Introduction

The unique and endangered flora of the *campos rupestres* occurs across three phytogeographic domains in Brazil: *Cerrado*, *Mata Atlântica*, and *Caatinga*. Harsh environmental conditions and discontinuity foster the occurrence of many microhabitats, promoting not only an incredibly high level of plant diversity, but also a high level of endemism (1). About 15% of the Brazilian flora occurs in the *campos rupestres*, in an area equivalent to less than 1% of the country's territory (2). Investigating this rich vegetation type is necessary to understand major questions concerning its origin, composition, and diversity (3). A paucity of studies in comparative phylogeography, dated phylogenies, and niche modeling prevents a better understanding of diversification patterns in the *campos rupestres* (2). Also, the role of the *campos rupestres* as putative interglacial refugia may be elucidated through the investigation of the region's biogeography and modeling of ancestral niches.

Moreover, a better understanding of this vegetation type benefits the development of informed conservation strategies; the *campos rupestres* have been under longstanding threat due to extraction of local flora, cattle-grazing, highway construction, tourism, and fire (4). Recent estimates suggest that the area occupied by the *campos rupestres* flora may diminish by 50% by the year 2080 (5). Thus, an important next step to provide crucial information for better conservation practices is to investigate predictions of future plant distributions through niche modeling and ecological forecasts.

There are several facets to my ongoing research. On a broad scale I want to understand the phylogenetic diversity, endemism and floristic turnover of the *campos rupestres*. A second key component of my comprehensive study of the *campos rupestres* and the focus of this proposal is the detailed analysis of a single plant clade as an exemplar. The large, pantropical angiosperm genus *Begonia* L. is represented in the *campos rupestres* by fewer than 20 species (6). Although several new species have been described, the genus still requires more attention in Brazil, where it has not been examined since the second half of the 19th century (6). Moonlight et al. (2018) produced the most complete phylogeny for *Begonia* based on both molecular and morphological characters including samples from 574 species. Circumscription of some of the groups was significantly modified to reflect monophyly. *Begonia* Section *Tetrachia* Brade now includes 16 species instead of only one as previously circumscribed based solely on morphological characters (7). However, 10 of the 16 species have not been included in the phylogeny, leading to a poorly understood clade.

Most species in *Tetrachia* occur in the *campos rupestres*, in all three phytogeographic domains (*Cerrado*, *Mata Atlântica*, and *Caatinga*). A few species occupy other vegetation types, such as forests, and some species occupy both forests and *campos rupestres*. Moreover, some species are widely distributed, while others are narrow endemics. *Begonia* Sect. *Tetrachia* is therefore an excellent model for investigating how geography drives evolution both among localities within a phytogeographic domain and/or vegetation types and among populations from different domains and/or vegetation types. Few plant clades from the *campos rupestres* present this pattern of distribution and comprise a modest number of species, enabling thorough analysis of diversification. Moreover, given its geographic distribution across phytogeographic domains, *Begonia* Sect. *Tetrachia* is an excellent model to investigate how climatic changes projected for 2080 will impact the flora of the *campos rupestres*. Sect. *Tetrachia* contains narrow endemics and widely distributed species, allowing for a broader and more complete analysis of putative shifts in niche. Finally, three species within Sect. *Tetrachia* are listed as endangered (*B. albidula* Brade, *B. ibitiocensis* E.L. Jacques & Mamede, *B. kuhlmannii* Brade) and one (*B. ruschii* L.

Kollmann) as critically endangered (8 – 11), making it even more urgent to understand this clade.

Objectives

The proposed project will address the following questions:
1 – How are the species within *Begonia* Sect. *Tetrachia* evolutionarily related? Does the current classification properly reflect the phylogeny?
2 – Are the species of *Begonia* Sect. *Tetrachia* that occur in the same phytogeographic domains and/or vegetation types more closely related than expected by chance, indicating phylogenetic clustering, or more distantly related, indicating phylogenetic overdispersion?
3 – Do diversification and niche evolution patterns in *Begonia* Sect. *Tetrachia* indicate that *campos rupestres* acted as an interglacial refugium, harboring cold- and dry-adapted plant species?
4 – How will future climatic changes projected for the year 2080 impact the future distribution of species within *Begonia* Sect. *Tetrachia*? What are the broader implications for the flora of the *campos rupestre*?

Material and Methods

To address these questions, a well-resolved and dated phylogeny of *Begonia* Sect. *Tetrachia* will be reconstructed. I will identify the biogeographic patterns that drive evolution in this group and model putative ancestral and current niches for all species. To conduct this study, a comprehensive sampling of *Begonia* Sect. *Tetrachia* is required, collecting multiple individuals per species, especially when these occur in different phytogeographic domains and/or vegetation types. Thus, field work will be paramount in obtaining the plant material needed for generating a complete and well-resolved phylogeny. DNA will be extracted from silica-dried leaf samples using a modified CTAB protocol (12), and target capture methods will be used to generate sequence data for ~100 nuclear loci as well as the entire plastid genome. To construct the phylogeny, both Bayesian and maximum likelihood methods will be used (e.g. MrBayes (13) and RAxML (14)) as well as multi-species coalescent methods that estimate a species tree from multiple gene trees (e.g. ASTRAL (15)). Other analyses will be performed to identify dates for major divergence events (e.g. BEAST (16)), diversification rates (e.g. Medusa (17)), and biogeographic patterns (e.g. BioGeoBears (18)). Lastly, niche models will be produced using MaxEnt (19) and the environmental and climatic layers available online (e.g. WorldClim (20)).

