A MACROEVOLUTIONARY PERSPECTIVE ON SPECIES INTERACTIONS IN THE CARNIVOROUS PITCHER PLANT GENUS *SARRACENIA*

by

JESSICA D. STEPHENS

(Under the Direction of Russell Malmberg)

ABSTRACT

How species interactions influence ecological and evolutionary processes has been a driving interest among biologists. My dissertation research has largely focused on understanding the ecological and evolutionary outcomes of plant interactions with insects and microbes, and how these interactions have potentially lead to the diversification of carnivorous plants. I have focused this work on the genus *Sarracenia* (pitcher plants). Pitcher plants are found in nutrient poor habitats and have evolved complex trapping structures used in attraction, retention, and digestion of prey. Within these trapping structures are communities of microbes that may be involved in digestion of prey. These plants are highly dependent on insects to obtain nutrients, and prey capture can directly affect seed production. This dependence on prey is predicted to create intense competition among sympatric species leading to strong selection on traits related to prey attraction and capture, as well as selection on the plant microbiome to facilitate the digestion of prey. Insight into the patterns and processes of these interactions requires an explicit understanding of evolutionary relationships of interest. Often species level relationships in groups that exhibit highly convergent traits are the result of recent radiations, which can complicate phylogenetic analyses. Therefore, I used a combination of target enrichment and

recently developed coalescent methods to resolve relationships in these ‘difficult’ groups. I have used these techniques to resolve species level relationships in both the genus *Sarracenia* and also *Helianthus.* The resulting *Sarracenia* phylogeny was then used to assess whether this group has evolved suites of trapping traits to attract specific prey types through a common garden approach. Results indicate there are in fact strong correlations among suites of traits and the prey captured for each species across the entire genus of *Sarracenia*. Together these data support the hypothesis of carnivorous syndromes within the genus *Sarracenia*. Additionally, these plants rely on their microbiota to digest prey. The diversity and structure of these microbial communities is largely unknown. I sequenced the microbiome across all *Sarracenia* species in a common garden approach to examine whether host species, season, and/or year structured communities. Results suggest that there are significant differences in microbial communities with the majority of variation explained by pitcher plant species. This suggests that the plant is exerting some selection pressure on the microbiome community. Future work examining the proteome of *Sarracenia* could elucidate this process. Furthermore, experimental approaches across all these interaction types can further our understanding of the evolutionary of carnivory.

INDEX WORDS: gene capture, coalescent, phylogenomics, species tree estimation, plant interactions, plant-insect interactions, plant-microbe interactions

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CHAPTER I:

INTRODUCTION

Species diversification through biotic interactions

Interspecific interactions are agents of natural selection that can drive species diversification (Darwin, 1859; van Valen, 1973). These interactions, ranging from antagonistic to mutualistic, can structure community dynamics (Wisz *et al*., 2013, Mougi and Kondoh, 2014; Bever *et al*., 2015) and subsequently influence evolutionary processes (Ehrlich and Raven, 1964; Futuyma and Agrawal, 2009). For instance, flowering plant biodiversity is thought to be partially a product of pollinator-plant interactions (Fenster *et al.*, 2004; Sargent, 2004). Likewise, herbivore interactions are hypothesized to have resulted in the extensive repertoire of plant defensive traits (Agrawal and Fishbein, 2006). Understanding the role biotic interactions have in shaping rates and patterns of speciation is therefore of great interest in ecology and evolutionary biology.

Carnivorous plants promote a diverse array of interactions; these plants are attacked by herbivores and largely rely on insects as pollinators to increase outcrossing, but they also must attract and capture insects to obtain vital nutrients (Folkerts, 1999). Carnivory in plants has evolved independently nine times resulting in roughly 583 species worldwide (Givnish, 2015). These plants have evolved complex trapping structures that are used to attract, capture, and digest prey and absorb nutrients in order to survive in nutrient poor habitats (Givnish *et al*., 1984; Juniper *et al.*, 1989). Because of this dependence on prey there is predicted to be strong selection on traits related to prey capture, especially among conspecifics, and this is thought to be the main

mechanism the has resulted in tremendous morphological diversity at both the infrafamilial and infrageneric level (Ellison and Gotelli, 2001; Ellison and Gotelli, 2009). In addition to insect interactions, carnivorous plants often harbor diverse and complex microbial communities hypothesized to facilitate the breakdown and digestion of insect prey, similar to animal guts (Koopman *et al.*, 2010; Sickel *et al.*, 2016).

Recent attention has begun to focus on the role that pathogens and other microbes have in shaping evolution. However, our current understanding of microbes as drivers of eukaryote evolution has mostly come from examining animal systems (Shapira, 2016). In these systems the microbiome is known to influence eukaryote development (Shin *et al*., 2011), behavior (Collins *et al*., 2012), and even host speciation (Brucker and Bordenstein, 2013). With that said, recent work examining the plant microbiome has established that microbes are a key to structuring plant communities and maintaining plant diversity (e.g., Bever *et al*., 2015) and can be linked to plant health and productivity (Berendsen *et al*., 2012). Furthermore, microbes can have downstream influences on other plant interactions, such as pollinator behavior (Good *et al*., 2014; Vannette and Fukami, 2016) and plant-plant interactions (Bever *et al*., 2015). These interactions, in addition to pollinator and herbivore interactions, highlight the complex biotic environment that plants must be able to navigate, often leading to different selection pressures for each interaction.

