

## Report: Les Mehrhoff Botanical Research Fund

### Phylogenetics and taxonomy of *Dichantherium*: a complex set of species-level problems in need of resolution

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#### Background

Taxonomy of *Dichantherium* (Poaceae) at the species level is a striking problem. Few authors agree on species circumscriptions in this genus, which has led to confusing and conflicting accounts, especially floristically. **The purpose of this study was to provide a phylogenetic framework for *Dichantherium* with dense taxon sampling and with multiple accessions per species (and those coming from across the range of each species) to test for monophyly of species and species complexes.**

To do this, extensive fieldwork was made across North America. With 300 samples (all vouchered), we sampled many populations from diverse geographic regions, focusing on eastern North America. For data acquisition, we took several approaches. First, we sequenced entire plastid genomes of 77 vouchered samples, utilizing Illumina HiSeq sequence data, then reference assembly to “genome skim” the plastid data. **The goal of this approach was to reconstruct deep phylogenetic relationships.** Second, for the full set of 300 collections, three DNA loci were targeted for amplification by PCR, followed by Sanger sequencing: ITS, *GBSSI*, and *rpl32-trnL*. **The goal of this approach was to assess species monophyly using intensive species-level sampling.**

#### Outcomes of this project

*Student mentorship and presentations* – Two undergraduates (Vignovich and Stonehouse) working in the Neubig lab at SIU received first hand research experience in the course of this project. These funds helped provide data for 6 different presentations this year: a native plant society talk and multiple scientific research presentations at both local (Illinois) and international (Savannah, Botanical Society of America) venues. Underlined names indicate students, bold name indicates PI, and asterisks indicate presenters:

- Vignovich W\*, Stonehouse G\*, Wood K, Abbott JR, Naczi R, Majure LC, **Neubig KM**. 2016. The origin and diversification of *Dichantherium* (Poaceae) in Hawaii. *Botanical Society of America, Savannah, Georgia [July 2016]*.
- Majure LC\*, **Neubig KM**, Abbott JR, Naczi R, Judd WS, Whitten WM. 2016. A preliminary look at phylogenetic relationships of *Dichantherium* (Paniceae: Poaceae). *Botanical Society of America, Savannah, Georgia [July 2016]*.
- Naczi R\*, **Neubig KM**, Majure LC, Abbott JR, Judd WS, Whitten WM. 2016. Systematics of the *Dichantherium ensifolium* clade (Poaceae: Paniceae). *Botanical Society of America, Savannah, Georgia [July 2016]*.
- Neubig KM\***, Vignovich W\*, Stonehouse G\*. 2015. Troubling panic grasses (*Dichantherium*, Poaceae): relationships, geography, and species complexes. *Illinois Native Plant Society, Carbondale, Illinois [April 2016]*.
- Stonehouse G\*, Vignovich W, Abbott JR, Naczi R, **Neubig KM**. 2016. Detecting interspecific hybrids in the panic grasses (*Dichantherium*, Poaceae). *UCARF, SIU [April 2016]*.

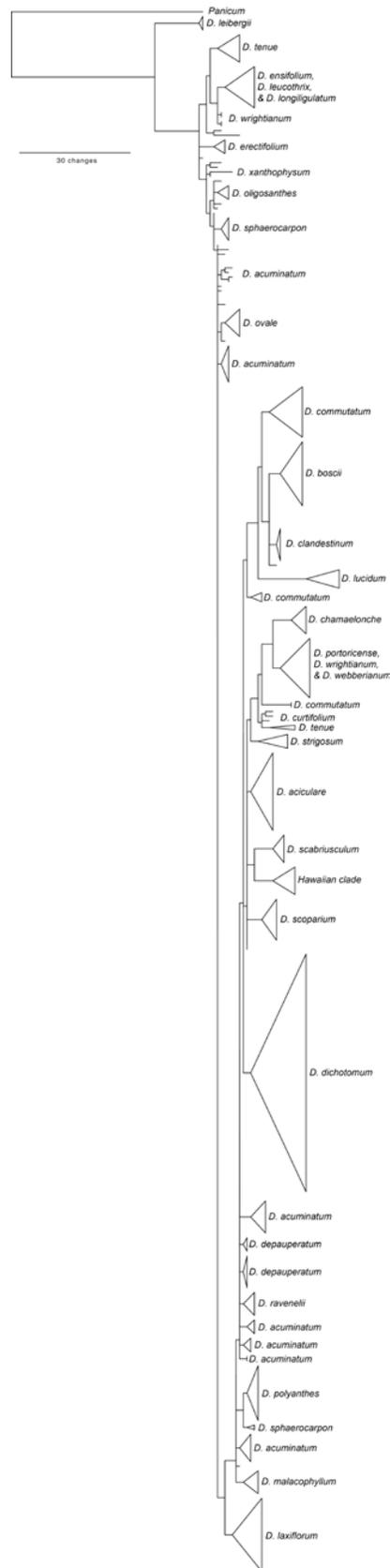
Vignovich W\*, Stonehouse G, Abbott JR, Naczi R, **Neubig KM**. 2016. The biogeography of the panic grasses (*Dichanthelium*, Poaceae): an unusual pattern of disjunct eastern North American and Hawaiian taxa. *UCARF, SIU [April 2016]*.

*Results* – With a two-pronged approach towards data acquisition, we were able to address the merit of these different approaches. Although “perfect” phylogenetic resolution among taxa is rarely possible, these new data are very useful at addressing two critical phylogenetic questions: what are the deeper relationships within the genus *Dichanthelium* and are the species and complexes monophyletic? The 3-locus phylogenetic tree (Fig. 1) has extensive taxon sampling, usually with multiple samples per species, and phylogenetic resolution at the species level, but poor resolution at broader levels. The monophyly of some species (e.g., *D. acuminatum*) is not supported with these data.

