

The progression of aridity in Africa and its effects on plant evolution.

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Background

Geophytes, terrestrial plants with an underground perennating organ, typically in the form of a bulb, corm, tuber or rhizome, occupy a variety of habitats, particularly those that experience seasonal periods of environmental stress such as freezing temperatures or drought (Raunkiaer 1934). Despite having arisen relatively recently, arid to semi-arid habitats harbor a high amount of biodiversity (Potter and Szatmari 2009), and represent one ecosystem where geophytes can be especially diverse. However, numerous dry-adapted plant clades, especially geophytes, remain poorly understood and the true diversity of many remains unknown, limiting our ability to fully understand the evolutionary dynamics of arid ecosystems. With the continued threat of climate change and expanding desertification, we must increase studies on groups that have evolved in arid climates in order to deepen our understanding of evolution within, and of, desert-like habitats. One such group is the Ledebouriinae, which are a member of the Scilloideae (Asparagaceae), and consists of three closely related taxa: *Ledebouria*, *Drimiopsis*, and *Resnova*.

Objectives

1) Improve our knowledge of Ledebouriinae phylogenetic relationships.

Previous phylogenetic studies have failed to provide resolution within the Ledebouriinae due to a lack of adequate taxon sampling and informative molecular markers (Lebatha et al. 2006; Pfosser 2012). Plastid genes have failed to provide resolution, leading us to suspect that putative rapid speciation leading to incomplete lineage sorting or hybridization has occurred in this group. Therefore, multiple, rapidly evolving nuclear markers, which are bi-parentally inherited, in addition to plastid genes are likely necessary to confidently clarify the historical evolution of the Ledebouriinae.

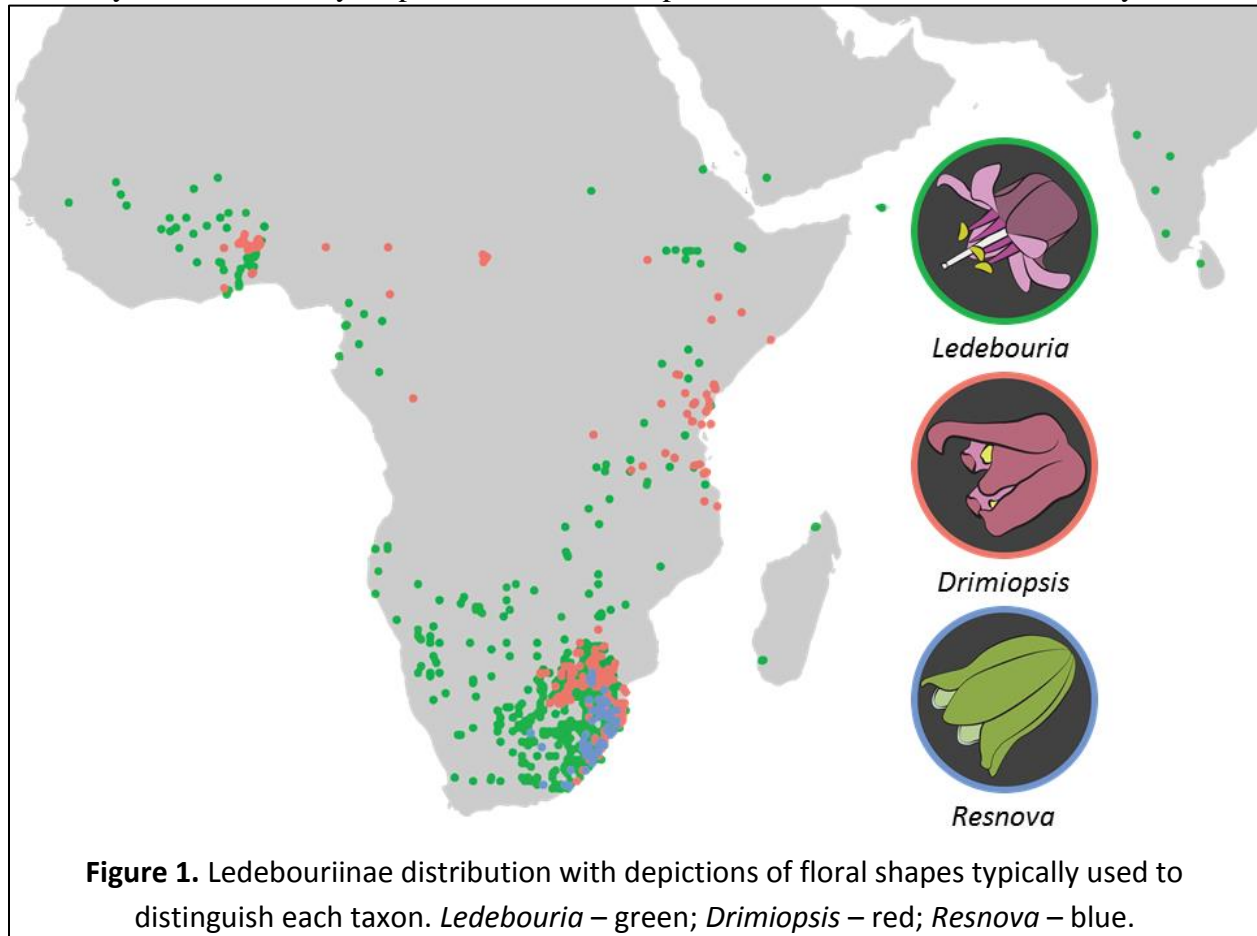
2) Discover the biogeographical history of the Ledebouriinae.