The North American carnivorous plant genus *Sarracenia* is a particularly attractive system to investigate and enhance our understanding of how plant-insect-microbe interactions may have shaped diversification in carnivorous plants. These largely sympatric species have evolved highly modified leaves (i.e. pitchers) used in trapping, retention, and digestion of insect prey. Furthermore, there have been substantial divergences in pitcher morphology within the genus in a relatively short period of time (~3 million years,) suggesting that the genus has

undergone rapid speciation possibly due to morphological diversification in trapping strategies (Ellison *et al.*, 2012). Traits related to the attractive zone (e.g., shape, color, trichome density, height, volatiles) vary significantly among species (Juniper *et al*., 1989; Jürgens *et al.*, 2009) and ecological assessments have noted prey specialization (Folkerts, 1992; Stephens *et al.*, 2015a). Pitchers are completely sterile upon development (Peterson *et al*., 2008) and are quickly colonized by a diverse assemblage of distinctive microbes thought to aid in the digestion of prey (Koopman *et al*., 2010). Thus, the genus *Sarracenia* is an excellent system for exploring how multispecies interactions may have contributed to species diversification within carnivorous plants using a macroevolutionary perspective.

Resolving species level phylogenies

Examination of patterns and distributions of traits across species as a way to infer potential processes or their effects on other traits must account for species relationships (Felsenstein, 1985); closely related species are more likely to share similar traits and characteristics, which can confound statistical measures assuming independence. Accounting for phylogenetic history strengthens statistical tests by identifying independent evolutionary events for traits of interest (Harvey and Pagel, 1991). In a comparative framework, if traits correlate in independently evolving lineages, then these character states are assumed to have evolved in concert lending support to an adaptive hypothesis. Given this dependence on a phylogenetic tree to determine areas of convergent evolution, it is vital in comparative methods to have a well-resolved phylogenetic tree.

Current sequencing and phylogenetic methods have contributed greatly to resolving species relationships and reconstructing the tree of life, however this is not without methodological challenges (Delsuc *et al*., 2005). In an ideal scenario, each gene sampled would

have the same evolutionary history and therefore be congruent with the species tree. However, speciation events are not abrupt, but often a continuous process resulting in genetic exchange among diverging groups. This can lead to discordance between gene trees and species trees through such factors as incomplete lineage sorting (ILS), horizontal gene transfer, and hybridization, all of which can result in an erroneously inferred species tree (Pamilo and Nei, 1988; Doyle, 1992).

Gene tree discordance can make species-level phylogenies especially difficult to resolve; as young or recently radiated groups increase the likelihood that genes retain ancestral polymorphisms (i.e., ILS) due to short branch lengths (Pamilo and Nei, 1988) and reticulation within gene trees (i.e., hybridization) is more probable with closely related species (Hennig, 1966). Given these potential sources of gene tree discordance, the use of multilocus data should increase nodal support values and implementing the multispecies coalescent can appropriately model the variation in gene histories (Maddison, 1997; Degnan and Rosenberg, 2009; Knowles, 2009; Liu *et al*., 2009). Luckily, both recent methodologies to increase multilocus sampling (see Heyduk *et al*., 2016) and programs to model the process of gene tree discordance are rapidly emerging (e.g., STEM-hy – Kubatko, 2009; MP-EST – Liu *et al*., 2010; PhyloNet – Yu *et al.*, 2011, Yu and Nakhleh, 2015), making resolving species level phylogenies more feasible than ever before.

Dissertation research

In this dissertation, I used the most recent methods of increasing multilocus sampling and coalescent approaches to resolve the species level relationships in both *Helianthus* (Chapter II; Stephens *et al.*, 2015b) and *Sarracenia* (Chapter III; Stephens *et al.,* 2015c). Using the subsequent *Sarracenia* phylogeny from Chapter III, I sought to examine whether species within

the genus exhibited suites of covarying trapping traits and whether those suites of traits were associated with the type of prey captured in Chapter IV. The broad objective of this chapter was to elucidate whether carnivorous plant diversification may have been potentially driven by the unique relationship of plant and insect prey. Chapter V of this dissertation examined another aspect of carnivorous plant interactions, specifically the unknown relationship between the microbiome community thought to aid in digestion of prey and the host species. In this chapter, I was interested in examining the broad scale patterns of the *Sarracenia* microbiome across host species, seasons, and years with the goal of providing insight into this unique host-microbiome relationship. Finally, I have included a teaching research chapter that resulted from a future faculty pedagogical course I taught as a graduate student (Chapter VI; Stephens *et al*., *accepted*). I designed a research component to this course to examine how future faculty respond to teaching feedback in hopes to improve and give recommendations to teaching training courses in the future.

References

Agrawal AA, Fishbein M. 2006. Plant defense syndromes. *Ecology* 87: S132–S149.