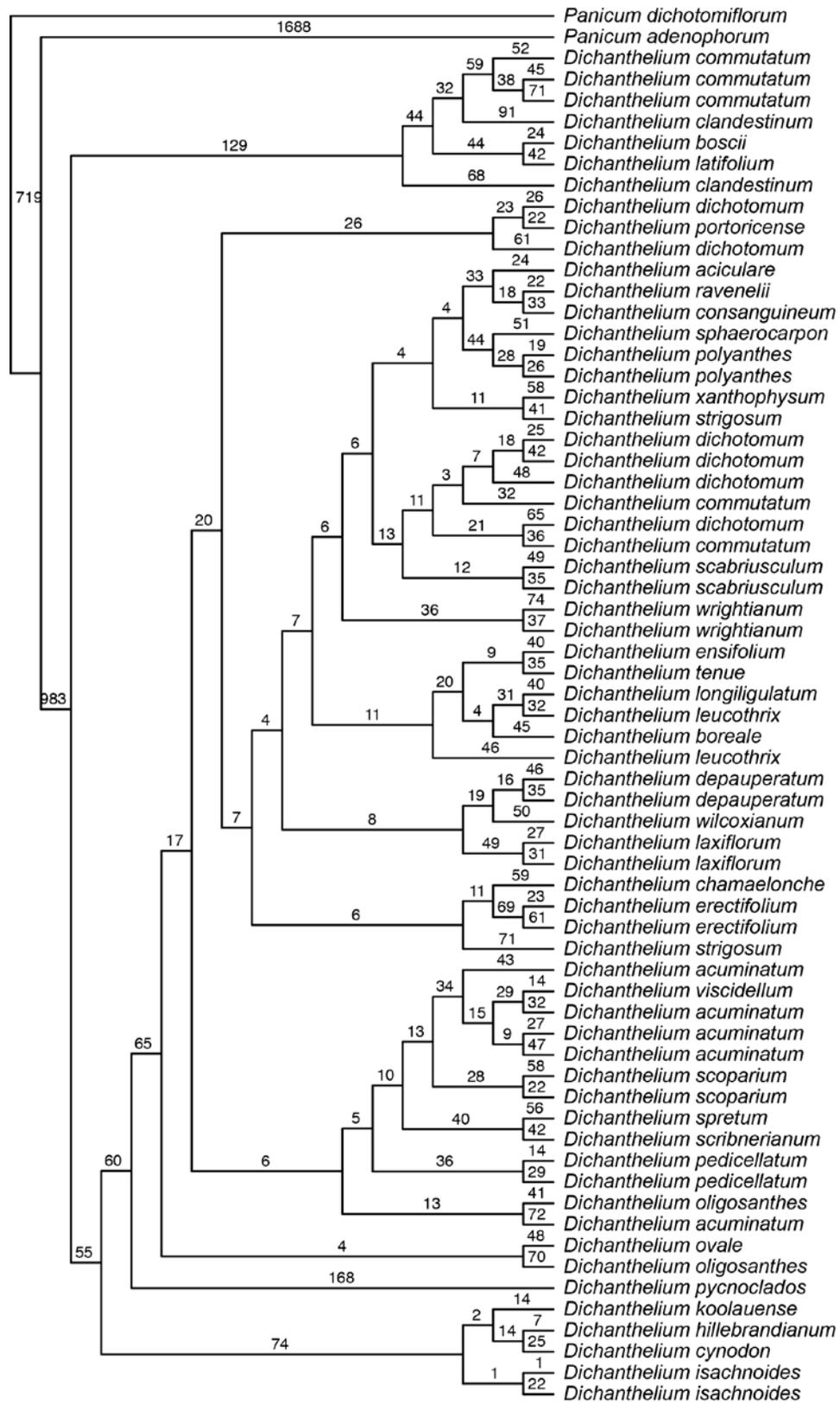
For the genome skimming approach, not all sequenced samples yielded usable data; only 66 out of 77 samples gave high sequence coverage and depth. The whole-plastome (plastid genome) phylogenetic tree (Fig. 2) includes most of the species in the genus, often with more than one sample per species, and there is greater resolution in the deeper nodes of the tree than in the 3-locus dataset. Each accession is represented by ~120,000 nucleotides, yet variation among some species groups is still remarkably low. Even more remarkably, for some species intraspecific variation is higher than interspecific variation (e.g., *D. depauperatum* & *D. scabriusculum*). Although we have gathered a great deal of data for both phases of this project, we are still in the preliminary analysis phase of this project. Also, we will likely target additional samples for genome skimming for critical taxon sampling based on phylogenetic results from the 3-locus dataset.

We expect that these cumulative DNA data will result in 2-4 peer-reviewed scientific publications.

Another critical outcome of this project is a practical consensus of alpha taxonomy at the species level within *Dichanthelium*. Collaborators Naczi and Abbott are developing a revision of Gleason and Cronquist's *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*. With these new data, we will be able to more objectively examine species circumscription in the group over the coming year.



**Figure 1.** Maximum parsimony cladogram including 300 OTUs for the 3 loci combined (ITS, *GBSSI*, *rpl32-trnL*) dataset. For a simplified presentation, many clades have been simplified to triangles.



**Figure 2.** Maximum parsimony cladogram (branch lengths shown above) of whole plastomes including 65 OTUs. Note that this is a large amount of data that will continue to be refined and edited; there are high levels of variation at both the interspecific and infraspecific levels.