Ledebouria occupy diverse habitats in arid to semi-arid environments throughout sub-Saharan Africa, Madagascar, the Arabian Peninsula, and India, whereas *Drimiopsis* is only widespread in sub-Saharan Africa, and *Resnova* appears to be restricted to the SE corner of Africa (Fig. 1). I hypothesize that the origin of *Ledebouria* is old enough that individuals were capable of utilizing land bridges that existed between Africa and the Arabian Peninsula, allowing it to further disperse to India (i.e., its current distribution is explained by vicariance) (Smíd et al. 2013). However, its arrival to Madagascar is due to long dispersal events as suggested by (Pfosser et al. 2003). Furthermore, I hypothesize that *Drimiopsis* is an African endemic due to a more recent origin and the reformation of the Red Sea thwarted any chance of dispersal to the Arabian Peninsula. Lastly, I hypothesize that the origin of *Resnova* is even more recent and of hybrid origin, explaining its current restricted distribution in SE Africa (blue, Fig. 1). Unfortunately, the answers as to the cause of this group's intriguing distribution have yet to be fully investigated since previous biogeographical studies were missing samples from critical geographical areas (e.g., Socotra, Eastern Africa).

3) Detail the expansion of aridity in sub-Saharan Africa.

Preliminary dating analyses of the Ledebouriinae have recovered an age estimate of approximately 19 MYA, which coincides with the onset of global cooling resulting in increased global aridity. Prior to this event, the ancestor of Ledebouriinae likely already possessed traits (e.g., an underground bulb) that would give all progeny an advantage in surviving these harsher

conditions. Therefore, I hypothesize that the Ledebouriinae evolved in SW Africa along with increased aridity. I propose SW Africa as an origin since our current understanding of Scilloideae relationships shows the Ledebouriinae as being sister to a clade that is largely found in the SW region of southern Africa (Pfosser et al. 2003). Additionally, this region is suggested as the first area of Africa where aridity took hold (Senut et al. 2009). If my hypothesis holds true, tracking the biogeographic dispersal of the Ledebouriinae within Africa would allow us to indirectly infer how aridity crept across the landscape and how this affected biodiversity.



Significance

In addition to addressing the above three objectives, a taxon-rich and well-resolved Ledebouriinae phylogeny will allow us to investigate important, broad evolutionary processes that influence the diversification of all life. Currently, we are acquiring genome size estimates of this clade, which will improve our capabilities of understanding genome evolution in geophytes, many of which are known for large amounts of DNA content (Veselý et al. 2011). Additionally, the Ledebouriinae are densely sympatric in SE Africa (see Fig. 1), and increased genomic resources will allow us to investigate factors promoting or inhibiting sympatric speciation. Furthermore, available studies have yet to provide comprehensive biogeographic analyses of arid-adapted African plant groups. Uncovering the historical response of organisms to a changing climate is incredibly important, especially since little is known about the mechanisms shaping them across the African landscape. Today, faced with a rapidly changing climate and continued land alterations, understanding the patterns of biodiversity and its historical response to environmental pressures is vital for long-term conservation efforts.

Methods

Over 250 living samples of Ledebouriinae from Namibia, South Africa, Tanzania and Zambia, in addition to dried material from Mozambique, West Africa, Socotra, Yemen, and Sri Lanka have been obtained with corresponding geolocation data. To resolve relationships between closely related species, I will target multiple, rapidly-evolving, single-copy loci spanning intron-exon boundaries. I propose to utilize Next-Generation Sequencing (Illumina; MiSeq) in combination with a hybridization-based targeted enrichment approach (using biotinylated single stranded RNA probes) to capture informative regions. Two Scilloideae transcriptomes from the 1KP project (Matasci et al. 2014) will be used to discover putative single-copy loci by using MarkerMiner (Chamala et al. 2015). Using HybPiper (Johnson et al. 2016), the MarkerMiner output will then be used to analyze seven readily available Scilloideae genome sequence skims (from Steele et al. 2012) in order to design baits, which will be built my MYBait. Library prep will be completed in-house. Sequencing will occur at the University of Florida's Interdisciplinary Center for Biotechnology (ICBR). Whole plastomes and whole mitogenomes will be assembled using closely related taxa as references, and used to assemble and filter out off-target reads from the Illumina data. Furthermore, morphological data will be gathered and analyzed both separately and in combination with the molecular data. This resulting dataset will be the first of its kind for the Scilloideae, and will be highly useful to both Scilloideae and Asparagaceae researchers. Molecular datasets will be analyzed using Maximum Likelihood (e.g., RAxML, Stamatakis 2014) and coalescent (e.g., *BEAST, Bouckaert et al. 2014; ASTRAL, Mirarab et al. 2014) approaches. By using the plethora of biogeographical models implemented in BioGeoBears (Matzke 2013), I will estimate ancestral ranges to investigate potential dispersal routes of the Ledebouriinae within and out of Africa. I will also investigate putative instances of hybridization and/or ILS using PhyloNetworks (Solís-Lemus et al. 2017). I will investigate ploidal evolution in the Ledebouriinae ($n = 5-33$) by gathering estimated genome sizes using flow cytometry (Dolezel et al. 2007). Morphological character evolution will be investigated using ancestral state reconstruction methods as implemented in the R package *ape*.

