Peeling Back the Layers: Complex Dynamics Shaping the Evolution of the Ledebouriinae (Scilloideae, Asparagaceae)

CODY COYOTEE HOWARD[1,2,*], ANDREW A. CROWL[3], TIMOTHY S. HARVEY[4], AND NICO CELLINESE[1,5,6]

[1] Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA

[2] Department of Biology, University of Florida, Gainesville, FL 32611, USA

[3] Duke University, Durham, NC 27708, USA

[4] Plantae Novae, Thousand Oaks, CA 91360, USA

[5] Biodiversity Institute, University of Florida, Gainesville, FL 32611, USA

[6] Genetics Institute, University of Florida, Gainesville, FL 32611, USA

* Correspondence to be sent to: Cody Coyotee Howard, Department of Biology, University of Florida, Gainesville, FL 32611, USA; Email: cchoward@ufl.edu

Abstract—The Ledebouriinae (Scilloideae, Asparagaceae) are a widespread group of bulbous geophytes found predominantly throughout seasonal climates in sub-Saharan Africa, with a handful of taxa in Madagascar, the Middle East, India, and Sri Lanka. Phylogenetic relationships within the group have been historically difficult to elucidate. Here, we provide the first phylogenomic perspective into the Ledebouriinae. Using the Angiosperms353 targeted enrichment probe set, we consistently recovered four major clades (i.e., two *Ledebouria* clades, *Drimiopsis*, and *Resnova*). The two *Ledebouria* clades closely align with geography, either

consisting almost entirely of sub-Saharan African taxa (*Ledebouria* Clade A), or East African and non-African taxa (*Ledebouria* Clade B). Our results suggest that the Ledebouriinae likely underwent a rapid radiation leading to rampant incomplete lineage sorting. We additionally find evidence for potential ancient hybridization between *Drimiopsis* and a subclade within *Ledebouria* Clade A. [Asparagaceae; *Drimiopsis*; geophytes; *Ledebouria*; monocots; *Resnova*; Scilloideae.]

Africa houses an enormous diversity of plants found across a range of habitats from tropical rainforests to arid landscapes (Linder 2014; Couvreur et al. 2020). Still, there remain numerous, understudied African taxa that may advance our ability to refine hypotheses regarding evolution within Africa (e.g., the Rand Flora (Pokorny et al. 2015), the “arid track” (Balinsky 1962)), or showcase their own distinct patterns. One such group is the hyacinths (Scilloideae, Asparagaceae; formerly Hyacinthaceae; APG IV (The Angiosperm Phylogeny Group 2016)), which consist of approximately 1,000 bulbous geophytes found throughout seasonal climates in Africa, as well as Madagascar, Europe, the Middle East, India, Sri Lanka, and South America (Speta 1998). Digging into the evolution of the Scilloideae has improved our understanding of historical biogeography in Africa (Buerki et al. 2012; Ali et al. 2013), polyploidy (Jang et al. 2018), and ecology (Johnson et al. 2001; Vogel and Müller-Doblies 2011). Unfortunately, the group remains largely understudied. To date, many clades within the Scilloideae have received varying degrees of phylogenetic and systematic attention (e.g., Ledebouriinae, Massonieae, Ornithogaloideae; (Pfosser et al. 2003; Lebatha et al. 2006; Martínez-Azorín et al. 2011); among many others), but phylogenomic applications are almost nonexistent (Steele et al. 2012). To showcase the value of focusing attention on understudied groups such as the Scilloideae, we

investigate the phylogenomic space of one of its subclades—the Ledebouriinae—the evolutionary relationships of which have been historically difficult to reconstruct.

The Ledebouriinae are widespread across sub-Saharan Africa, found predominantly within the "arid track" (Balinsky 1962), with just a handful of taxa in Madagascar, Yemen, India and Sri Lanka (Fig. 1a; (Venter 1993; Giranje and Nandikar 2016). The current center of diversity is the Limpopo, Mpumalanga, and KwaZulu-Natal regions of South Africa (Venter 1993), with a secondary center in East Africa (Lebatha 2004; Lebatha et al. 2006).

