continent (Ali et al., 2012, 2013; Buerki et al., 2012; Pfosser, 2012). However, most inferences have been made using a broad view of each groups' distribution (i.e., in biogeographical studies sub-Saharan Africa has been subdivided into one to three areas) (Ali et al., 2012, 2013; Buerki et al., 2012; Pfosser, 2012). Focusing on widespread groups within the Scilloideae using a more detailed approach to the regionalization of Africa as well as a greater taxon sampling, may provide refined insights into the biogeographical processes that have impacted the dispersal of plants across and out of Africa.

The Ledebouriinae are an ideal group to study because they are widespread within sub-Saharan Africa, with a handful of taxa found in Madagascar, Socotra, Yemen, India, and Sri Lanka (Giranje & Nandikar, 2016; Venter, 1993) (Figure 1). This distribution is unique within Scilloideae since many sympatric lineages with Ledebouriinae are also found in northern Africa (Pfosser, 2012; Speta, 1998a), but the Ledebouriinae are absent from the Scilloideae-rich Mediterranean Basin (Venter, 2008) (Figure 1). In sub-Saharan Africa, the Ledebouriinae are predominantly found within more seasonal landscapes, with highest diversity in the Limpopo, Mpumalanga, and KwaZulu-Natal regions of South Africa (Venter, 1993), yet some occurrences are documented from more wet, tropical regions (Figure 1). Much diversity within the Ledebouriinae, however, remains undescribed to science (Howard, 2014; Howard et al., 2022). Additionally, an expanded phylogenomic analysis of the group suggests that a complex biogeographical history awaits to be thoroughly examined (Howard et al., 2022). Previous dating analyses have estimated the origin of the Ledebouriinae sometime within the last 25 myr in sub-Saharan Africa (Ali et al., 2012; Buerki et al., 2012). However, this clade was not the focus of study and therefore, taxon sampling was low. Additionally, results were unable to provide fine-

scale biogeographical patterns since sub-Saharan Africa was considered and analyzed as one large area. A more detailed categorization of the various regions in the continent would provide a better understanding of the biogeographical history of the group. For example, Ali et al. (2012) found that Malagasy and Indian *Ledebouria* were sister to one another, which led the authors to invoke long-distance dispersals from Madagascar to India. However, as stated by the authors, Ledebouriinae samples from eastern, western, and northern sub-Saharan Africa were absent, limiting confidence in these conclusions (Ali et al., 2012). Furthermore, the phylogenetic reconstructions of Pfosser et al. (2012) suggested two independent dispersal events to Madagascar, but low phylogenetic resolution diminished confidence in this hypothesis. A recent phylogenomic analysis of the Ledebouriinae provided the framework to suggest (without testing) multiple dispersals to Madagascar, and a potential migration out of Africa via the Arabian Peninsula into India (Howard et al., 2022).

The widespread distribution of the Ledebouriinae provides us with the opportunity to refine our understanding of evolution and biogeography within Africa, potentially during a time of extensive climatic, geologic, and habitat change on the continent. Here, we investigate the timing and historical dispersal of the Ledebouriinae both within and out of Africa. We ask: 1) Which region within Africa acted as the putative origin of the group, and 2) What roles have dispersal and vicariance played in the evolutionary history of the Ledebouriinae, if any? Our specific focus on these questions and the use of an increased sampling of the Ledebouriinae allow for a more thorough investigation into the evolutionary history of the group and improve our knowledge on patterns and processes within Africa.

2 METHODS

2.1 Phylogenetic analysis

Ledebouriinae samples were obtained from the field, private collections, and herbarium vouchers (Howard et al., 2022). DNA extractions were performed using a modified CTAB protocol, followed by high throughput sequencing on an Illumina HiSeq using the Angiosperms353 universal probe set (Johnson et al., 2019). Raw reads were cleaned using SECAPR (Andermann et al., 2018), sequences were pulled using hybpiper (Johnson et al., 2016) and aligned using MAFFT v.7 (Katoh & Standley, 2013). See Howard et al. (2022) for more details on Ledebouriinae sequence acquisition and analysis as well as data used.

We incorporated outgroup taxa from the Plant and Fungal Tree of Life project (Baker et al., 2022) and the 1KP dataset (Matasci et al., 2014). Exons were only available for the outgroup taxa included to estimate divergence times using fossils and secondary calibration points. Phylogenetic reconstruction including the Ledebouriinae plus outgroups was performed on a concatenated, partitioned supermatrix of exons with 10% gappy/ambiguous sites removed using phyx (Brown et al., 2017). This matrix was analyzed using IQ-Tree v.2-rc1 (Nguyen et al., 2015) with 1000 ultrafast bootstraps (Nguyen et al., 2015) with 1000 ultrafast bootstraps, a best-fit partitioning scheme using the greedy algorithm of PartitionFinder (Lanfear et al., 2012), and a relaxed clustering percentage of 10 (Lanfear et al., 2014), followed by phylogenetic reconstruction (-m TESTMERGE). However, the exon-only dataset returned low support for many nodes within the Ledebouriinae. Therefore, we reran the IQ-Tree analysis with a topological constraint tree (-g) that was previously built from a supercontig (i.e., exons + introns) dataset of the Ledebouriinae (Howard et al., 2022).

2.2 Time calibration

We incorporated eight outgroup fossil calibration points, each with a minimum age specified in Iles et al. (2015) (Table S1). A secondary calibration point at the crown node of monocots was inferred between 131–135 mya based on previous analyses (Givnish et al., 2018; Magallón et al., 2015).

Given the size of the dataset, we used penalized likelihood as implemented in treePL (Smith & O'Meara, 2012) for time calibration. To incorporate uncertainty around age estimation, we took a multi-tiered approach (see https://github.com/sunray1/treepl) similar to previous studies estimating divergence times using large phylogenetic datasets (Emberts et al., 2020; Li et al., 2019; Magallón et al., 2015). We generated 100 bootstrap replicates of our original exon supermatrix alignment using RAxML v.8.2.0 (f -j option) (Stamatakis, 2014). A maximum likelihood tree for each replicate with a corresponding partition file was then reconstructed using a topological constraint (i.e., the phylogeny from the IQ-Tree analysis) to ensure consistent calibration point placement, and a GTRGAMMA model of evolution. The resulting 100 "best trees" were rooted on *Acorus gramineus* using phyx (Brown et al., 2017). In Step 1, each replicate tree had a priming step completed with a random seed number and the thorough command invoked. Step 2 was performed three independent times to assess convergence on the best smoothing parameter for each individual tree. Step 2 also included the individual outputs from each tree's previous priming step (e.g., optad, moredetail, etc.) as well as the cross-validation (CV) steps, which were set to cvstart=10000, cvstop= .00000000000001, cvmultstep=0.09. Lastly, Step 3 summarized each individual tree's CV output to determine and scale each tree using the appropriate smoothing parameter. The 100 ultrametric phylogenies were

summarized using TreeAnnotator v.1.10.4 (Bouckaert et al., 2014) to obtain a maximum clade credibility tree with 95% confidence intervals around each node and median node heights.

2.3 Biogeographical analysis

The biogeographical regionalization of Linder et al. (2012) was used for categorizing the location of each sub-Saharan African Ledebouriinae accession. The exact distribution of populations/taxa represented by many field-collected individuals (i.e., collections made by C.C. Howard) remains to be fully assessed since they are undescribed species and/or are only known from one locality. Additionally, many described species are currently known as occurring in small geographic ranges or even single mountain tops (Lebatha, 2004; Venter, 2008). Consequently, most samples were assigned only to known areas of occurrence. Accessions found outside of Africa were coded according to their respective areas of occurrence: Madagascar, Yemen, Socotra, or Sri Lanka). Another reason we coded each taxon using this approach is exemplified by *Ledebouria revoluta*, reported from the Cape Region of South Africa to Sri Lanka (Mwafongo et al., 2017; Venter, 1993). This widespread taxon likely represents a species complex with a wide range of morphological variation (Brita Stedje, 1998), and therefore, often this name is associated with individuals whose species identification is difficult (Mwafongo et al., 2017). Consequently, and not surprisingly, *L. revoluta* is an ambiguous entity as evidenced by its polyphyly within the Ledebouriinae (Howard et al., 2022). In total, 14 geographic regions were used to categorize all terminals. To estimate ancestral ranges, we compared the maximum likelihood implementations of three biogeographical models, DEC, DIVA-like, and BAYAREA-like as implemented in BioGeoBEARS (Matzke, 2013a, 2013b, 2014) in R (R Core Team, 2016). We used log-likelihood and AICc values to determine the best model between the three. Given the debate surrounding the use of models with the +j parameter (Matzke, 2022; Ree &

Sanmartín, 2018), we decided to not include them and, additionally, their implementation tends to infer a high number of jump dispersals, which are unlikely biological scenarios for the majority of the Ledebouriinae (see Discussion).