Berendsen RL, Pieterse CMJ, Bakker PAHM. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* 17: 478–486.

Bever JD, Mangan SA, Alexander HM. 2015. Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics* 46: 305–325.

Brucker RM, Bordenstein SR. 2013. The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science* 341: 661–669.

Collins SM, Surette M, Bercik P. 2012. The interplay between the intestinal microbiota and the brain. *Nature Reviews Microbiology* 10: 735–742.

Darwin C. 1859. *On the Origin of Species by Means of Natural Selection.* John Murray, London.

Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology and Evolution* 24: 332–340.

Delsuc F, Brinkmann H, Philippe H. 2005. Phylogenomics and the reconstruction of the tree of life. *Nature Reviews* 6: 361–375.

Doyle JJ. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. *Systematic Botany* 17:144–163.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586–608.

Ellison AM, Gotelli NJ. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology & Evolution* 16: 623–629.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants–Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* 60: 19–42.

Ellison AM, Butler ED, Hicks EJ, Naczi RFC, Calie PJ, Bell CD, Davis CC. 2012. Phylogeny and biogeography of the carnivorous plant family Sarraceniaceae. *PLoS ONE* 7(6): e39291. doi:10.1371/journal.pone.0039291.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375–403.

Folkerts DR. 1992. Interactions of pitcher plants (*Sarracenia*: Sarraceniaceae) with their arthropod prey in the southeastern United States. PhD Dissertation. University of Georgia, Athens, GA

Folkerts DR. 1999. Pitcher plant wetlands of the southeastern United States. In: Batzer DP, Rader RB, Wissinger SA., eds. *Invertebrates in Freshwater Wetlands of North America: Ecology and Management.* New York, NY: John Wiley & Sons, Inc., 247–276.

Futuyma DJ, Agrawal AA. 2009. Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences, USA* 106:18054–18061.

Givnish TJ, Burkhardt EL, Happel RE, Weintraub JW. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist* 124: 479–497.

Givnish TJ. 2015. New evidence on the origin of carnivorous plants. *Proceedings of the National Academy of Sciences, USA* 112: 10–11.

Good AP, Gauthier M-PL, Vannette RL, Fukami T. 2014. Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut. *PLoS ONE* 9:e86494.

Harvey PH, Pagel MD. 1991. Why worry about phylogeny? In: *The Comparative Method in Evolutionary Biology*. Pg. 35–49. Oxford University Press Inc. New York.

Hennig W. 1966. Phylogenetic Systematics. Illinois Press, Urbana-Champaign (IL).

Heyduk K, Stephens JD, Faircloth BC, Glenn TC. 2016. Targeted DNA region re-sequencing. In: *Field Guidelines for Genetic Experimental Designs in High-Throughput Sequencing.* Eds. Aransay AM, Trueba JLL. Pg. 43–68. Springer International Publishing, Switzerland.

Juniper BE, Robins RJ, Joel DM. 1989. *The Carnivorous Plants*. Academic Press, New York, NY. 353 pp.

Jürgens A, El-Sayed AM, Suckling DM. 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology* 23: 875–887.

Knowles LL. 2009. Estimating species trees: Methods of phylogenetic analysis when there is incongruence across genes. *Systematic Biology* 58: 463–467.

Koopman MM, Fuselier DM, Hird S, Carstens BC. (2010). The carnivorous pale pitcher plant harbors diverse, distinct, and time-dependent bacterial communities. *Applied and Environmental Microbiology* **76**:1851–1860.

Kubatko LS. 2009. Identifying hybridization events in the presence of coalescence via model selection. *Systematic Biology* 58: 478–488.

Liu L, Yu L, Pearl DK, Edwards SV. 2009. Estimating species phylogenies using coalescence times among sequences. *Systematic Biology* 58: 468–477.

Liu L, Yu L, Edwards SV. 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. *BMC Evolutionary Biology* doi:10.1186/1471-2148-10-302.

Maddison WP. 1997. Gene trees in species trees. *Systematic Biology* 46: 523–536.

Mougi A, Kondoh M. 2014. Stability of competition-anatagonism-mutualism hybrid community and the role of community network structure. *Journal of Theoretical Biology* 360: 54–58.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Molecular Biol. Evol.* 5: 568–583.

Peterson CN, Day S, Wolfe BE, Ellison AM, Kolter R, Pringle A. 2008. A keystone predator controls bacteria diversity in the pitcher-plant (*Sarracenia purpurea*) microecosystem. *Environmental Microbiology* 10: 2257–2266.

Sargent RD. 2004. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society of London B* 271: 603–608.

Shapira M. 2016. Gut microbiotas and host evolution: Scaling up symbiosis. *Trends in Ecology and Evolution* 31: 539–549

Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, *et al*. 2011. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 6056: 670–674.

Sickel W, Grafe TU, Meuche I, Steffan-Dewenter I, Keller A. 2016. Bacterial diversity and community structure in two Bornean *Nepenthes* species with differences in nitrogen acquisition strategies. *Microbial Ecology* 71: 938–953.