Schedule:

Current stage: specimens have been obtained; flow cytometry is currently underway; DNA extractions are underway; MarkerMiner analyses are underway; HybPiper steps will occur once MarkerMiner output has been analyzed

Summer 2018 – specimen vouchering, DNA extraction, probe design

Fall 2018 – Library prep, DNA sequencing

Spring 2018 – Data analysis

Summer 2019 – Manuscript preparation

Literature Cited

Bouckaert R., et al. 2014. PLoS Comput. Biol. 10:e1003537; Chamala S., et al. 2015. Appl. Plant Sci. 3; Dolezel J., et al. 2007. Nat. Protoc. 2:2233–2244; Johnson M.G., et al. 2016. Appl. Plant Sci. 4; Lebatha P., et al. 2006. Taxon. 55:643–652; Matasci N., et al. 2014. Data access for the 1,000 Plants (1KP) project. Gigascience. 3:17; Matzke N.J. 2013. R package, version 0.2. 1; Mirarab S., et al. 2014. Bioinformatics. 30:i541–8; Pfosser M., et al. 2003. J Plant Res. 116:115–132; Pfosser M. 2012. Plant Ecol. Evol. 145:65–72; Potter P.E., Szatmari P. 2009. Earth-Science Reviews. 96:279–295; Raunkiaer C. 1934; Senut B., et al. 2009. Comptes Rendus Geoscience. 341:591–602; Smíd J., et al. 2013. PLoS One. 8:e64018; Solís-Lemus C., et al. 2017. Mol. Biol. Evol. 34:3292–3298; Stamatakis A. 2014. Bioinformatics. 30:1312–1313; Steele P.R., et al. 2012. Am. J. Bot. 99:330–348; Veselý P., et al. 2011. Ann. Bot. 109:65–75.

Budget

Funds from the SSB GSRA will be applied towards purchasing MYBaits probes. Sequencing will occur at the University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR). **All necessary specimen samples for testing my hypotheses have been obtained.**

Costs

Illumina sequencing (1 lane) = \$2,800

MYBaits Probes = \$2,000

Total: \$4,800.00

Requested: \$2,000.00

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EDUCATION

- In progress* Ph.D. Botany — University of Florida
Faculty supervisor: Dr. Nico Cellinese
- 2009 B.S. Horticulture Science, German minor — University of Arkansas, Fayetteville
Advisor: Dr. Jon T. Lindstrom (deceased)

PROFESSIONAL EXPERIENCE

- 2009 – 2014 Conservation Technician — Huntington Botanical Gardens

GRANTS & AWARDS (totaling \$17,200 USD)

- 2018 John Paul Olowo Memorial Fund Research Grant (\$300)
- 2017 Botanical Society of America Travel Award (\$2,400)
International Botanical Congress Excellent Scholar/Outstanding Student Award (\$1,200)
- 2016 University of Florida Research Abroad for Graduate Students Award (\$7,000)
American Society of Plant Taxonomist (\$750)
Mary Sue Ittner Bulb Research Grant (Pacific Bulb Society) (\$250)
Cactus & Succulent Society of America Research Grant (\$2,000)
University of Florida David Graduate Fellowship in Botany (\$300)
- 2015 Florida Museum of Natural History Travel Award (\$1,000)
- 2013 Mary Sue Ittner Bulb Research Grant (Pacific Bulb Society) (\$500)
- 2011 Cactus & Succulent Society of America Research Grant (\$1,500)

PRESENTATIONS

Oral Presentations

- 2017 **Howard CC**, J Beaulieu, N Cellinese. The Monocotyledonous Underground: excavating the evolutionary history of geophytes. *International Botanical Congress* (China).
- 2016 **Howard CC**, and N. Cellinese. Buried Treasures: the wonderful underground life of bulbs. *Botany Conference*.

Posters

- 2017 **Howard CC**, E Sessa, J Leebens-Mack, CD Specht, N Cellinese. Geophytic Organisms — Ontogeny & Phylogeny (GOOPhy). *International Botanical Congress* (China).
- 2016 **Howard CC** and N Cellinese. Combing Africa: preliminary attempts at untangling the historical evolution of the Ledebouriinae (Scilloideae, Asparagaceae). *Evolution Conference*.

EDITORIAL DUTIES

- Cactus & Succulent Journal* — Assistant Editor 2013 – present