2.3.1 Biogeographical uncertainty

Within a subclade of *Ledebouria* Clade A, phylogenetic relationships are poorly supported (Figure 2, node a) (Howard et al., 2022). However, given the potential rapid radiation along the backbone of this group, we wanted to explore the biogeography of this clade since it may contain an interesting history. To compensate for the uncertainty, we incorporated topological and branch depth variability into our biogeographical analysis by using a random sampling of trees from the posterior of a Bayesian analysis for the subclade of interest (Ceccarelli et al., 2019; Magalhaes et al., 2021). To begin, we reduced our total alignment to the most clock-like genes using SortaDate (Smith et al., 2018). This was done due to failure to reach convergence when using the entire alignment (data not shown). We kept genes that were at least 10% concordant (bipartition >0.1), had a tree length greater than 7.24, and had root-to-tip variation of less than 0.009. Cutoff values for the latter two were determined using the median values for all gene trees, as previously reported (Pillon et al., 2021). Of the remaining alignments, we included only taxa within the subclade of interest (Figure 2b) and removed duplicate Ledebouriinae outgroup taxa as well as those putatively duplicate within the Ledebouriinae ingroup (indicated by * in Figure 2). Each gene was then aligned using MAFFT (Katoh & Standley, 2013) invoking the --auto option. Sites with 5% gaps/ambiguity were removed using phyx (Brown et al., 2017). Due to failure to reach convergence using a partitioned, concatenated alignment in BEAST v1.10.4 (Drummond & Rambaut, 2007), we instead input an unpartitioned, concatenated alignment using a GTR+GAMMA model of

substitution, a speciation birth-death prior, and a lognormal distribution with an offset 17.1, mean 1.0, stdev 1.0 (95% range 17.24–19.88) on the root node of the subclade. This initial value was chosen based on the treePL divergence time estimates for this node. We ran an MCMC chain of 300k generations sampling every 10k generations. Convergence was assessed using Tracer v1.7.1 (Rambaut et al., 2018) to ensure all ESS values were above 200. We removed 50% of trees as burnin and used the remaining trees in the analysis. We randomly sampled 100 trees after burnin, and performed a DIVA-like analysis (i.e., the best model) on each tree followed by stochastic mapping using BioGeoBEARS (Matzke, 2013a). Trees were summarized to obtain an average number of biogeographical events within and between each region included in the analysis. Scripts for running the analysis can be found on github (https://github.com/ivanlfm/BGB_BSM_multiple_trees). The rationale for incorporating phylogenetic uncertainty was developed by Ceccarelli et al. (2019) and Magalhaes et al. (2021).

3 RESULTS

3.1 Phylogenetic relationships and age estimates

We recovered a polyphyletic *Ledebouria*, and a monophyletic *Drimiopsis* and *Resnova*, which is congruent with a recent study (Howard et al., 2022). However, shallow-level relationships differed compared to Howard et al. (2022), particularly within the *Ledebouria* clades, and especially within *Ledebouria* Clade A. This is due to using a topological constraint (-g), which searches tree parameter space, over using a fixed topology (-te) which does not perform a tree search (see http://www.iqtree.org/doc/Command-Reference). Given differences in the inputs between Howard et al. (2022) and our exons-only datasets, we preferred a topological constraint allowing for a tree search over forcing a fixed topology.

Within a penalized likelihood framework, the Scilloideae originated approximately 52.0 my (95% HPD 51.2 – 54.5) (Figure 2; Table 1). We recovered a median crown age estimate for the Ledebouriinae of 28.0 myr (95% HPD 26.1–28.3), with age estimates of the four major Ledebouriinae clades soon thereafter. The split between *Drimiopsis* and *Ledebouria* Clade B was estimated at 27.1 mya (95% HPD 25.2–27.5). The crown of *Drimiopsis* was dated to 22.1 mya (95% HPD 20.5–23.5), and *Ledebouria* Clade B was dated at 26.1 mya (95% HPD 24.0–26.5). The split between *Resnova* and *Ledebouria* Clade A occurred at 26.3 mya (95% HPD 23.7–25.2). The *Resnova* crown was dated at 17.9 mya (95% HPD 16.6–18.5), and *Ledebouria* Clade A was estimated at 25.0 mya (95% HPD 23.0–25.4) (Table 1).

3.2 Biogeography

The DIVA-like model produced the most likely ancestral range estimates among the models (LnL -220.54) (Table 2). The regions Natal+Zambezian were reconstructed as the ancestral range for the Ledebouriinae (p = .53) and *Drimopsis* (p = .84), while Zambezian was reconstructed for *Drimiopsis* + *Ledebouria* Clade B (p = .95) and *Ledebouria* Clade B (p = .98). Within *Ledebouria* Clade B, the model favors a widespread distribution of Yemen+Zambezian (p = .55) followed by subsequent reconstructions containing various combinations of Zambezian and other regions, or Zambezian alone (Figures 3 and S2). Within *Ledebouria* Clade B we also recover one of the two dispersals of the Ledebouriinae to Madagascar (Figure 3).

The Kalahari+Natal regions were recovered as the ancestral range for *Resnova* + *Ledebouria* Clade A (p = .42), whereas the Natal region was recovered for *Resnova* (p = .99), and Kalahari for *Ledebouria* Clade A (p = .51) (Figures 3 and S2). Within *Ledebouria* Clade A, we find an additional dispersal to Madagascar (Figure 3). Additionally, in summarizing the 10 biogeographic stochastic reconstructions (see section 2.3.1), we recovered a steady rise in the

number of lineages in the Zambezian and Kalahari regions from ~15 myr onwards in a subclade of *Ledebouria* Clade A (Figure 3b). Additionally, despite some differences in topology between these results and those of Howard et al. (2022), both studies overall share clades that largely reflect geography. We recover two subclades of mostly Namibian taxa, a subclade containing mostly South African taxa, and a subclade containing a mixture of Zambian, Tanzanian, and Zimbabwean taxa (Figures 2 and 3).

4 DISCUSSION

4.1 Broad biogeographical patterns

Here, we present the best and most comprehensive sampling of the Ledebouriinae to date (representing ~30% of described Ledebouriinae taxa (POWO, 2019) plus numerous undescribed taxa) which has provided greater insights into the historical biogeography of this widespread, bulbous lineage than previously uncovered. Our results suggest a rapid radiation along the backbone of the Ledebouriinae, estimated between ~28–26 mya (Figure 2; Table 1) in southeastern, sub-Saharan Africa (Figures 3, S1, S2). This region corresponds somewhat to the current center of diversity of the Ledebouriinae, which is at the intersection of the Natal-Kalahari-Zambezian biogeographical regions (Lebatha, 2004; Manning, 2020; Venter, 1993). The four major subclades (*Ledebouria* Clade A, *Ledebouria* Clade B, *Resnova*, and *Drimiopsis*) originated in neighboring regions to one another soon thereafter (Table 1; Figure 3). During this timeframe in Africa, major shifts in climate and habitat composition have been inferred at both local and continental scales, and at shallow and deep phylogenetic scales (Couvreur et al., 2021; Gizaw et al., 2021; Hagen et al., 2021; Kandziora et al., 2022; Pokorny et al., 2015). From the Eocene-Oligocene transition, global cooling promoted the expansion of seasonal and arid climates as well as savanna, grassland, and fire-prone habitats in Africa, with greater increases

during the Miocene (Senut et al., 2009; Sepulchre et al., 2006). Furthermore, the Great Escarpment underwent renewed uplift in the early Miocene, which increased habitat heterogeneity that was coupled with an intensification of global cooling and aridification that collectively led to lineage radiation and dispersal in many African taxa (Cowling et al., 2009; Galley et al., 2007; Neumann & Bamford, 2015; Partridge & Maud, 1987). Being bulbous geophytes, the Ledebouriinae may have been “pre-adapted” for these climatic and environmental changes, and therefore diversified in response (Howard et al., 2020). Overall, our biogeographic reconstructions and divergence time estimations lead us to hypothesize that the expansion of seasonal habitats and extensive orogenic activity in southern, sub-Saharan Africa spurred the radiation of the Ledebouriinae and promoted additional radiations and dispersals over time.