Stephens JD, Godwin RL, Folkerts DR. 2015a. Distinctions in pitcher morphology and prey capture of the Okefenokee variety within the carnivorous plant species *Sarracenia minor*. *Southeastern Naturalist* 14: 254–266**.**

Stephens JD, Rogers WL, Mason C, Donovan L, Malmberg RL. 2015b. Species tree estimation of diploid *Helianthus* (Asteraceae) using target enrichment. *American Journal of Botany* 102: 921–941.

Stephens JD, Rogers WL, Heyduk K, Cruse-Sander JM, Determann RO, Glenn TC, Malmberg RL. 2015c. Resolving phylogenetic relationships of the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Molecular Phylogenetics and Evolution* 85: 76–87.

Stephens JD, Battle D, Gormally C, Brickman P. *Accepted.* Show me the way: Future faculty prefer directive feedback when trying active learning approaches. *Journal of College Science Teaching.*

van Valen L. 1973. A new evolutionary law. *Evolutionary Theory* 1: 1–30.

Vannette RL, Fukami T. 2016. Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar composition by pollinators. *Ecology* 97: 1410–1419.

Wisz MS, Pottier J, Kissling WD, et al. 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. *Biological Reviews* 88:15–30.

Yu Y, Cuong T, Degnan JH, Nakhleh L. 2011. Coalescent histories on phylogenetic networks and detection of hybridization despite incomplete lineage sorting. *Systematic Biology* 60: 138–149.

Yu Y, Nakhleh L. 2015. A distance-based method for inferring phylogenetic networks in the presence of incomplete lineage sorting. *Bioinformatics Research and Applications* 9096: 378–389.

CHAPTER II:

SPECIES TREE ESTIMATION OF DIPLOID *HELIANTHUS* (ASTERACEAE) USING TARGET ENRICHMENT[1]

[1] Stephens JD, Rogers WL, Mason CM, Donovan LA, Malmberg RL. 2015. Species tree estimation of diploid *Helianthus* (Asteraceae) using target enrichment. American Journal of Botany 102: 921–941. http://www.amjbot.org/content/102/6/910.short

Abstract

Premise of the study: The sunflower genus *Helianthus* has long been recognized as economically significant, containing species of agricultural and horticultural importance. Additionally, this genus displays a large range of phenotypic and genetic variation, making *Helianthus* a useful system for studying evolutionary and ecological processes. Here we present the most robust *Helianthus* phylogeny to date, laying the foundation for future studies of this genus.

Methods: We used a target enrichment approach across 37 diploid *Helianthus* species/subspecies with a total of 103 accessions. This technique garnered 170 genes used for both coalescent and concatenation analyses. The resulting phylogeny was additionally used to examine the evolution of life history and growth form across the genus.

Key results: Coalescent and concatenation approaches were largely congruent, resolving a large annual clade and two large perennial clades. However, several relationships deeper within the phylogeny were more weakly supported and incongruent among analyses, including the placement of *H. agrestis*, *H. cusickii*, *H. gracilentus*, *H. mollis*, and *H. occidentalis*.

Conclusions: The current phylogeny supports three major clades, including a large annual clade, a southeastern perennial clade, and another clade of primarily large-statured perennials. Relationships among taxa are more consistent with early phylogenies of the genus using morphological and crossing data than recent efforts using single genes, highlighting the difficulties of phylogenetic estimation in genera known for reticulate evolution. Additionally, conflict and low support at deeper nodes among the perennials may suggest a rapid radiation and/or ancient introgression within the genus.

Introduction

Sunflowers are among the most charismatic flowering plants, instantly recognizable the world over and the subject of art for centuries. The cultivated sunflower (*Helianthus annuus* L.) is of agricultural importance as a major oilseed and confectionery crop, and additionally is of horticultural importance to the cut flower industry and as a widespread favorite of gardeners. Both wild species and crop-wild hybrids are also noxious weeds across a number of regions worldwide (Rehorek, 1997; Seiler et al., 2008; Cantamutto et al., 2010; Muller et al., 2011). As a result of these economic interests, sunflowers have long been studied in the contexts of genetic research and breeding efforts, and in recent decades have emerged as a key system for the study of ecological and evolutionary processes thanks in part to their enormous genetic and phenotypic variation (Kane et al., 2013).

The genus *Helianthus* is a diverse assemblage of approximately fifty species, with a much larger number of subspecies and varieties (Heiser et al., 1969; Schilling and Heiser, 1981). The genus includes both diploids and polyploids, both annuals and perennials, as well as wide variation in size and growth form (Schilling and Heiser, 1981). All *Helianthus* are native to North America, with the majority of species occurring primarily within the continental United States (Heiser et al., 1969). Members of *Helianthus* occupy a broad range of habitats, including deserts, wetlands, prairies, forests, rock outcrops, coastal dunes, and a variety of disturbed environments (Heiser et al., 1969). The genus is placed within the subtribe Helianthineae within tribe Heliantheae of the subfamily Asteroideae in the family Asteraceae (Robinson, 1981; Panero and Funk, 2002), with the most likely sister genus being *Phoebanthus* (Schilling, 2001; Schilling and Panero, 2002; Mandel et al., 2014). A large and varied number of efforts have been made to understand the phylogenetic relationships within the genus over the past four decades, using