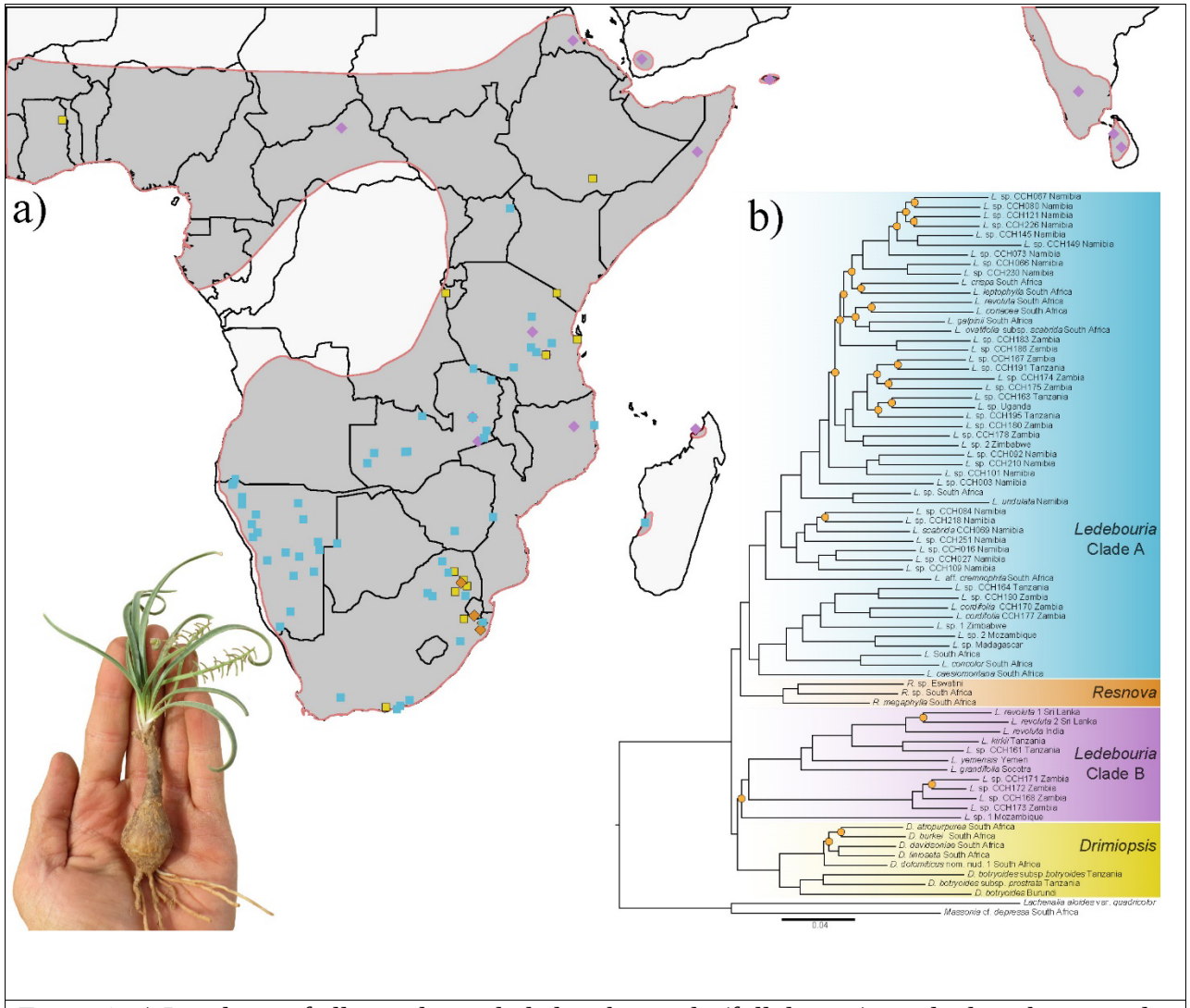

Figure 1. a) Localities of all samples included in this study (full dataset) overlaid on the general

distribution of the Ledebouriinae (dark gray polygon). Shapes and colors correspond to clades shown in (b): *Ledebouria* Clade A (blue squares), *Ledebouria* clade B (purple diamonds), *Resnova* (outlined orange diamonds), *Drimiopsis* (outlined yellow squares). b) Maximum likelihood phylogenetic reconstruction of the Taxa70 dataset with the four major clades labeled. Orange circles indicate nodes with SH-aLRT and ultrafast bootstrap values *below* 80 and 95, respectively.

Taxonomically, the group currently consists of *Ledebouria* Roth with two sections: *Drimiopsis* (Lindl. & Paxton) J.C. Manning & Goldblatt and *Resnova* (Van der Merwe) J.C. Manning & Goldblatt (Manning et al. 2003; Manning and Goldblatt 2012; Manning 2020). However, taxonomic concepts still vary and a lack of consistency in the hypotheses generated by phylogenetic studies has resulted in disagreements regarding the nature of the Ledebouriinae and whether this group includes one or more distinct entities (Manning et al. 2003; Lebatha et al. 2006). Historically, *Ledebouria* and *Drimiopsis* Lindl. & Paxton were classified as separate taxa, and *Drimiopsis* enjoyed its own identity for some time (Lebatha et al. 2006; Müller-Doblies and Müller-Doblies 2008; Goldblatt et al. 2012). The status of *Resnova* van der Merwe, on the other hand, has always been in question (Manning et al. 2003; Lebatha et al. 2006; Goldblatt et al. 2012; Manning 2020). There are currently 53 *Ledebouria*, 16 *Drimiopsis*, and five *Resnova* species accepted (The Plant List 2013), with the majority of systematic work occurring in South Africa (Venter 1993). Thus far, difficulties in reconstructing the phylogenetic history of the Ledebouriinae have stemmed from morphological homoplasy (Lebatha et al. 2006), the use of a small number of molecular markers with low information content (Manning et al. 2003), the lack of robust phylogenetic methods (Pfosser et al. 2003; Wetschnig et al. 2007), and the inclusion of a small fraction of the taxonomic and geographic diversity of the group. These limitations

constrain our ability to detect confounding factors that have likely impacted the evolutionary history of the Ledebouriinae, which may be why it has been difficult to confidently reconstruct it.