4.2 A tale of two *Ledebouria*

We estimate that the two *Ledebouria* lineages originated at slightly different times (Figure 2; Table 1) in neighboring regions (i.e., Kalahari vs Zambezian), which lead to subsequent divergent biogeographic histories (Figure 3). In general, the two *Ledebouria* overlap in eastern Africa with a geographical divide between southern and northern sub-Saharan Africa (Figure 2) (Howard et al., 2022). The divergent evolutionary histories and current overlapping distribution of the two *Ledebouria* may have been influenced by the expansion and contraction of wet, tropical, forested habitats in eastern Africa that repeatedly split a historically more widespread distribution (Couvreur, 2015; Couvreur et al., 2021). These past habitat fluctuations have created a mosaic of climates and vegetation types in eastern Africa, and have influenced the evolutionary trajectory of countless lineages (Dagallier et al., 2020; Lorenzen et al., 2012). Other lineages adapted to seasonal and arid habitats share similar distributions with the two *Ledebouria* (Grace et al., 2015; Jürgens, 1997). For example, the two lineages overlap geographically with

that of stapeliads (Ceropegieae, Apocynaceae)—*Ledebouria* Clade B overlaps with the Northern grade, whereas *Ledebouria* Clade A overlaps with the Pan-African clade (see Fig. 3 in Bruyns et al. (2014)). We also find two dispersals of *Ledebouria* to Madagascar (Figure 3), which had been previously hypothesized (Pfosser, 2012). Madagascar and mainland Africa have been separated since at least the Paleocene (Couvreur et al., 2021), which suggests long distance dispersal from Africa to Madagascar led to the origin of Ledebouriinae on the island.

4.2.1 The Voyage of *Ledebouria* Out of Africa, or *Ledebouria* Clade B

Previous studies invoked long distance dispersal from Madagascar to India to explain the distribution of *Ledebouria* in Asia (Ali et al., 2012); however, we find little support for this hypothesis. Reconstructions place the ancestral area of *Ledebouria* Clade B in the Zambezian region followed by migration into other regions, such as Yemen and India, between ~20–15 mya (Figures 2 and 3). During this time, the Arabian Peninsula and Socotra were connected or in close proximity with mainland Africa (Edgell, 2006; Fleitmann et al., 2004; Jacobs, 2004; Rögl, 1999), which would have allowed overland dispersal to these regions. Pollen and wood fossils indicate historical widespread distributions between eastern Africa and south Asia of other closely related taxa (Bonnefille, 2010; Morgan et al., 1994). Additionally, fossils indicate that forested habitats were present in the Arabian Peninsula during the Eocene and Oligocene, that later gave way to more open, xeric, grassland habitats in the Miocene (Jacobs et al., 1999; Whybrow & McClure, 1980). The modern-day equivalents of these conditions are commonly inhabited by *Ledebouria* (Venter, 1993). Given the historical environments present in Africa and south Asia during the Late Oligocene/Early Miocene, the ancestor of *Ledebouria yemenensis* + *L. nossibeensis* likely had a widespread distribution that was fragmented as aridification increased during the Miocene. Additionally, vicariance likely promoted allopatric speciation of

Socotran *Ledebouria* as the islands drifted away from mainland Africa over time. Vicariance has been invoked for other lineages with hypothesized widespread historical ranges between Africa, the Arabian Peninsula, Socotra, and/or southern Asia, including *Isodon* (Lamiaceae) (Yu et al., 2014), *Aganope* (Fabaceae) (Sirichamorn et al., 2014), *Searsia* (Anacardiaceae) (Yang et al., 2016), *Smilax* (Smilacaceae) (Chen et al., 2014), and several reptiles (Main et al., 2022; Smíd et al., 2013; Tamar et al., 2016), many of which have similar age estimates and distributions as *Ledebouria* Clade B. In other groups, such as the Urgineoideae (Asparagaceae) (Ali et al., 2013) and *Uvaria* (Annonaceae) (Zhou et al., 2011), dispersals from Africa to south Asia by way of the Arabian Peninsula have been hypothesized. Furthermore, *Ledebouria* seed has limited dispersal capacity, which occurs primarily via sheet water flow (i.e., travels only short distances and is rainfall dependent) (Venter, 1993). In years of limited rainfall and/or in flat terrains, seeds and seedlings can be found surrounding the parent plant (CCH, pers. obs.), and the ephemeral nature of the seeds in some taxa warrants germination soon after ripening (CCH and TSH, pers. obs.). Thus, our age estimates and biogeographical reconstructions as well as the limited dispersal ability of *Ledebouria* lead us to hypothesize that Clade B dispersed out of Africa to south Asia via overland migration through the Arabian Peninsula. This historical, widespread distribution was then fragmented by Miocene-driven aridification. Today, *L. yemenensis*, the only Ledebouriinae currently recorded from the Arabian Peninsula, likely represents a relic of this once widespread distribution as it is endemic to the cooler, higher elevation of the Yemeni Highlands surrounded by arid lowlands.

4.2.2 Within Africa—*Ledebouria* Clade A

Our results suggest that *Ledebouria* Clade A originated slightly later and in a more southerly region than that of *Ledebouria* Clade B (Table 1; Figures 2 and 3). Based on our

dataset, *Ledebouria* Clade A arose ~25–23 mya and remained predominantly within sub-equatorial Africa (Figures 2 and 3). Despite the low resolution within some areas of Clade A (Howard et al., 2022), we recovered multiple subclades that reflect geographic affinity (Figure 2). For example, we see a subclade of mixed geographic composition (i.e., *L. caesiomontana* + *L.* sp. 12 CCH190) (node c, Figure 2) that suggests a widespread distribution between the Kalahari and Zambezian regions that diverged and lead to *in situ* speciation in the Zambezian region followed by a dispersal to Madagascar (Figure 3). We recovered an additional subclade of predominantly Zambezian taxa (i.e., *L.* sp. 37 CCH169 + *L.* sp. 24 Uganda) (subclade in node a, Figure 2) with evidence of a dispersal to the Sudanian region (i.e., *L.* sp. 24 Uganda). Furthermore, two subclades of predominantly Namibian taxa (i.e., *L.* sp. 14 CCH109 + *L.* sp. 18 CCH218 (node b, Figure 2), and *L.* sp. 7 CCH066 + *L.* sp. 32 CCH145 (subclade in node a, Figure 2)) suggest multiple dispersals to the region followed by *in situ* speciation (Figure 3). Lastly, a subclade of mostly South African taxa (i.e., *L. galpinii* + *L. coriacea*) (subclade in node a, Figure 2) was recovered (Figure 3). Overall, our results suggest that within a relatively short time frame (~20–15 mya), a complex history of dispersals followed by *in situ* speciation events occurred within *Ledebouria* Clade A in southern Africa (Figure 3; Table S2).

From the Miocene onwards, complex interactions between mountain uplift and climate change have promoted diversification across lineages within southern Africa (García-Aloy et al., 2017; Maswanganye et al., 2017; Neumann & Bamford, 2015; Nielsen et al., 2018). *Ledebouria* Clade A may have originated in southeastern Africa then dispersed and diversified as the region grew more heterogeneous and arid. For example, we found an instance of potentially rapid radiation within *Ledebouria* Clade A between 20–17 mya (Figure 3b), which corresponds to a clade with consistently poor phylogenetic resolution (Howard et al., 2022). This subclade

contains taxa from across southern sub-Saharan Africa with geographic signals within (i.e., clades of South African, Namibian, and Zambian taxa) (Figures 2 and 3). It is possible that the Middle Miocene Climatic Optimum or Middle Miocene Climate Transition (Zachos et al., 2001) coupled with renewed uplift of the Great Escarpment spurred this radiation, and we see a steady rise in lineage accumulation from ~20–15 mya (Figure 3b). During the Middle Miocene, the vegetation of South Africa was likely tropical, which gave way to more xerophytic shrubland by Late Miocene due to a global cooling climate (Pound et al., 2012). This time period is associated with diversification in the Crassulaceae (Lu et al., 2022) and also the expansion of C4 grasslands/savannas at a global scale (Bobe, 2006; Senut et al., 2009). Current biogeographical patterns of *Aloe* suggest dispersal from southern Africa to East Africa as seasonality and aridification increased across the continent within the past ~16 my (Grace et al., 2015). *Monsonia* (Geraniaceae) share similar distributional patterns as *Ledebouria* Clade A with multiple lineages comprised of mainly southwestern or southeastern taxa (i.e., southern African split between eastern and western clades), which radiated within the past ~20–15 myr (García-Aloy et al., 2017). Overall, we find a fascinating, albeit more complicated, evolutionary history of *Ledebouria* Clade A within southern sub-Saharan Africa that remains to be fully resolved.

