The promises and pitfalls of herbarium phylogenomics:
A case study in a Neotropical plant radiation

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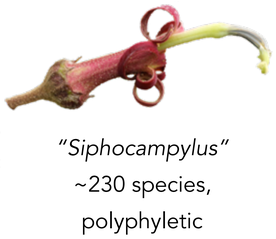
Background

- Herbaria have the ability to offer **wide phylogenetic sampling for relatively little effort** compared to field sampling
  - Historically, **degraded specimens' DNA** served as a barrier to Sanger sequencing
  - Recently, the **target enrichment**, or sequence capture, method has been **particularly effective** for animals specimens from natural history collections[1,2]

Burmeistera ~110 species, monophyletic



"Siphocampylus" ~230 species, polyphyletic

"Centropogon" ~210 species, polyphyletic

- **Centropogonid clade** of the Neotropical bellflowers (Campanulaceae: Lobelioideae)[3]:
  - ~550 species within *Burmeistera, Centropogon*, and *Siphocampylus* resulting from a **rapid radiation** centered in the Andes[4]
  - **Diverse floral morphology** which evolved in response to their hummingbird and bat pollinators

Questions:

- Can DNA from herbarium samples be used in phylogenomic analyses?
  - If yes, to what extent?
  - What **data quality standards** need to be used?
  - Will relationships **support prior findings?**

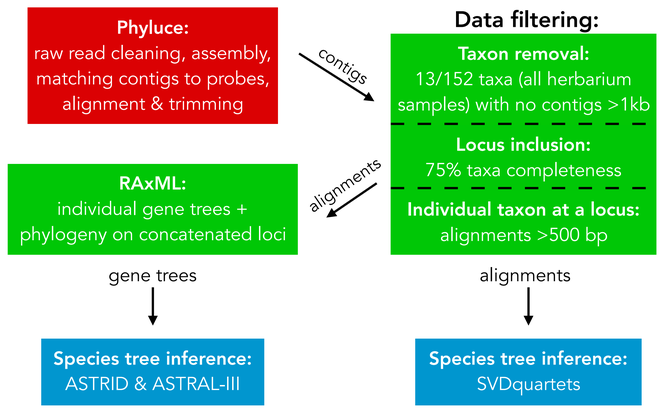
Acknowledgments

Thanks to the Grinnell College Careers, Life, and Service Center, which provided an Open Research Grant for this project. Additionally, my gratitude goes to Brant Faircloth, Carl Oliveros, Jessie Salter, and Genna Mount. Floral photographs are graciously provided by Laura Lagomarsino. This work was completed using LSU High Performance Computing.

Methods: sequencing

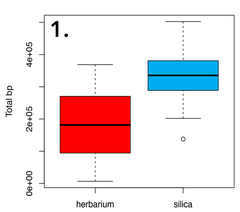
- Taxa selected to include species-level sampling in *Burmeistera* & to reflect phylogenetic diversity of centropogonid clade
  - **108 silica collected tissue, 44 herbarium samples**
- **Targeted sequence capture**, using probes designed from transcriptomes and shotgun sequences from *Burmeistera*
  - Final probe set targeted **745 loci**, with Illumina HiSeq 300 PE150 sequencing performed by RAPiD Genomics

Methods: bioinformatics pipeline



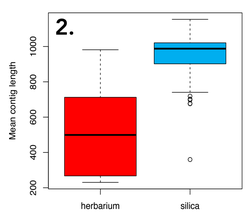
Results: herbarium efficacy

Total assembled bp
Mean 181k vs. 332k



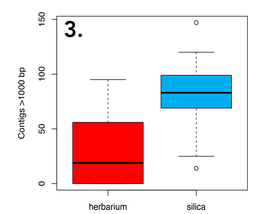
t=-8.60, p=1.086e-11

Avg. contig length (bp)
Mean 525 vs. 951



t=-10.86, p=8.44e-15

contigs >1kb
Mean 30 vs. 83

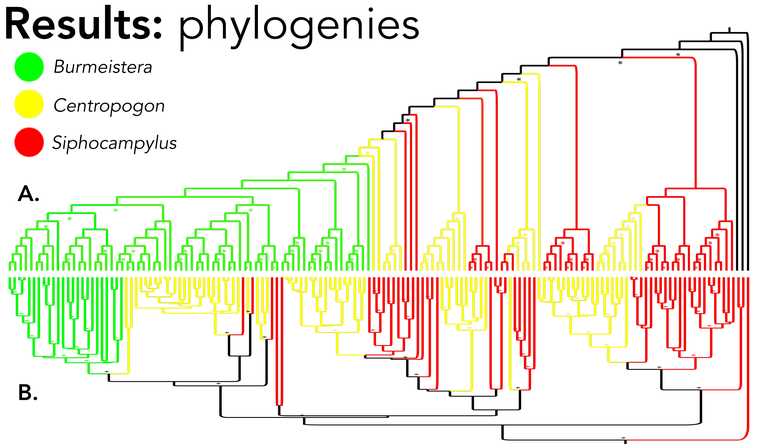


t=-10.34, p=4.18e-15

Herbarium samples underperformed compared to silica-dried samples

- Significant correlations between specimen age and each summary statistic (**1.** p=2.86e-08 **2.** p=1.06e-11 **3.** p=4.33e-09)

Results: phylogenies



A. Sequence capture phylogeny inferred with ASTRAL-III. **B.** 5 plastid gene phylogeny inferred with BEAST, Lagomarsino et al. 2014[3]. 90% bootstrap or posterior support is denoted with asterisks for both.

Discussion

- **Data quality filtering steps integral** to improving resolution and support of inferred trees
  - **Poor-quality specimens** (low-quality input DNA and no assembled contigs > 1kb) **attracted to the root**
  - All 13 taxa removed (no contigs > 1kb) from herbaria → **70.5% herbarium success rate**
  - Correlation between gene tree long outlier branch lengths and length of a taxon's alignment at a locus
- Our phylogenetic results (Fig A.) are **largely consistent** with relationships as previously understood[3]
  - **Better resolution** in phylogenetic relationships at a **deeper scale**
  - *Burmeistera* monophyletic, *Centropogon* and *Siphocampylus* polyphyletic with 7 and 9 subclades each

Herbarium specimens are a rich source of phylogenetic data, but they are not a silver bullet

References

[1] McCormack, J. E., Tsai, W. L. E. & Faircloth, B. C. Sequence capture of ultraconserved elements from bird museum specimens. *Mol. Ecol. Resour.* 16, 1189–1203 (2016).

[2] Blaimer, B. B., Lloyd, M. W., Guillory, W. X. & Brady, S. G. Sequence capture and phylogenetic utility of genomic ultraconserved elements obtained from pinned insect specimens. *PLoS One* 11, 1–20 (2016).

[3] Lagomarsino, L. P. *et al.* Phylogeny, classification, and fruit evolution of the species-rich Neotropical bellflowers (Campanulaceae: Lobelioideae). *Am. J. Bot.* 101, 2097–2112 (2014).

[4] Lagomarsino, L. P., Condamine, F. L., Antonelli, A., Mulch, A. & Davis, C. C. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytol.* 210, 1430–1442 (2016).