Based on previous dating analyses (Ali et al. 2012; Buerki et al. 2012), we hypothesize that the Ledebouriinae have experienced a rapid radiation(s). These studies suggest that the group originated during a time (i.e., within the last 30 My) when Africa was undergoing major climatic and geological shifts (e.g., increasing seasonality, orogenic activity) (Partridge and Maud 1987; Jacobs 2004; Bobe 2006), and the bulbous geophytic habit may have allowed the Ledebouriinae to capitalize on such conditions (Howard et al. 2019). Additionally, hybridization and polyploidy within and between groups has been documented (Stedje and Nordal 1987; Lebatha et al. 2006), which present further complications in reconstructing the evolutionary history of the group. Therefore, to recover a well-supported phylogenetic hypothesis for the Ledebouriinae, the use of a large, genomic dataset consisting of rapidly evolving markers is likely needed. Unfortunately, the group lacks the necessary genomic resources to develop a custom probe set. However, broadly applicable targeted-capture kits (Johnson et al. 2019; Breinholt et al. 2021) have been instrumental in bringing understudied groups, like the Ledebouriinae, to the phylogenomic playing field (Dodsworth et al. 2019).

Here, we apply a HybSeq/targeted enrichment approach using the Angiosperms353 probe set, which has been effective in uncovering relationships at both deep and shallow scales (Larridon et al. 2019). The use of this probe set circumvents the lack of genomic resources that plagues the Ledebouriinae and allows future studies to leverage our dataset, which will enable continual refinement of the phylogenetic understanding of this group as well as the Scilloideae. We include samples from across the distribution of the group using both field-collected and

museum-based specimens to investigate putative lineages within the Ledebouriinae (i.e., *Ledebouria*, *Drimiopsis* and *Resnova*) as well as potential reasons for discordance.

MATERIALS AND METHODS

Taxon Sampling and Sequence Assembly

Plants of Ledebouriinae were collected from the field in 2012 (Namibia), 2014 (Namibia), and 2017 (Tanzania, Zambia, and Namibia). Leaves of each sample were dried in silica gel for DNA extractions. Herbarium material from four institutions (Missouri Botanical Gardens, Uppsala's Evolutionsmuseet Botanik, Sweden Museum of Natural History, and The Royal Botanic Gardens, Kew, DNA Bank, https://www.kew.org/data/dnaBank/) were also included. Additionally, silica-dried leaf material from specimens with known provenance were generously donated from private collections. Our final dataset included 94 Ledebouriinae samples from across the distribution of the group as well as two outgroup taxa (*Massonia* cf. *depressa* Houtt. and *Lachenalia aloides* var. *quadricolor* (Jacq.) Engl.). See Supplementary Table 1 for the specimen list with associated collection and locality information. Targeted capture was performed using the Angiosperms353 probe set on an Illumina HiSeq 2000 followed by sequence assembly and alignment.

Phylogenetic Analyses

We constructed two datasets composed of supercontig sequences (i.e., consensus sequences for each targeted locus including intronic and exonic regions). The full dataset included all samples regardless of missing data. The second dataset included taxa with less than 70% missing data in the supermatrix alignment (i.e., <= 70% gaps/ambiguities; referred to as Taxa70). Maximum likelihood phylogenetic reconstruction was performed using IQ-Tree v2.0-rc1 (Minh et al. 2020). We quantified discordance and concordance using 1) gene concordance

factors (gCF; percentage of genes supporting the input topology) and site concordance factors (sCF; percentage of sites informative for a branch) as implemented in IQ-Tree v2.0-rc1, and 2) Quartet Sampling (QS), which provides unique and more revealing information pertaining to discordance often prevalent in phylogenomic datasets (Pease et al. 2018). We further quantified gene tree discordance within the Taxa70 dataset using DiscoVista (Sayyari et al. 2018). To obtain a species tree while also accounting for potential instances of incomplete lineage sorting, we used ASTRAL III v5.6.2 (Zhang et al. 2018) as well as singular value decomposition quartet species-tree estimation (SVDQuartets) (Chifman and Kubatko 2014) implemented in PAUP* v4.0a (Swofford 2001). Lastly, we investigated potential instances of hybridization on randomly subsampled phylogenies of ten taxa using SNaQ (Solís-Lemus et al. 2017). See Supplementary Methods for an expanded explanation of methodology.

RESULTS

Excluding the full dataset, which included samples with large amounts of missing data, the major clades received overall high support according to SH-aLRT, ultrafast bootstrap (UFBoot), and local posterior probability (LPP) (Table 1, Fig. 2, Supplementary Figs. 1 – 4).