4.3 Limited, but intriguing insights into *Drimiopsis*

Based on our limited sampling of *Drimiopsis*, we find additional evidence for a southern and northern sub-Saharan African divide (Figures 2 and 3), which is also seen in ungulates (Lorenzen et al., 2012), *Lycaon pictus* (Canidae) (Marsden et al., 2012), *Encephalartos* (Cycadaceae) (Mankga et al., 2020), and *Agama* (Reptilia) (Leaché et al., 2014). The eastern/northern sub-Saharan African *Drimiopsis* arose ~19–17 mya (Figure 2), during a time of increased mountain building in the region (~17 and ~13.5 mya; (Wichura et al., 2015) that was

followed by a shift towards a more arid climate (Bonnefille, 2010; Linder, 2017; Morgan et al., 1994; Senut et al., 2009). Several East African *Drimiopsis* have overall more succulent leaves compared to their southern African relatives (Lebatha, 2004), which may have been selected for as eastern Africa experienced greater aridification compared to the southeast. Additionally, East African *Drimiopsis* are known polyploids (Lebatha, 2004; B Stedje & Nordal, 1987), which may have further spurred evolution in this region since polyploidy may provide an advantage in particular environmental conditions (e.g., aridity) (Manzaneda et al., 2012; Sonnleitner et al., 2010). In contrast, South African *Drimiopsis* occur in moist and shady habitats, and are not found in the drier portions of southwestern Africa (Lebatha, 2004). Given the estimated age of South African *Drimiopsis* (~13.6 mya) (Figure 2), uplift of the Great Escarpment may have increased aridity in southwest Africa (Bobe, 2006; Partridge & Maud, 1987) that hindered *Drimiopsis* establishment in this region, but at the same time promoted diversification in southeastern Africa leading to its high diversity in the area (Lebatha, 2004). However, far greater sampling of *Drimiopsis* from its entire distribution is needed to test hypotheses regarding their evolutionary history. Ideally, an expanded phylogenetic framework would be coupled with ploidal inferences, anatomical studies, and experimental manipulations to fully assess the relationship between historical biogeography and morphological evolution.

4.4 Where to sample next?

Our taxon sampling heavily consisted of southern, sub-Saharan African taxa (Figure 1), and largely excluded *Resnova*. Therefore, increased sampling of *Resnova* as well as *Ledebouria* and *Drimiopsis* from western, central, and northern Africa will provide further insights into the biogeographical history of the Ledebouriinae. For example, *Ledebouria* occurs in northern Angola (Gregory Jongsma, pers. obs.) and Gabon (Figure 1). This leads us to wonder whether a

continuous distribution was once present along the central/western coast of Africa, or if the Central African tropical forests have consistently obstructed Ledebouriinae dispersal within the coastal, wet tropics. Furthermore, a *Ledebouria* accession from Uganda is nested within *Ledebouria* Clade A, which suggests that by increasing sampling from northern/western Africa we may uncover additional biogeographic events. Lastly, multiple, morphologically distinct *Ledebouria* taxa are recorded from Socotra and surrounding islands (Miller & Alexander, 2010), and India (Giranje & Nandikar, 2016). Incorporating these into future phylogenetic studies may highlight further dispersals, or bolster support for allopatric speciation/vicariance as a major evolutionary process leading to the current distribution of the group outside of Africa.

5 CONCLUSION

The Ledebouriinae are a widespread, bulbous lineage of monocots that remain poorly understood taxonomically due to a high degree of phenotypic plasticity and significant under collection of specimens. Our study provides a pivotal first step towards refining our understanding of historical evolution and biodiversity within this fascinating lineage. The results of our analyses highlight the complex biogeographic history of the Ledebouriinae within and outside of sub-Saharan Africa. We find that the Ledebouriinae evolved within the past ~30 myr, which suggests the group radiated in response to increasing climatic seasonality and orogenic activity since. We find evidence that vicariance led to the current distribution of *Ledebouria* in Asia and Socotra, with overland dispersal out of Africa into Asia likely occurring via the Arabian Peninsula. We also find evidence of two independent dispersals to Madagascar. Within Africa, we find evidence of a northern and southern sub-Saharan division between the two *Ledebouria* lineages and within *Drimiopsis*. We also uncover a complex history of dispersals and *in situ* speciation events within southern Africa in *Ledebouria* Clade A, a clade that warrants improved

phylogenetic resolution to further elucidate the processes affecting its current distribution. In conclusion, our study shows the value of increasing research focus on understudied lineages inhabiting seasonal and arid habitats.

Tables

Table 1. Comparison of median age estimates and associated uncertainty (95% highest probability density (HPD)) for each major Ledebouriinae clade using penalized likelihood as implemented in treePL.

Clade	median estimate	95% HPD
Scilloideae	52.2	51.2 – 54.5
Ledebouriinae	28.0	26.1 – 28.3
Ledebouria Clade A	25.0	23.0 – 25.4
Resnova	17.9	16.6 – 18.5
Clade A + *Resnova*	26.3	24.5 – 26.7
Drimiopsis	22.1	20.5 – 23.5
Ledebouria Clade B	26.1	24.0 – 26.5
Clade B + *Drimiopsis*	27.1	25.2 – 27.5

Table 2. Statistical outputs for each model from BioGeoBEARS. log-likelihood (LnL); rate of dispersal (*d*); rate of extinction (*e*); Akaike's Information Criterion (AIC)

Model	**LnL**	**Number of parameters**	***d***	***e***	***j***	**AIC**
DEC	-267.1	2	0.01	0.01	0	538.4
DIVA-like	**-220.5**	**2**	**0.0027**	**0.009**	**0**	**445.2**
BAYAREA-like	-232.8	2	0.0036	0.04	0	469.8

Figures

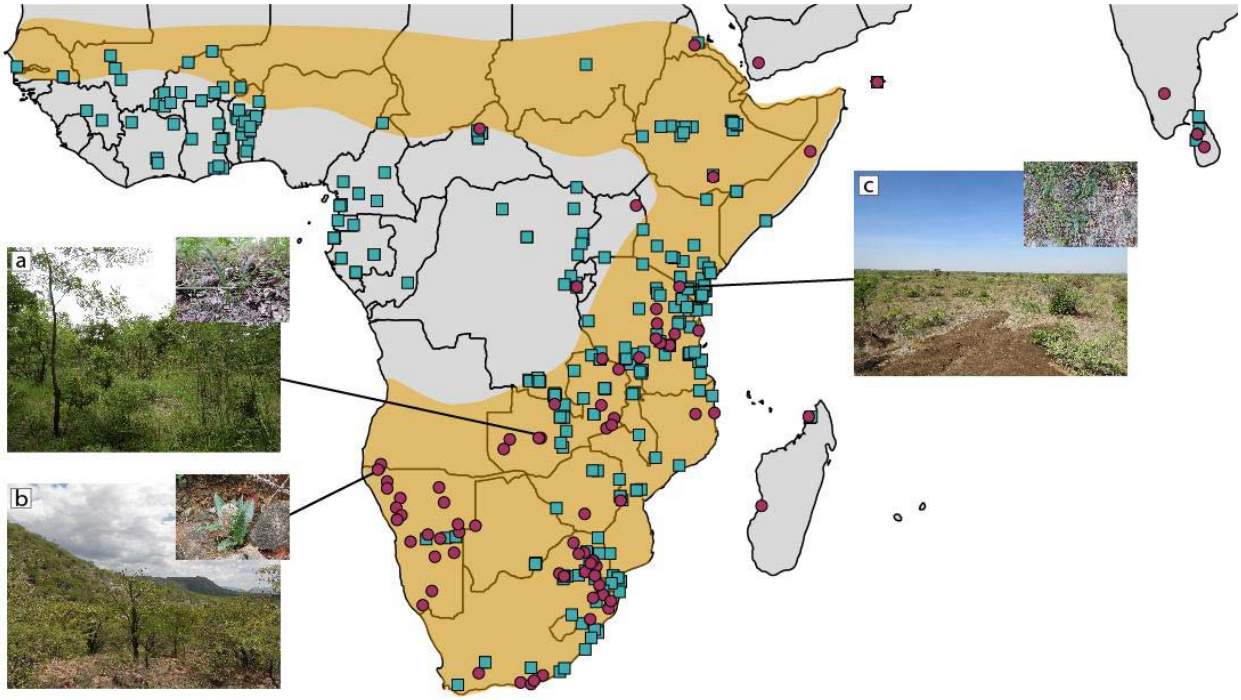


Figure 1. General distribution of the seasonal and arid ecosystems of sub-Saharan Africa (light orange polygon) underlain by the distribution of the Ledebouriinae displayed using GBIF specimen occurrence data (teal squres; GBIF.org (22 March 2022) GBIF Occurrence Download https://doi.org/10.15468/dl.xmccae). Collection localities of samples used in this study are denoted with maroon circles. Examples of habitats and associated Ledebouriinae taxa are displayed in the images. a) *Ledebouria* sp. 8 CCH186, b) *Ledebouria* sp. 15 CCH027, c) *Drimiopsis botryoides* subsp. *botryoides* CCH153. See Figure 2 for phylogenetic placement of these example taxa. General distribution of seasonal landscapes adapted from Balinsky (1962), Jürgens (1997), and Bellstedt et al. (2012).