morphology and crossing data (Schilling and Heiser, 1981), phytochemistry (Schilling, 1983; Spring and Schilling, 1989; Spring and Schilling, 1990; Spring and Schilling, 1991), isozyme data (Rieseberg et al., 1991), chloroplast restriction sites (Rieseberg et al., 1991; Schilling, 1997), nuclear restriction sites (Rieseberg, 1991; Gentzbittel et al, 1992; Schilling et al., 1998), and more recently sequence data (Schilling et al., 2001; Timme et al., 2007a). Results of these efforts are discordant and are characterized by low sequence divergence, widespread polytomies, poor branch support, lack of species resolution, and repeated swapping of taxa placement among studies. Timme et al. (2007a) is by far the most well-resolved large-scale phylogeny thus far in terms of the placement of the species into reasonably well-supported clades. However, a large proportion of accessions within species were unresolved, and low bootstrap support was prevalent toward the tips. Timme et al. (2007a) also wrestled with the difficulties of phylogenetic reconstruction under the extensive reticulate evolution within the genus, and showed that polyploidization, hybrid speciation, and horizontal gene transfer are likely common within *Helianthus*. These difficulties highlight the limitations of single-gene sequence data for phylogenetic inference in groups of species with complex histories, and the need for more data to enhance our understanding of the phylogenetic relationships within this genus.

Recent studies of small groups of species have made progress by explicitly considering the processes of hybridization and polyploidy. In one study of the annual clade (sect. *Helianthus*), eleven nuclear loci achieved good resolution of species when known homoploid hybrids were excluded, but not when they were included (Moody and Rieseberg, 2012). Another study successfully used genome skimming to tease apart the effects of polyploidy and hybridization in the origins of the Jerusalem artichoke (*Helianthus tuberosus*) within a complex of eastern perennial sunflowers (Bock et al., 2014). These two studies underscore the difficulties

of working with polyploids and hybrids, and present potential avenues for progress in small clades. However, the large-scale phylogenetic relationships across *Helianthus* still remain tentative.

Here we seek to improve upon previous efforts and clarify the phylogenetic relationships among the diploid non-hybrid members of the genus *Helianthus* using target enrichment to obtain hundreds of informative loci (Mamanova et al., 2010). This technique has proven to be effective at capturing numerous informative single copy loci for phylogenetic analyses in previous studies focusing on plants (Weitemier et al., 2014; Heyduk et al., 2015; Stephens et al., 2015). By concentrating on the diploid non-hybrids, we avoid the major complications to phylogenetic reconstruction posed by polyploidy and contemporary hybrid speciation while providing a better understanding of the evolution of the major clades and the majority of species in the genus. Based on previous work (particularly Timme et al., 2007a) we know that the polyploid members of the genus likely arose through a handful of events in different major clades; therefore, resolving the relationships among the diploids will provide a solid scaffold for further detailed studies of the polyploids and the dynamics of their origins. Furthermore, the origins of the confirmed diploid hybrids have been well studied (*H. anomalus*, *H. paradoxus,* and *H. deserticola*; see Rieseberg et al., 2006), and the remaining putative diploid hybrids have either been refuted as hybrids (*H. verticillatus*; see Ellis et al., 2006), or remain putative but have only two possible parents (*H. simulans*; see Timme et al., 2007a). In this study we present a well-resolved phylogeny of 37 diploid species and subspecies of *Helianthus*.

Methods

Taxon sampling

All diploid non-hybrid *Helianthus* species were targeted for inclusion in this study, as well as the sole diploid member of the sister genus *Phoebanthus* as an outgroup. For species with recognized infraspecific taxa, multiple subspecies were included. For 28 taxa under ongoing study in the Donovan lab, 2-4 populations of each species were included and sampled from across the geographic range of each species. For the other 13 taxa, one population was included. Populations for all taxa were selected to avoid known contemporary hybrid zones as much as was possible. Excluding polyploids, hybrid species, and known hybrid zones improves our power to untangle the diploid backbone of the genus.

Seeds for all taxa were obtained through either direct collections from wild populations, or from existing accessions with the UDSA Germplasm Resources Information Network (GRIN). Seeds from wild-collected populations were subsequently submitted to GRIN to establish accessions (Table S3.1). Sampled plants were either grown from seed in the summers of 2012 and 2013 as part of a large multi-year common garden experiment, or grown from seed in a growth chamber in the fall of 2013. Leaf tissue was snap-frozen in liquid nitrogen and stored at -80°C until DNA extraction. Due to the fact that sampling for a large proportion of taxa occurred as part of a physiological experiment, herbarium vouchers were not able to be initially collected for all taxa, but are currently being produced. This is an exception to standard AJB policy. However, seeds for all accessions save two are available through USDA GRIN (Table S3.1).