	Dataset	*Ledebouria* Clade A + *Resnova*	*Ledebouria* Clade A	*Resnova*	*Ledebouria* Clade B + *Drimiopsis*	*Ledebouria* Clade B	*Drimiopsis*
UFBoot	Full	97	90	100	93	76	100
	Taxa70	100	100	100	95	70	100
	L. sp. 1 Moz removed	100	100	100	100	97.2	100
SH-aLRT	Full	100	97.7	100	98.9	15.9	100
	Taxa70	100	99	100	99.6	14.3	100
	L. sp. 1 Moz removed	100	98.8	100	100	100	100
LPP	Full	1	0.97	0.34	0.91	0.62	1
	Taxa 70	1	0.95	1	0.96	0.74	1
	L. sp. 1 Moz removed	1	0.93	1	0.98	1	1
gCF	Full	3.67	2.56	44.2	3.57	2.78	26.8
	Taxa70	4.67	2.56	46.6	4.87	3.47	32.7
	L. sp. 1 Moz removed	5.67	2.56	47	4.55	16	34
sCF	Full	38.8	34.3	59.7	32.4	36.1	44.8
	Taxa70	40	37.7	65.1	34.3	36.5	55.5
	L. sp. 1 Moz removed	39.5	38	64.3	33.9	38.5	54.1
QC	Full	0.035	0.031	0.32	0.019	-0.017	0.37
	Taxa70	-0.0045	0.025	0.33	0.0081	0.031	0.27
	L. sp. 1 Moz removed	0.05	0.023	0.39	0.047	0.026	0.33
QD	Full	0.81	0.76	0.95	0.92	0.77	0.47
	Taxa70	0.88	0.83	0.77	0.86	0.85	0.55
	L. sp. 1 Moz removed	0.74	1	0.92	0.86	0.98	0.43
QI	Full	0.34	0.41	0.54	0.3	0.33	0.48
	Taxa70	0.33	0.38	0.58	0.31	0.31	0.5
	L. sp. 1 Moz removed	0.34	0.39	0.57	0.27	0.32	0.55

Table 1. Support value measures, gene concordance factor (gCF), site concordance factor (sCF), and quartet sampling measures for the four major clades recovered. Full shows values for the full dataset, Taxa70 reports values for the Taxa70 dataset, and *L*. sp. 1 Moz removed shows values when *Ledebouria* sp. 1 Mozambique is removed from the analyses. Support measures are ultrafast bootstrap (UFBoot), SH-like approximate likelihood ratio test (SH-aLRT), and local posterior probability (LPP). Support measures are those recovered using the GENESITE partition resampling measure in IQ-Tree. Gene concordance factor (gCF) is expressed as the percentage of genes that support a given topology, site concordance factor (sCF) is expressed as the number of sites informative for a branch. Quartet sampling measures include quartet concordance (QC), quartet differential (QD), and quartet informativeness (QI). See Supplementary Methods or Pease et al. (2018) for a detailed explanation of these measures.