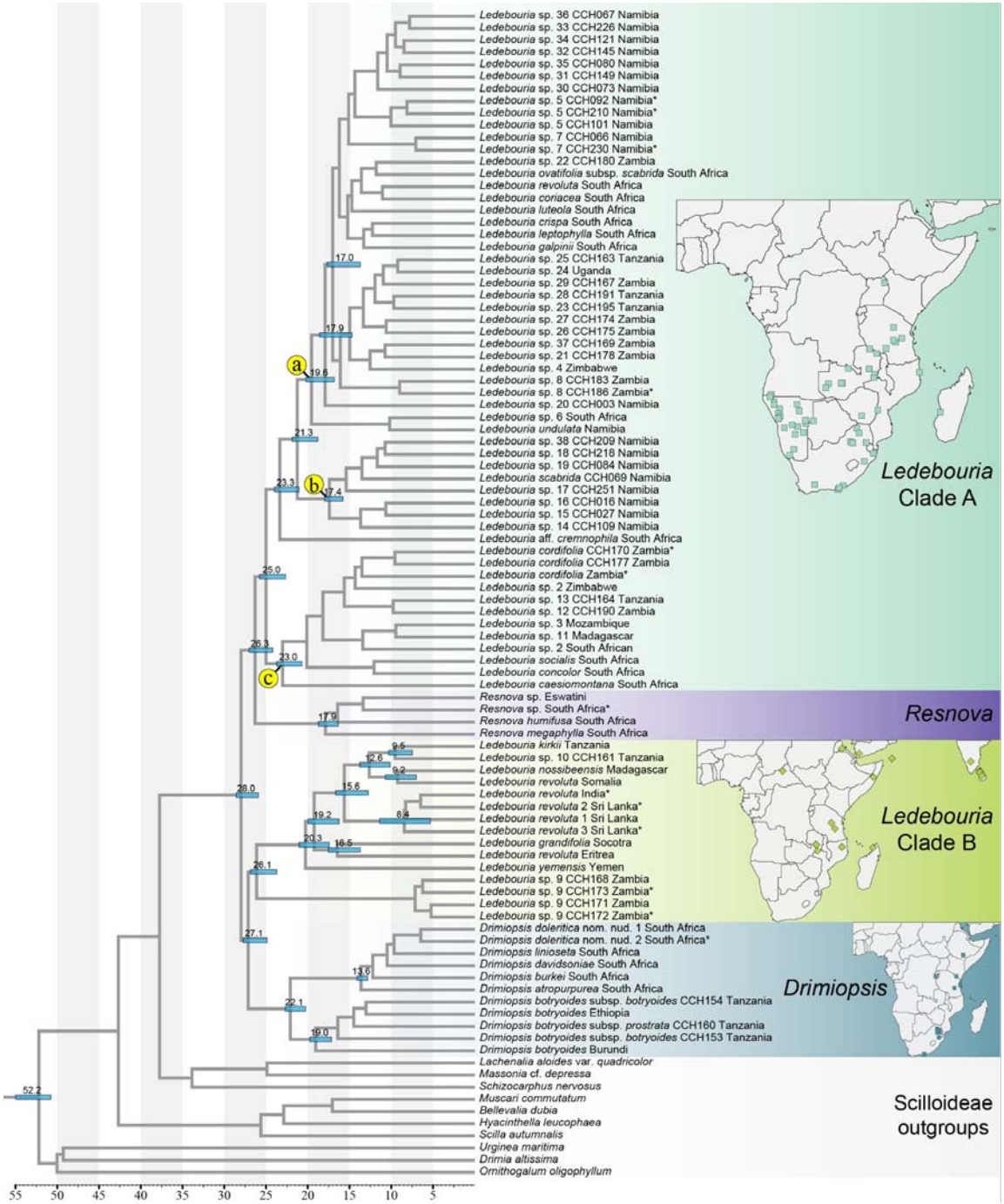
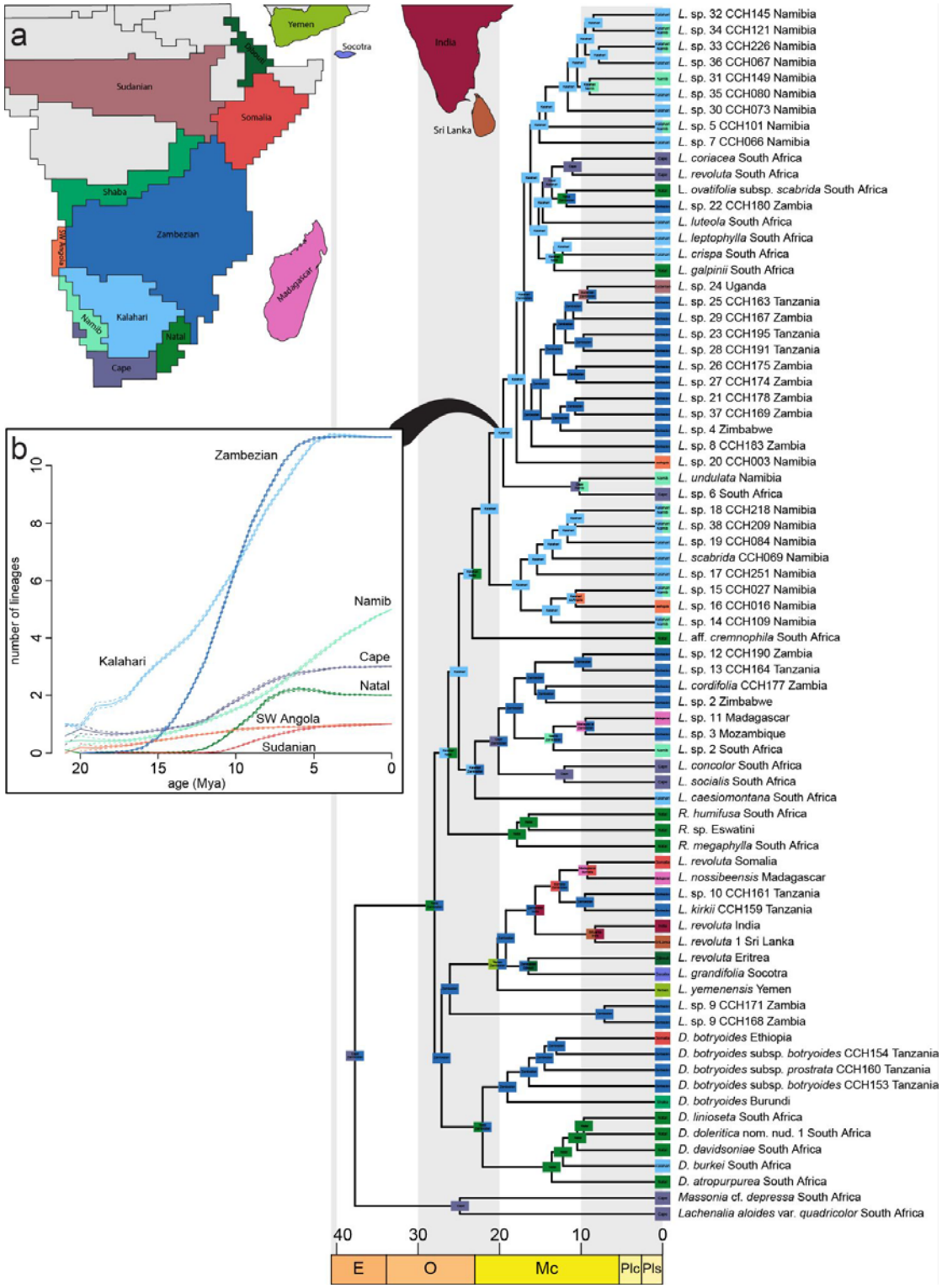