Significance and Broader Impacts

Understanding the evolutionary history of these *Begonia* species has broad implications – it will provide insight into the complex diversification processes that led to the rich and endemic flora of the *campos rupestres*, and this study will serve as an example for other plant lineages that occupy the same area. Furthermore, projecting niches for all *Begonia* species using future climate predictions will enable analysis of the vulnerability of these species, and by extension of the unique *campos rupestres* ecosystem, to climate change. Field work for this project will be conducted in collaboration with Dr. Peter Moonlight, a specialist on *Begonia*, who has contributed to this project since the very start. Collected material will be added to the herbaria at the State University of Campinas (UEC), where I have forged collaborators (Dr. Anete Pereira de Souza) and at the Florida Museum of Natural History (FLAS). These international collaborations will strengthen community building both between important institutions (University of Florida, State University of Campinas and Royal Botanic Garden Edinburgh) and between researchers at different life stages, promoting valuable information exchange and scientific growth. The results of this study, particularly in concert with my ongoing work on understanding the extraction of local flora from the *campos rupestres* by local communities, will help shape conservation priorities in this species-rich and unique environment.

Relevant Information

This project has received funding from the American Society of Plant Taxonomists ($1,200), The Botanical Society of America ($1,500) and the Biology Department of the University of Florida ($800).

Literature cited

[1] **Fernandes GW. 2016.** in Fernandes, Geraldo Wilson (ed.), *Ecology and Conservation of Mountaintop grasslands in Brazil*, Springer International Publishing, pp. 3–14. [2] **Silveira FAO et al. 2016.** *Plant and Soil* **403** (1): 129–152. [3] **Morellato LPC, Silveira FAO. 2018.** *Flora*. **238**: 1–10. [4] **Giulietti AM et al. 1997.** in Davis, SD; Heywood, VH; Herrera-Macbryde, O; Villa-Lobos, J; Hamilton, AC (eds.). *Centres of plant diversity: a guide and strategy for their conservation*. Cambridge: IUCN Publication Unit. pp. 397–404. [5] **Bitencourt C et al. 2016.** *Flora* **218**: 1–10. [6] **Moonlight PW et al. 2018.** *Taxon* **67**: 267–323. [7] **Doorenbos J et al. 1998.** *Agricultural University Wageningen Papers* **98**: 1–266. [8] **CNCFlora.** *Begonia albidula* in Lista Vermelha da flora brasileira versão 2012.2 Centro Nacional de Conservação da Flora. Available at <http://cncflora.jbrj.gov.br/portal/pt-br/profile/Begonia albidula>. Acess in January 15th, 2020. [9] **CNCFlora.** *Begonia ibitiocensis* in Lista Vermelha da flora brasileira versão 2012.2 Centro Nacional de Conservação da Flora. Available at <http://cncflora.jbrj.gov.br/portal/pt-br/profile/Begonia ibitiocensis>. Acess in January 15th, 2020. [10] **CNCFlora.** *Begonia kuhlmannii* in Lista Vermelha da flora brasileira versão 2012.2 Centro Nacional de Conservação da Flora. Available at <http://cncflora.jbrj.gov.br/portal/pt-br/profile/Begonia kuhlmannii>. Acess in January 15th, 2020. [11] **CNCFlora.** *Begonia ruschii* in Lista Vermelha da flora brasileira versão 2012.2 Centro Nacional de Conservação da Flora. Available at <http://cncflora.jbrj.gov.br/portal/pt-br/profile/Begonia ruschii>. Acess in January 15th, 2020. [12] **Doyle JJ, Doyle JL. 1987.** *Phytochemical Bulletin* **19**: 11–15. [13] **Ronquist F et al. 2012.** *Systematic Biology* **61**: 539–542. [14] **Stamatakis A. 2014.** *Bioinformatics* **30**: 1312–1313. [15] **Chao Z et al. 2018.** *BMC Bioinformatics* **19** (S6): 153. [16] **Suchard et al. 2018.** *Virus Evolution.* **4**, vey016. [17] **Alfaro ME et al. 2009.** Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the Natural Academy of Sciences USA* **106**: 13410–13414. [18] **Matzke, NJ. 2018.** published on GitHub on November 6, 2018. [19] **Phillips SJ et al. 2017.** *Ecography* **40**: 887–893. [20] **Fick SE, Hijmans RJ. 2017.** *International Journal of Climatology* **37**: 4302–4315.