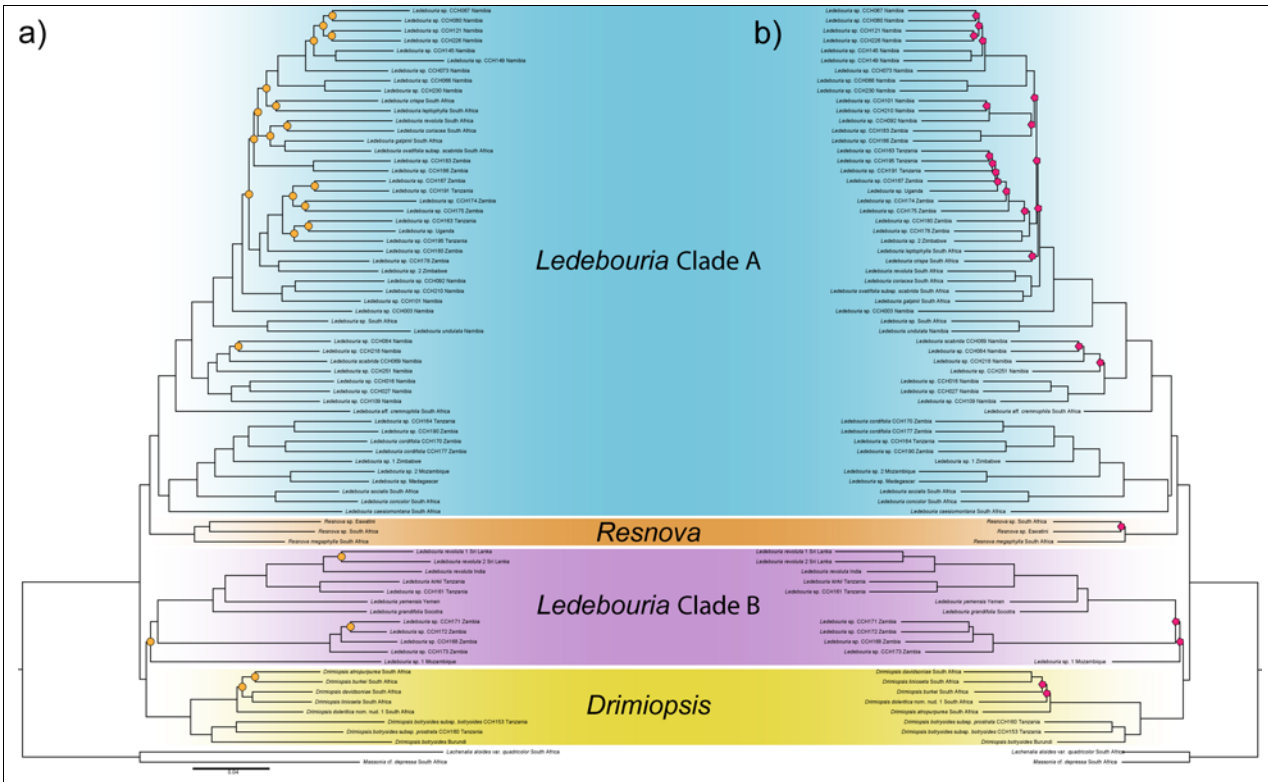

Figure 2. Phylogenetic reconstruction of the Taxa70 datasets using maximum likelihood as implemented in IQ-Tree (a), and species tree estimation as implemented in ASTRAL III (b). a) Orange circles indicate nodes with SH-aLRT and ultrafast bootstrap values below 80 and 95, respectively. b) Pink polygons indicate nodes with local posterior probability (LPP) below .95. Note, the same four major clades are recovered in both, with differences in relationships of subclades and tips between the two analyses, particularly within *Ledebouria* Clade A.

Our analyses consistently recovered a paraphyletic *Ledebouria*, with each *Ledebouria* clade sister to either *Resnova* or *Drimiopsis* (Fig. 2). However, two results warrant attention. First, in the full dataset, we found a paraphyletic *Resnova* (Supplementary Fig. 1) and recovered low LPP for *Resnova* (Supplementary Fig. 2). After the removal of one herbarium sample found in only four alignments (i.e., *Resnova lachenalioides*, which was a sample that consistently jumped around potentially due to missing data [compare Supplementary Figs. 1, 2, 9]), we found

increased support for a monophyletic *Resnova* across all measures (Table 1, Fig. 2, Supplementary Figs. 3, 4). Second, *Drimiopsis* + *Ledebouria* Clade B recovered overall high support except in the full dataset (Table 1, Supplementary Figs. 1, 2). Using all samples, we found low support for *Ledebouria* Clade B in terms of the placement of *Ledebouria* sp. 1 Mozambique (Fig. 2, Table 1, Supplementary Figs. 1, 2). The subclade sister to this sample shows higher but still low support in the full dataset (Fig. 2, Supplementary Figs. 1, 2). In the Taxa70 dataset, we find stronger support for *Drimiopsis* + *Ledebouria* Clade B (Supplementary Figs. 3, 4). Support increases further for *Drimiopsis* + *Ledebouria* Clade B as well as *Ledebouria* Clade B alone after the removal of *Ledebouria* sp. 1 Mozambique (Table 1, Supplementary Figs. 5, 6).

We found relatively low gene (gCF) and site (sCF) concordance factors for many nodes in both the full and Taxa70 datasets (Table 1; Supplementary Figs. 1, 3). Quartet concordance values were mostly positive for the four major clades (Table 1; Supplementary Figs. 7, 8). Quartet differential (QD) indicated low proportions of an alternative evolutionary history (i.e., values nearer to 1) (Table 1; Supplementary Figs. 7, 8). Quartet informativeness (QI) indicated low informativeness of the quartets for the topology, reflecting the results of sCF (Table 1; Supplementary Figs. 7, 8). DiscoVista reported only one gene tree that supports a monophyletic *Ledebouria* (Fig. 3b; Supplementary Table 2).