Figure 2. Divergence times within the Ledebouriinae as estimated using penalized likelihood in treePL. For each clade, collection localities are overlaid on a map of Africa, Madagascar, and Asia to aid with discussions of geographical patterns. Clades labeled with a, b, and c in yellow are clades discussed within the manuscript. Numbers above blue bars denote median age estimates. Blue bars denote 95% highest posterior density (95% HPD). * indicate samples excluded from subsequent biogeographical analyses. Not all nodes are annotated with estimated divergences times for illustrative simplicity. For a fully annotated phylogeny, see Figure S1.


a
Yemen
Socotra
Sudanian
Somalia
Shaba
Zambezian
Kalahari
Namib
Natal
Cape
Madagascar
India
Sri Lanka
b
Zambezian
Namib
Kalahari
Cape
Natal
SW Angola
Sudanian
number of lineages
age (Mya)
L. sp. 32 CCH145 Namibia
L. sp. 34 CCH121 Namibia
L. sp. 33 CCH226 Namibia
L. sp. 36 CCH067 Namibia
L. sp. 31 CCH149 Namibia
L. sp. 35 CCH080 Namibia
L. sp. 30 CCH073 Namibia
L. sp. 5 CCH101 Namibia
L. sp. 7 CCH066 Namibia
L. coriacea South Africa
L. revoluta South Africa
L. ovatifolia subsp. scabrida South Africa
L. sp. 22 CCH180 Zambia
L. luteola South Africa
L. leptophylla South Africa
L. crispa South Africa
L. galpinii South Africa
L. sp. 24 Uganda
L. sp. 25 CCH163 Tanzania
L. sp. 29 CCH167 Zambia
L. sp. 23 CCH195 Tanzania
L. sp. 28 CCH191 Tanzania
L. sp. 26 CCH175 Zambia
L. sp. 27 CCH174 Zambia
L. sp. 21 CCH178 Zambia
L. sp. 37 CCH169 Zambia
L. sp. 4 Zimbabwe
L. sp. 8 CCH183 Zambia
L. sp. 20 CCH003 Namibia
L. undulata Namibia
L. sp. 6 South Africa
L. sp. 18 CCH218 Namibia
L. sp. 38 CCH209 Namibia
L. sp. 19 CCH084 Namibia
L. scabrida CCH069 Namibia
L. sp. 17 CCH251 Namibia
L. sp. 15 CCH027 Namibia
L. sp. 16 CCH016 Namibia
L. sp. 14 CCH109 Namibia
L. aff. cremnophila South Africa
L. sp. 12 CCH190 Zambia
L. sp. 13 CCH164 Tanzania
L. cordifolia CCH177 Zambia
L. sp. 2 Zimbabwe
L. sp. 11 Madagascar
L. sp. 3 Mozambique
L. sp. 2 South Africa
L. concolor South Africa
L. socialis South Africa
L. caesiomontana South Africa
R. humifusa South Africa
R. sp. Eswatini
R. megaphylla South Africa
L. revoluta Somalia
L. nossibeensis Madagascar
L. sp. 10 CCH161 Tanzania
L. kirkii CCH159 Tanzania
L. revoluta India
L. revoluta 1 Sri Lanka
L. revoluta Eritrea
L. grandifolia Socotra
L. yemenensis Yemen
L. sp. 9 CCH171 Zambia
L. sp. 9 CCH168 Zambia
D. botryoides Ethiopia
D. botryoides subsp. botryoides CCH154 Tanzania
D. botryoides subsp. prostrata CCH160 Tanzania
D. botryoides subsp. botryoides CCH153 Tanzania
D. botryoides Burundi
D. linioseta South Africa
D. doleritica nom. nud. 1 South Africa
D. davidsoniae South Africa
D. burkei South Africa
D. atropurpurea South Africa
Massonia cf. depressa South Africa
Lachenalia aloides var. quadricolor South Africa
40
30
20
10
0
E
O
Mc
Plc
Pls

513

Figure 3. Biogeographical reconstruction using the DIVA-like model as implemented in BioGeoBEARS and plotted using RevGadgets. a) Regionalization scheme used in the classification of taxa for analysis. b) Summary of 10 stochastic mappings from a posterior distribution of trees including only taxa from the indicated clade (solid black line). For pie chart probabilities at each node, see Figure S2.

Data Availability

Raw reads from PAFTOL are available in ENA (accession PRJEB35285) and assembled reads from www.treeoflife.kew.org. Raw reads of the Ledebouriinae are available from the SRA (accession PRJNA721471). Alignments, species trees, biogeography inputs/outputs are all available on Dryad (*doi: currently in process*) (but try https://doi.org/10.5061/dryad.r4xgxd2gw).

Biosketch

Cody Coyotee Howard is an Assistant Professor at Oklahoma State University broadly interested in geophyte evolution and biogeography, and he has a particular fascination with bulbous plants. The Ledebouriinae represent one bulbous lineage that has captivated him for years, and these plants will continue to do so for many more.

Author contributions: Conceptualization CCH, TSH, NC; Formal analysis, CCH and ARZ; Investigation, CCH and ARZ; Resources, TSH, LN, DC, LN, PM, ARZ, NC; Writing — Original Draft, CCH and NC; Writing — Review & Editing, all authors; Visualization, CCH; Funding acquisition, CCH and NC.

Supporting information

Table S1. Fossil and secondary calibration points used in the penalized likelihood (treePL) analysis. With the exception of monocots, all calibrations were set as minimum values in the analysis.

node	minimum calibration	source
Zingiberaceae	72.1	Iles et al., 2015
Typhaceae	51.66	Iles, et al. 2015
Cyperaceae	47.0	Iles, et al. 2015
Amaryllidaceae	48.88	Pigg et al., 2018
Coryphoideae	83.6	Iles, et al. 2015
Agavoideae	14.5	Iles, et al. 2015
Xanthorrhoeaceae	38.0	Iles, et al. 2015
Goodyerinae	15.0	Iles, et al. 2015
Monocots	131 – 135	Magallón et al., 2015

Table S2. Top ten most common transition types found in a subclade of *Ledebouria* Clade A (Figure 3b) using stochastic mapping of biogeographic events.

starting_range	ending_range	average_n_events	SD	transition_type
K	K	23.432	2.121	in situ speciation
Z	Z	19.252	1.297	in situ speciation
K	KN	3.393	0.517	dispersal
T	T	2.764	2.089	in situ speciation

C	C	1.29	1.912	in situ speciation
KZ	K	1.09	0.307	vicariance or subset sympatry or extinction
KZ	Z	1.09	0.307	vicariance or subset sympatry or extinction
K	KZ	1.029	0.215	dispersal
CK	C	0.912	0.703	vicariance or subset sympatry or extinction

Figure S1. Full dated phylogeny with 95% highest posterior density plots shown.

Figure S2. Biogeographical reconstruction using the DIVA-like model with nodes showing the probable range for each region at that node.