Probe design

RNA from four *Helianthus* species (*H. annuus, H. argophyllus, H. porteri, H. verticillatus*) was extracted using a Spectrum Plant Total RNA kit (Sigma-Aldrich #STRN50-1KT, St. Louis, Missouri, USA). These four species were selected based on previous taxonomic placements to examine variation between closely related species (i.e., *H. annuus* and *H. argophyllus*) and more distantly related species (i.e., *H. porteri* and *H. verticillatus*). RNA extracted from these species was submitted to the Georgia Genomic Facility (Athens, Georgia, USA) for cDNA construction and normalization; sequencing was on an Illumina HiSeq PE150. Subsequent bioinformatics analysis was as follows: highly repetitive sequences were removed using RepeatMasker (http://www.repeatmasker.org/) and then the sequences were clustered using cd-hit-est v4.6.1 (Li and Godzik, 2006; Fu et al., 2012); possible paralogs were removed by a BLAST search (Altshul et al., 1997) across all clusters comparing their *H. annuus* sequences, with a stringent e-value cut off of $< 3 \times 10^{-20}$; only clusters that did not have duplicate *H. annuus* sequences were retained; clusters that were compliments of each other were also removed to insure only a single strand of each cDNA was used. The retained clusters were then aligned using T-Coffee v10.00.r1613 (Notredame et al., 2000) for visual inspection. For probe design, the *H. annuus* sequence was used as a reference for comparison to the other sequences within each cluster to target areas with single nucleotide polymorphisms (SNPs). Specifically, *H. annuus* sequences where the other three showed the highest levels of SNP variation were used; this yielded 598 targets for probe design. Approximately three 120-mer oligonucleotide probes were designed for each target and commercially synthesized per the manufacturer's probe design specifications by Mycroarray® (http://www.mycroarray.com; Ann Arbor, Michigan, USA) into a custom MYbaits kit used for library capture procedures.

DNA extraction, library preparation, and sequncing

DNA extractions were conducted following the protocol described in Lodhi et al. (1994) with similar modifications to those used in Stephens et al. (2015). Extractions were initially performed on approximately eight individuals in 93 populations across 40 species/subspecies of *Helianthus* and three populations of the outgroup, *Phoebanthus tenuifolius*, resulting in ~690 individuals. In most cases, the four most desirable extractions from each population were selected and used for further analyses with the exception of one population containing only three individuals; desirable is defined as those samples with the highest 260/280 and 260/230 readings and concentrations of greater than 50ng/µl using a NanoDrop 2000 (Thermo Scientific, Wilmington, Delaware, USA). Unfortunately, we were unable to successfully extract DNA from *H. glaucophyllus*; therefore, this species was excluded from phylogenetic analyses. Each sample was treated with RNAase A (Sigma-Aldrich #R4642-10MG, St. Louis, Missouri, USA) to rid samples of unwanted RNA sequences. At the end of the extraction procedures, 208 samples were sheared to 180-500bps in length using an S220-Focused ultrasonicator (Covaris, Inc., Woburn, Massachusetts, USA). Libraries were prepared using KAPA LTP library preparation kits (#KK8232, KAPA Biosystems, Inc., Wilmington, Massachusetts, USA). Dual-index oligonucleotide barcodes were designed then manufactured by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA) and used during the amplification step in order to pool samples later. The MYbaits capture protocol was performed per manufacturer's instruction. A blocking oligonucleotide with eight inosines at the index location was implemented to reduce daisy chaining during hybridization (c.f., Faircloth et al., 2012). Subsequent pooled libraries were sequenced on an Illumina HiSeq PE 2500 by the Duke Center for Genomic and Computational Biology.

Assembly and alignment

Assessment of read quality was conducted in FastQC v0.10.1 (http://www.bioinformatics.babraham.ac.uk/). All demultiplexed pooled reads were then trimmed with a Phred score of <20 at the 3' end to a minimum length of 40bp in FastX v 0.013.2 (http://hannonlab.cshl.edu/fastx_toolkit/). Removal of Illumina adapter contaminants was done using FAR v2.15.

Assembly of sequence reads was performed using a combination of a reference-based approach in VELVET v 1.2.08 (Zerbino and Birney, 2008) with the Columbus extension and *de novo* based approach in Trinity v 20140717 (Grabherr et al., 2011). Prior to Velvet assembly, an average k-mer length of 25 was determined from values obtained for each library via KmerGenie v 1.5393 (Chikhi and Medvedev, 2013). The reference used for assembly consisted of the targeted genes used for probe design. Poorly supported isoforms (<1% per-component read support) from the Trinity assembly were removed using RSEM in Bowtie 1.1.0 (Langmead et al., 2009). The resulting contigs that had at least 95% identity over 20bp from both Velvet and Trinity were merged using CAP3 v 10/2011 (Huang and Madan, 1999). Heterozygosity was ignored in both assemblies as we felt that the addition of multiple accessions and populations, as well as multiple target genes would effectively capture within species diversity in a phylogenetic context. Assembly was successful for all but two species (*H. pumilus* and *H. petiolaris* ssp. *fallax*); therefore, these two were not included in the phylogenetic analyses. Specifically, *H. petiolaris* ssp. *fallax* was poorly sequenced and *H. pumilus* had few contigs that matched the targets.