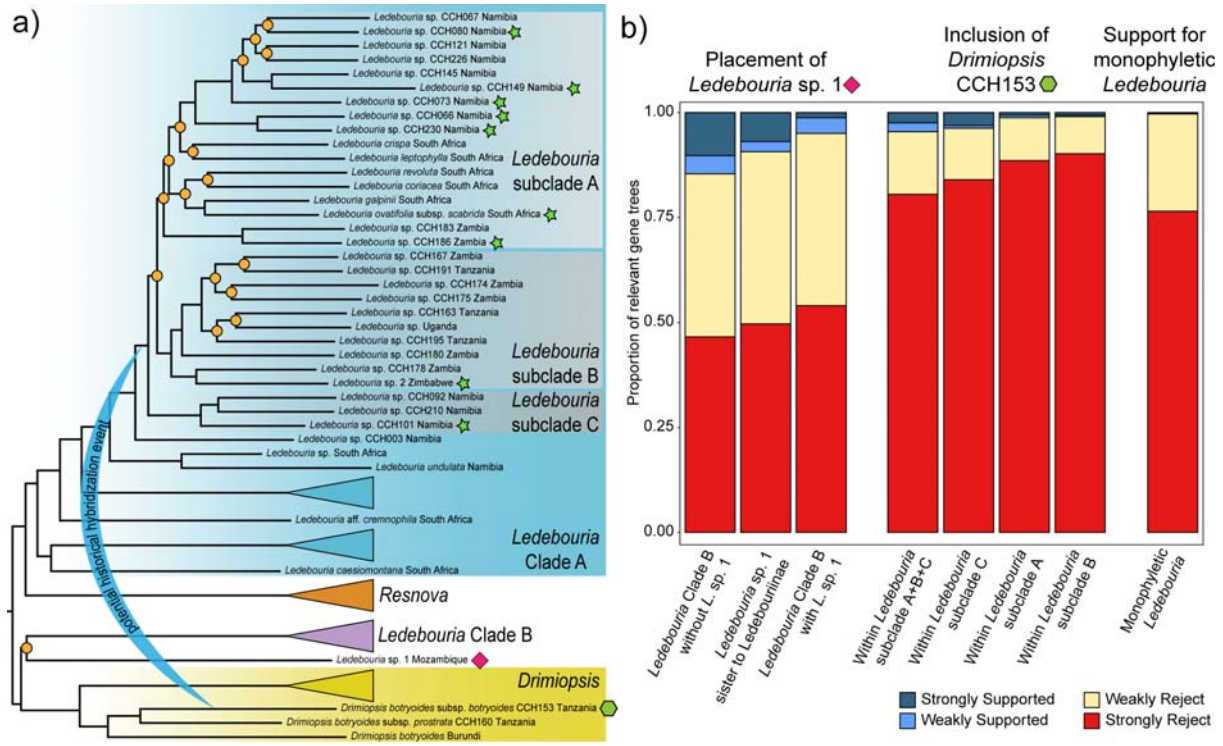

Figure 3. Summary of hypothesized hybridization and phylogenetic discordance. a) Maximum likelihood reconstruction with select clades collapsed for clarity; see Fig. 1 for the full Taxa70 phylogeny. Green stars indicate taxa identified as potentially involved in a hybridization event with *Drimiopsis botryoides* subsp. *botryoides* CCH153 [green polygon] in randomly subsampled replicate analyses. Given these results, we hypothesize an ancient hybridization event involving an ancestor of this subclade. Orange circles indicate nodes with SH-aLRT and ultrafast bootstrap values below 80 and 95, respectively. b) The number of relevant gene trees (i.e., those with the clade(s) of interest) that support or reject clades of interest. The first three bars show the number of gene trees that support the placement of *Ledebouria* sp. 1 Mozambique [phylogenetic placement marked by a pink diamond in a] in relation to *Ledebouria* Clade B and the Ledebouriinae. The middle four bars show the number of genes that support the placement of *Drimiopsis botryoides* subsp. *botryoides* CCH153 within the subclade of *Ledebouria* Clade A where an ancient hybridization event may have occurred. The last bar shows the number of gene trees that support a monophyletic *Ledebouria*.

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A surprisingly high number of gene trees placed *Drimiopsis botryoides* subsp. *botryoides* CCH153 within a subclade of *Ledebouria* Clade A (Fig. 3b; Supplementary Table 2). The DiscoVista results complement the results from SNaQ, which placed a potential hybridization event between the same *Drimiopsis* sample and several taxa within *Ledebouria* Clade A (Fig. 3a; Supplementary Fig. 12). Interestingly, this *Ledebouria* subclade also has the lowest supported nodes within the entire phylogeny (Fig. 2, 3a).

For both the full dataset and Taxa70 dataset, SVDQuartets returned the same overall results as IQ-Tree and ASTRAL with a few notable exceptions (Supplementary Figs. 9 – 12). Low bootstrap values were found across the full dataset phylogeny, and *Resnova lachenalioides* was placed within *Ledebouria* Clade B (Supplementary Fig. 9, 10). In both datasets, SVDQuartets placed *Ledebouria* sp. 1 Mozambique sister to the remaining Ledebouriinae (Supplemental Figs. 9 – 12). This complements the results from DiscoVista, which reported more gene trees supporting *Ledebouria* sp. 1 Mozambique as sister to the Ledebouriinae than those that included it within the clade (Fig. 3b; Supplementary Table 2). The Taxa70 SVDQuartets bootstrap tree placed *Drimiopsis* as sister to *Resnova* + *Ledebouria* Clade A, but with the lowest bootstrap support of all the nodes (Supplemental Fig. 12). Lastly, SVDQuartets placed *Drimiopsis botryoides* subsp. *botryoides* CCH153, the same sample implicated in hybridization (Fig. 3a; Supplementary Fig. 13), within *Ledebouria* Clade A (Supplemental Figs. 9 – 12).