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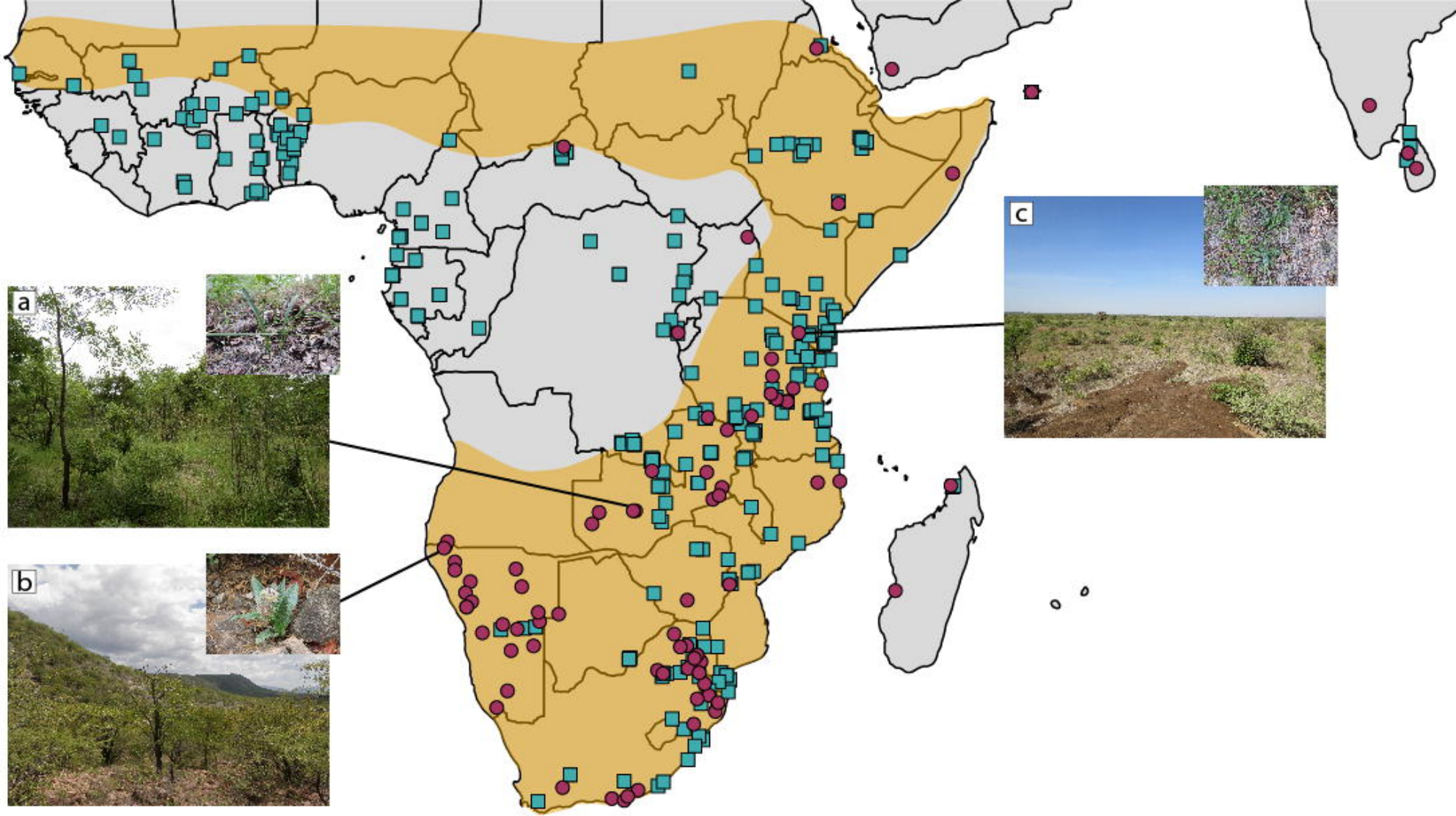
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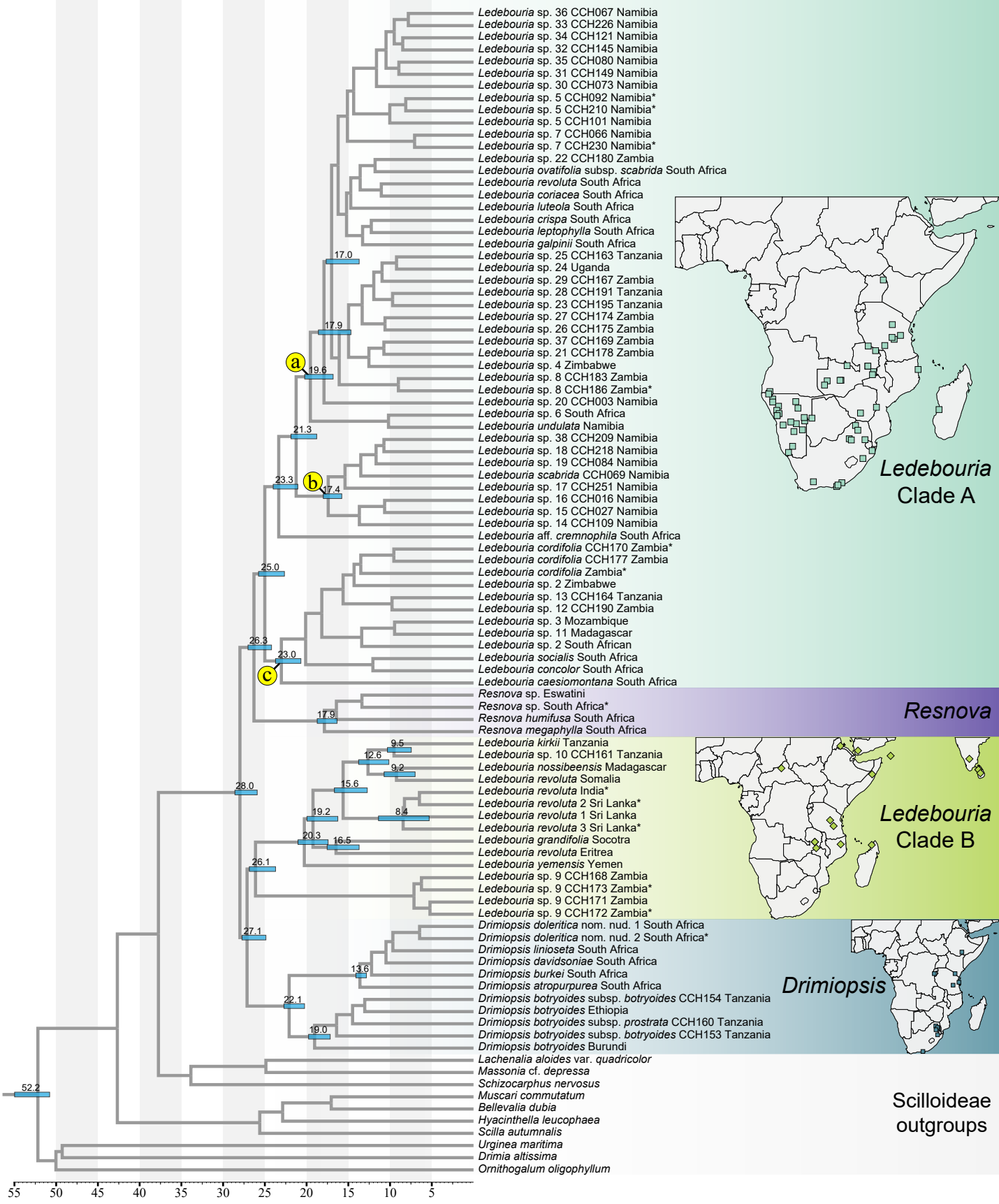
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Ledebouria revoluta South Africa
Ledebouria coriacea South Africa
Ledebouria luteola South Africa
Ledebouria crispa South Africa
Ledebouria leptophylla South Africa
Ledebouria galpinii South Africa
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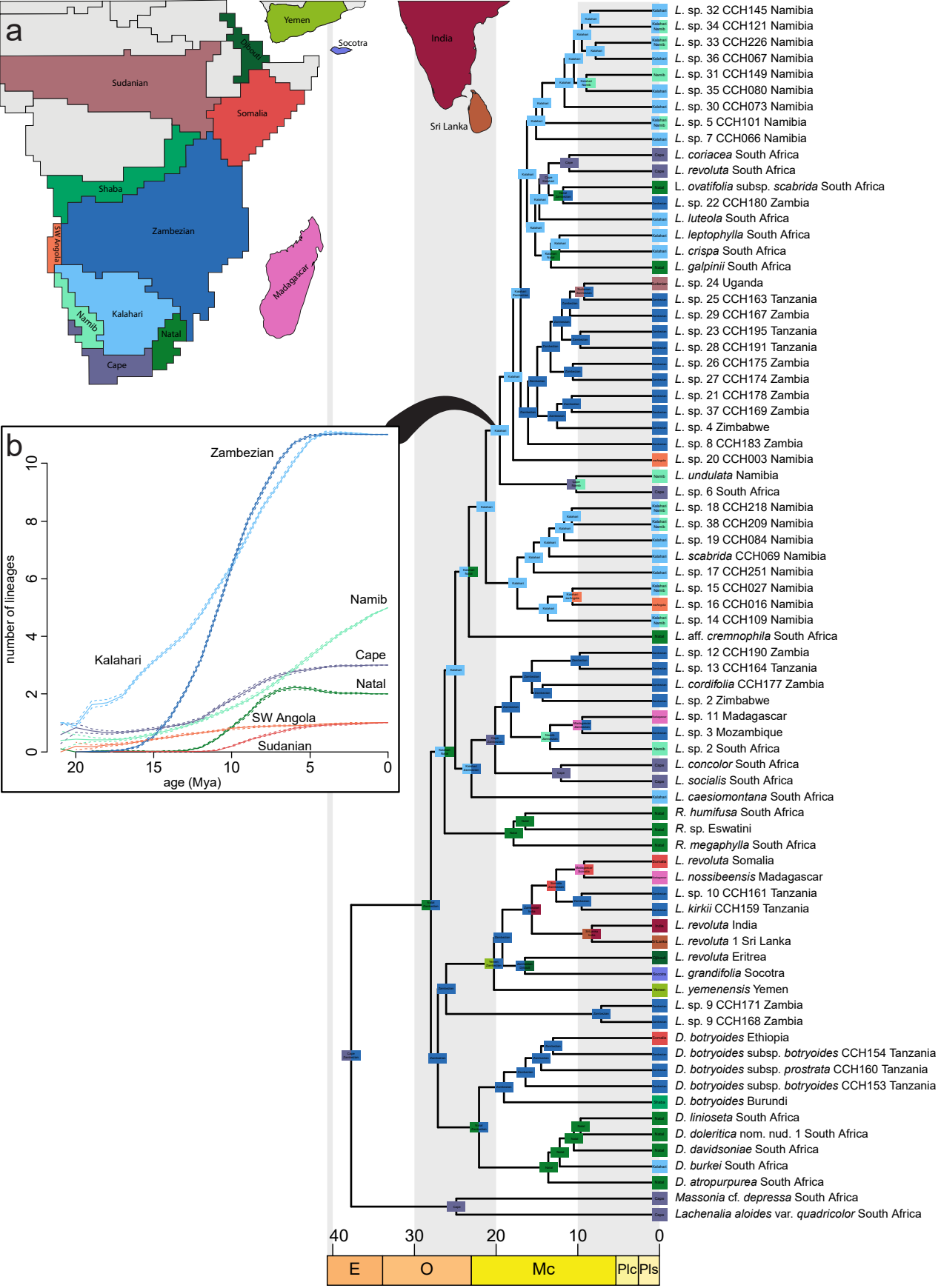
Ledebouria sp. 28 CCH191 Tanzania
Ledebouria sp. 23 CCH195 Tanzania
Ledebouria sp. 27 CCH174 Zambia
Ledebouria sp. 26 CCH175 Zambia
Ledebouria sp. 37 CCH169 Zambia
Ledebouria sp. 21 CCH178 Zambia
Ledebouria sp. 4 Zimbabwe
Ledebouria sp. 8 CCH183 Zambia
Ledebouria sp. 8 CCH186 Zambia*
Ledebouria sp. 20 CCH003 Namibia
Ledebouria sp. 6 South Africa
Ledebouria undulata Namibia
Ledebouria sp. 38 CCH209 Namibia
Ledebouria sp. 18 CCH218 Namibia
Ledebouria sp. 19 CCH084 Namibia
Ledebouria scabrida CCH069 Namibia
Ledebouria sp. 17 CCH251 Namibia
Ledebouria sp. 16 CCH016 Namibia
Ledebouria sp. 15 CCH027 Namibia
Ledebouria sp. 14 CCH109 Namibia
Ledebouria aff. cremnophila South Africa
Ledebouria cordifolia CCH170 Zambia*
Ledebouria cordifolia CCH177 Zambia
Ledebouria cordifolia Zambia*
Ledebouria sp. 2 Zimbabwe
Ledebouria sp. 13 CCH164 Tanzania
Ledebouria sp. 12 CCH190 Zambia
Ledebouria sp. 3 Mozambique
Ledebouria sp. 11 Madagascar
Ledebouria sp. 2 South African
Ledebouria socialis South Africa
Ledebouria concolor South Africa
Ledebouria caesiomontana South Africa
Resnova sp. Eswatini
Resnova sp. South Africa*
Resnova humifusa South Africa
Resnova megaphylla South Africa
Ledebouria kirkii Tanzania
Ledebouria sp. 10 CCH161 Tanzania
Ledebouria nossibeensis Madagascar
Ledebouria revoluta Somalia
Ledebouria revoluta India*
Ledebouria revoluta 2 Sri Lanka*
Ledebouria revoluta 1 Sri Lanka
Ledebouria revoluta 3 Sri Lanka*
Ledebouria grandifolia Socotra
Ledebouria revoluta Eritrea
Ledebouria yemensis Yemen
Ledebouria sp. 9 CCH168 Zambia
Ledebouria sp. 9 CCH173 Zambia*
Ledebouria sp. 9 CCH171 Zambia
Ledebouria sp. 9 CCH172 Zambia*
Drimiopsis doleritica nom. nud. 1 South Africa
Drimiopsis doleritica nom. nud. 2 South Africa*
Drimiopsis linioseta South Africa
Drimiopsis davidsoniae South Africa
Drimiopsis burkei South Africa
Drimiopsis atropurpurea South Africa
Drimiopsis botryoides subsp. botryoides CCH154 Tanzania
Drimiopsis botryoides Ethiopia
Drimiopsis botryoides subsp. prostrata CCH160 Tanzania
Drimiopsis botryoides subsp. botryoides CCH153 Tanzania
Drimiopsis botryoides Burundi
Lachenalia aloides var. quadricolor
Massonia cf. depressa
Schizocarphus nervosus
Muscari commutatum
Bellevalia dubia
Hyacinthella leucophaea
Scilla autumnalis
Urginea maritima
Drimia altissima
Ornithogalum oligophyllum
Ledebouria Clade A
Resnova
Ledebouria Clade B
Drimiopsis
Scilloideae outgroups
a
b
c
17.0
17.9
19.6
21.3
23.3
17.4
25.0
26.3
23.0
17.9
9.5
12.6
9.2
15.6
28.0
19.2
8.4
20.3
16.5
26.1
27.1
13.6
22.1
19.0
52.2
55
50
45
40
35
30
25
20
15
10
5