Merged contigs that had a 1:1 match to the gene targets used for probe design were extracted. In addition, instances where two contigs from an accession had best hits to the same

target, but were non-overlapping (possibly due to intron regions) were extracted and concatenated. The resulting contigs which had a BLAST hit to the same gene were merged, renamed based on gene target, parsed into gene files, and aligned via Prank v100802 (Löytynoja and Goldman, 2008). Poorly aligned regions were filtered from the subsequent aligned gene files using Gblocks v0.91b (Castresana, 2000). Genes selected for downstream analyses had an average pairwise distance of less than 0.35, at least 50% of the accessions present, no more than 45% missing data, and had at least one representative from within the outgroup.

Coalescent analyses and ancestral state reconstruction

Gene trees were estimated from 500 bootstraps in RAxML v 04262012 (Stamatakis, 2006) under the GTRGAMMA model, determined from five randomly selected genes in JModelTest 2.1.1 using AIC (Darriba et al., 2012). Due to the unmanageable computational time and size of tree space that increases with numbers of taxa, we sampled a subset of the taxa for species tree estimations. Specifically, we selected at least one individual per population for each species and included all outgroup individuals for a total of 103 individuals across the 38 species/subspecies. Individuals selected for species tree analyses had the highest number of assembled contigs (Table S3.1). Subsequent gene trees were used for species tree estimations in Maximum Pseudolikelihood Estimation of the Species Tree v1.4 (MP-EST) (Liu et al., 2010). This method estimates species trees while accounting for gene tree discordance resulting from incomplete lineage sorting. We used three approaches in MP-EST: one in which accessions were grouped by their taxonomic designations, a second in which accessions were grouped by populations within species, and lastly an accession tree where all accessions are treated as terminal taxa. The latter two estimations were used to examine population and accession reciprocal monophyly within their taxonomic groupings.

In addition to species tree estimation, we were interested in examining the evolution of life history and growth form (i.e. annual vs. perennial; erect vs. basal rosette). However, MP-EST outputs branch lengths in coalescent units with all terminal tips set to a default of 9; which is not ideal for ancestral state reconstruction or other phylogenetic comparative methods (e.g. Felsenstein, 1985; Martins and Hansen, 1997). Therefore, branch lengths were estimated using a similar approach as Song et al. (2012). Specifically, we used RAxML v 04262012 (Stamatakis, 2006) with a fixed topology determined from MP-EST to generate 100 bootstrap replicates to estimate branch lengths in substitution units. To map life history and growth form, we performed stochastic character mapping (Bollback, 2006; Huelsenbeck et al., 2003) on the substitution unit tree using 1000 simulations with the *make.simmap* function in the package *phytools* (Revell, 2012) in R (R Development Core Team, 2008).

Chloroplast, concatenation tree, and conflict measures

While chloroplast genes were not targeted in our enrichment, an average of 34 321 trimmed reads mapped back to the *Helianthus annuus* complete chloroplast genome (Timme et al., 2007b) using Bowtie2 v2.2.1 (Langmead and Salzberg, 2012). Trimmed reads that mapped to the genome were extracted for each accession using SAMtools (Li et al., 2009). Reads were then assembled using the reference-based assembler YASRA (Ratan, 2009) using a 3x coverage cutoff. Assembled contigs were then concatenated for each accession. Alignment of resulting chloroplast sequences was conducted in MAFFT v7.029-e (Katoh and Standley, 2013). Poorly aligned regions were removed using with Gblocks v0.91b (Castresana, 2000) followed by visual inspection in Geneious v7.0.6 (http://www.geneious.com/). Finally, the chloroplast tree was estimated under a GTRGAMMA model with 500 bootstraps in RAxML v04/26/2012 (Stamatakis, 2006).

In addition to the chloroplast and the MP-EST trees, we used RAxML with 500 bootstraps to estimate a species tree from all 170 nuclear genes concatenated into a 'supergene' of 106 862 bp. The resulting tree was then compared to the MP-EST accession tree. Areas of conflict between tree estimation methodologies (nodes labeled in Fig. 2.1) were queried to assess discordance among genes using custom Perl scripts (Heyduk et al., 2015; Stephens et al., 2015; see https://github.com/kheyduk/). Gene tree discordance at queried nodes was classified as follows: a gene tree which had the node present with bootstrap support >80 "strongly agreed"; trees with support on the queried node between 50 and 80 "weakly agreed"; trees with support between 20 and 50 "weakly conflicted"; and trees with bootstrap support of less than 20 "strongly conflicted."

Results

Assembly and gene trees

The 103 accessions selected for species tree estimation had an average of 2.2 million trimmed reads. Across the 103 accessions there was an average of 84 868 contigs after Velvet assembly and 9720 contigs after Trinity assembly. Contigs from the two assemblies merged into an average of 6771 contigs per accession with roughly 501 matching the 598 gene targets. The subsequent contig targets across the 103 accessions had an approximate coverage of 11x with an N50 of 709bp (Table S3.1). It should be noted that 11x coverage is lower than other target enrichment studies (McCormack et al., 2013; Heyduk et al., 2015) most likely due to off-target sequencing. However, a sequencing error would have to occur at a sufficiently early stage to become the majority sequence in the assembly. Given the multiple individuals sampled per population, and the large number of targets sequenced, it is likely these rare errors would have minimal effect on the final phylogeny.