DISCUSSION

We provide the first phylogenomic insights into the Ledebouriinae. Four major clades were recovered: *Ledebouria* Clade A, *Resnova*, *Drimiopsis*, and *Ledebouria* Clade B (Figs. 1b, 2, Supplementary Figs. 1 – 6). The low number of informative genes coupled with coalescence

and concordance analyses suggest that incomplete lineage sorting due to rapid radiations may have played a significant role in the group's evolutionary history. Additionally, historical hybridization may be contributing to substantial phylogenetic discordance within the group, particularly within a subclade of *Ledebouria* Clade A (Fig. 3). Past phylogenetic studies of the Ledebouriinae have recovered conflicting signal or low resolution along the backbone of the group (Manning et al. 2003; Pfosser et al. 2003; Lebatha et al. 2006; Wetschnig et al. 2007; Pfosser 2012). The focus of these studies has largely been on whether *Drimiopsis* and *Resnova* constitute two distinct taxa or are deeply nested within *Ledebouria* (Manning et al. 2003; Lebatha et al. 2006; Manning and Goldblatt 2012). Our findings suggest that delimitation of *Drimiopsis* and *Resnova* has been a misguided focus since both have easily distinguishing characteristics, such as tepal shape, stamen positioning, and non-stipitate ovaries (Lebatha 2004; Lebatha et al. 2006). Perhaps a more appropriate goal is to uncover diversity within *Ledebouria*, and we recommend attention should now shift to delimiting and interpreting the history of its two independent lineages. Reconfiguring both the broad- and fine-scale taxonomy of the Ledebouriinae is beyond the scope of this work, but our results lead us to question the status of *Ledebouria* sensu Manning (2003).

The current geographic distribution of the Ledebouriinae is reflected within the phylogeny and provides clues as to potential historical factors that have shaped their evolution. For example, the two *Ledebouria* clades overlap geographically in eastern Africa, which now serves as a melting pot, center of diversity, area of endemism, and biogeographical hub for many different African plants and animals (Lebatha 2004; Zhou et al. 2011; Lorenzen et al. 2012; Dagallier et al. 2020). Similar influences may have also shaped the current diversity and distribution of *Drimiopsis*, which, based on our limited sampling, consist of two clades: a South

African clade, and a northern African clade (Fig. 1). These *Drimiopsis* clades are distinguishable by morphological traits and different ploidy level (Stedje and Nordal 1987; Lebatha 2004). Surprisingly, SVDQuartets consistently, albeit with low support, placed the East African *Drimiopsis botryoides* subsp. *botryoides* (CCH153) within *Ledebouria* Clade A. Our SNaQ analyses implicate this taxon in a potential hybridization event involving an ancestral taxon within *Ledebouria* Clade A (Fig. 3a; Supplementary Fig. 12), and 15 gene trees support its placement within this subclade and a further 48 gene trees weakly reject its placement in the subclade (Fig. 3b; Supplementary Table 2). Today, sympatric populations of all Ledebouriinae can be found in sub-Saharan Africa, with the highest sympatry in eastern South Africa (Venter 1993; Lebatha 2004). Therefore, it is not unreasonable to hypothesize that ancestral populations of some *Drimiopsis* and *Ledebouria* Clade A may have overlapped in distribution leading to potential hybridization. East African *Drimiopsis* are known polyploids (Stedje and Nordal 1987), which lead us to suspect that hybridization may be at play in these lineages. However, more extensive sampling of *Drimiopsis* is needed to fully test any hypotheses regarding historical hybridization within the Ledebouriinae.

Ledebouriinae taxa are continually being described (Hankey et al. 2014; Cumming 2018; Hankey 2020), and much remains to be uncovered about their diversity (Howard 2014). This is exemplified by *Ledebouria* sp. 1 Mozambique, whose placement has 1) low support in the IQ-Tree and ASTRAL trees (Fig. 2), 2) has different placement in the IQ-Tree and SVDQuartet trees (Fig. 2a, Supplementary Figs. 9 – 10), 3) has the lowest quartet fidelity value (i.e., a measure for rogue taxa) of all the tips (Taxa70 dataset; Supplementary Fig. 14), 4) is found sister to the Ledebouriinae in several gene trees (Fig. 3b; Supplementary Table 2), and 5) exhibits unique morphological characteristics compared to currently known Ledebouriinae (e.g., seeds

possess a rostrate hilum giving them a distinctive pointed appearance; Supplementary Fig. 15). Whether this sample represents a distinct lineage remains to be understood.

Our results showcase the value of extending the phylogenomic repertoire to groups with unexplored potential. Our use of the Angiosperms353 probe set was vital for uncovering a revealing Ledebouriinae phylogeny, but a custom probe set may prove even more informative. Our analyses suggest that gene tree conflict due to incomplete lineage sorting and hybridization is prevalent within the phylogeny (Table 1; Fig. 3), and several short backbone branches suggest rapid radiations (Fig. 2). Increasing *Ledebouria* samples from Central and West Africa as well as significantly improving sampling of *Resnova* and *Drimiopsis* will be necessary to fully capture the intricate genomic evolution of the group. The role that incomplete lineage sorting and hybridization have played in historically obscuring the evolutionary history of the Ledebouriinae present an exciting opportunity for further study.