a
Yemen
Djibouti
Socotra
India
Sudanian
Somalia
Sri Lanka
Shaba
Zambezian
SW Angola
Madagascar
Kalahari
Namib
Natal
Cape
b
Zambezian
Namib
Cape
Natal
Kalahari
SW Angola
Sudanian
number of lineages
age (Mya)
L. sp. 32 CCH145 Namibia
L. sp. 34 CCH121 Namibia
L. sp. 33 CCH226 Namibia
L. sp. 36 CCH067 Namibia
L. sp. 31 CCH149 Namibia
L. sp. 35 CCH080 Namibia
L. sp. 30 CCH073 Namibia
L. sp. 5 CCH101 Namibia
L. sp. 7 CCH066 Namibia
L. coriacea South Africa
L. revoluta South Africa
L. ovatifolia subsp. scabrida South Africa
L. sp. 22 CCH180 Zambia
L. luteola South Africa
L. leptophylla South Africa
L. crispa South Africa
L. galpinii South Africa
L. sp. 24 Uganda
L. sp. 25 CCH163 Tanzania
L. sp. 29 CCH167 Zambia
L. sp. 23 CCH195 Tanzania
L. sp. 28 CCH191 Tanzania
L. sp. 26 CCH175 Zambia
L. sp. 27 CCH174 Zambia
L. sp. 21 CCH178 Zambia
L. sp. 37 CCH169 Zambia
L. sp. 4 Zimbabwe
L. sp. 8 CCH183 Zambia
L. sp. 20 CCH003 Namibia
L. undulata Namibia
L. sp. 6 South Africa
L. sp. 18 CCH218 Namibia
L. sp. 38 CCH209 Namibia
L. sp. 19 CCH084 Namibia
L. scabrida CCH069 Namibia
L. sp. 17 CCH251 Namibia
L. sp. 15 CCH027 Namibia
L. sp. 16 CCH016 Namibia
L. sp. 14 CCH109 Namibia
L. aff. cremnophila South Africa
L. sp. 12 CCH190 Zambia
L. sp. 13 CCH164 Tanzania
L. cordifolia CCH177 Zambia
L. sp. 2 Zimbabwe
L. sp. 11 Madagascar
L. sp. 3 Mozambique
L. sp. 2 South Africa
L. concolor South Africa
L. socialis South Africa
L. caesiomontana South Africa
R. humifusa South Africa
R. sp. Eswatini
R. megaphylla South Africa
L. revoluta Somalia
L. nossibeensis Madagascar
L. sp. 10 CCH161 Tanzania
L. kirkii CCH159 Tanzania
L. revoluta India
L. revoluta 1 Sri Lanka
L. revoluta Eritrea
L. grandifolia Socotra
L. yemenensis Yemen
L. sp. 9 CCH171 Zambia
L. sp. 9 CCH168 Zambia
D. botryoides Ethiopia
D. botryoides subsp. botryoides CCH154 Tanzania
D. botryoides subsp. prostrata CCH160 Tanzania
D. botryoides subsp. botryoides CCH153 Tanzania
D. botryoides Burundi
D. linioseta South Africa
D. doleritica nom. nud. 1 South Africa
D. davidsoniae South Africa
D. burkei South Africa
D. atropurpurea South Africa
Massonia cf. depressa South Africa
Lachenalia aloides var. quadricolor South Africa
40
30
20
10
0
E
O
Mc
Plc
Pls