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Disclosure Statement

The authors have no conflict of interests to disclose.

Data Availability Statement

The raw genomic reads for each sample used in this study are available on the SRA (BioProject ID: PRJNA721471). Supplementary Methods, Results, and Figures as well as alignment files, individual gene trees, and output files for each analysis are available on Dryad (DOI: *upon acceptance*).

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SUPPLEMENTARY TABLES

Supplementary Table 1. List of specimens used in this study. Specimens include only those returned after adjusting for sequences coverage and removing sequences with paralogy warnings.

Supplementary Table 2. Number of gene trees that support or reject defined clades in DiscoVista. See Fig. 3 for clades/samples defined in the analysis.

Supplementary Table 3. SNaQ likelihood values for each of the ten randomly sampled subsets of the Ledebouriinae phylogeny when allowing 0 (H0) – 2 (H2) hybrid edges. Supplementary Fig. 13 shows the ten subset phylogenies.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Maximum likelihood phylogenetic reconstruction using IQ-Tree of the full dataset. Branch labels show SH-aLRT support value/Ultrafast bootstrap support value/gene concordance factor/site concordance factor. Strong support is branches with SH-aLRT and UFBoot values above 80 and 95, respectively.

Supplementary Figure 2. Species tree estimation of the full dataset inferred from ASTRAL. Branch labels indicate local posterior probability (LPP) support values.

Supplementary Figure 3. Maximum likelihood phylogenetic reconstruction using IQ-Tree of the Taxa70 dataset. Branch labels show SH-aLRT support value/Ultrafast bootstrap support

value/gene concordance factor/site concordance factor. Strong support is branches with SH-aLRT and UFBoot values above 80 and 95, respectively.

Supplementary Figure 4. Species tree estimation of the Taxa70 dataset inferred from ASTRAL. Branch labels indicate local posterior probability (LPP) support values.

Supplementary Figure 5. Maximum likelihood phylogenetic reconstruction using IQ-Tree of the Taxa70 dataset with *Ledebouria* sp. 1 Mozambique removed from the analysis. Branch labels show SH-aLRT support value/Ultrafast bootstrap support value/gene concordance factor/site concordance factor. Strong support is branches with SH-aLRT and UFBoot values above 80 and 95, respectively.

Supplementary Figure 6. Species tree estimation of the Taxa70 dataset with *Ledebouria* sp. 1 Mozambique removed inferred from ASTRAL. Branch labels indicate local posterior probability (LPP) support values.

Supplementary Figure 7. Maximum likelihood phylogeny with quartet sampling values drawn on branches using the full dataset. Values on branches are quartet concordance (QC)/quartet differential (QD)/quartet informativeness (QI).

Supplementary Figure 8. Maximum likelihood phylogeny with quartet sampling values drawn on branches using the Taxa70 dataset. Values on branches are quartet concordance (QC)/quartet differential (QD)/quartet informativeness (QI).

Supplementary Figure 9. Species tree estimation of the full dataset using singular value decomposition quartet species-tree estimation (SVDQuartets). Phylogeny was reconstructed using an exhaustive search.

Supplementary Figure 10. Species tree estimation of the full dataset using singular value decomposition quartet species-tree estimation (SVDQuartets). Phylogeny was reconstructed using an exhaustive search. Branch labels indicate bootstrap support from 100 bootstrap replicates.

Supplementary Figure 11. Species tree estimation of the Taxa70 dataset using singular value decomposition quartet species-tree estimation (SVDQuartets).

Supplementary Figure 12. Species tree estimation of the Taxa70 dataset using singular value decomposition quartet species-tree estimation (SVDQuartets). Phylogeny was reconstructed using an exhaustive search. Branch labels indicate bootstrap support from 100 bootstrap replicates.

Supplementary Figure 13. SNaQ results from ten randomly sampled subsets of the Ledebouriinae phylogeny. Network 1 reports results from analyses that allowed 1 hybrid edge (h=1), and network 2 reports results from analyses that allowed up to 2 hybrid edges (h=2) for each replicate. Dark blue lines indicate the major hybrid edge, light blue lines indicate the minor

edge. Inheritance probabilities are provided on the edges. Gray lines connect branches that are rotated across the two phylogenies.

Supplementary Figure 14. Maximum likelihood phylogeny showing quartet fidelity (QF) values for each of the samples in the Taxa70 dataset. Values represent a measure of taxon “rogueness”, that is, a measure of how much a tip jumps around a phylogeny.

Supplementary Figure 15. *Ledebouria* sp. 1 Mozambique (a) showing the pronounced rostrate hilum that sets it apart from other Ledebouriinae (e.g., image b) that typically have more spherical seeds.