Removal of poor alignments, genes with >50% missing data, and those genes missing outgroup sequence resulted in 170 genes for phylogenetic analyses. Of the 170 genes, 11 (7%) had all ingroup taxa represented, 29 (17%) had one missing taxon, 36 (21%) had two missing taxa, 26 (15%) had three missing taxa, 22 (13%) had four missing taxa, 14 (8%) had five missing taxa, and the remaining 32 genes (19%) had between six and thirteen missing taxa. On average there were 75 accessions per gene that were approximately 629bp in length. Taken together this totaled 106 862bp with 22 885 (21.4%) variable sites and 11 407 (10.7%) parsimony-informative sites within the ingroups. In addition to nuclear data, we were able to recover an average of 91 868bp (72.6%) of the 126 471bp (excluding one copy of the inverted repeat) in the *Helianthus* chloroplast across the 103 accessions (Table S2.2). Of the 96 789bp, 4519 (4.7%) were variable sites and 1063 (1.1%) were parsimony-informative within the ingroups. Short reads for all sequenced individuals (Table 2.1) were deposited in the NCBI Sequence Read Archive (BioProject PRJNA277479). Gene trees, alignments, and species trees have all been deposited in Dryad http://dx.doi.org/10.5061/dryad.4n28n. Annotated partial chloroplast genomes (Table S2.2) were deposited in Verdant (http://verdant.iplantcollaborative.org).

Coalescent analyses and ancestral state reconstruction

All MP-EST analyses (i.e., species tree, population tree, and accession tree) support *H. porteri* as sister to all *Helianthus* species with high bootstrap support (Figs. 2.1, 2.2, S2.1, see Supplemental Data), which is consistent with the concatenation tree (Fig. 2.2). Also consistent with the concatenation tree was the annual clade (Figs. 2.1, 2.2) consisting of *H. annuus*, *H. argophyllus*, *H. debilis* subspecies, *H. exilis*, *H. neglectus*, *H. niveus* subspecies, *H. petiolaris*, and *H. praecox* subspecies across all MP-EST analyses. The specific relationships within this clade were well supported at the species tree level (>91BS), population level (>81BS), and

accession level (>81BS) with the exception of intraspecific relationships among *H. praecox* subspecies. The population and accession MP-EST trees were unable to resolve the relationships among *H. neglectus* and *H. petiolaris* populations and accessions resulting in a polytomy. In addition, both the population and accession level MP-EST trees did not support reciprocal monophyly for *H. praecox* ssp. *runyonii* accessions and populations.

The other clade recovered by MP-EST population and accession analyses consists of a strongly supported (87 and 88BS; respectively), largely perennial clade (Figs. 2.1, 2.2, S2.1). The MP-EST species tree analysis recovered *H. gracilentus* and the annual *H. agrestis* as sister to other members within the clade (Fig. 2.1); however, population and accession level analyses were unable to resolve this relationship (Fig. 2.2, S2.1). In addition, the placement of *H. mollis* and *H. occidentalis* differed across all MP-EST analyses. The species level tree was unable to resolve their relationship to other *Helianthus* species, placing these two species in a polytomy with two large clades containing all other perennial *Helianthus* except *H. gracilentus*. The population and accession trees placed *H. mollis* sister to a clade consisting of *H. angustifolius*, *H. atrorubens*, *H. carnosus*, *H. floridanus*, *H. heterophyllus*, *H. longifolius*, *H. radula*, and *H. silphioides* (hereafter the southeastern perennial clade, see Fig. 2.1), albeit with very low support (51 and 52BS; respectively). Both the population and accession analyses placed *H. occidentalis* sister to a clade consisting of *H. arizonensis*, *H. cusickii*, *H. divaricatus*, *H. giganteus*, *H. grosseserratus*, *H. laciniatus*, *H. maximiliani*, *H. microcephalus*, *H. nuttallii* spp. *nuttallii*, *H. salicifolius*, and *H. verticillatus* (hereafter the large perennial clade, see Fig. 2.1) again with very low support (56 and 55BS; respectively). Relationships within the southeastern perennial clade were consistent across all MP-EST analyses with mostly strong bootstrap support with the exception of *H. angustifolius/floridanus* and *H. heterophyllus* having bootstrap support of <78

across analyses (Figs. 2.1, 2.2, S2.1). In addition, population and accession level analyses did not recover reciprocal monophyly for populations and accessions of *H. angustifolius* and *H. floridanus* (Fig. 2.2, S2.1).

Within the large perennial clade, all MP-EST analyses recovered *H. arizonensis* and *H. laciniatus* as sister species at very high support (>99BS) and this clade as sister to *H. divaricatus*, *H. giganteus, H. grosseserratus, H. maximiliani, H. microcephalus, H. nuttallii* spp. *nuttallii*, *H. salicifolius*, and *H. verticillatus* and *H. cusickii*, with bootstrap support of <70BS (Figs. 2.1, 2.2, S2.1). Moreover, there was very low support (<53BS) for the placement of *H. cusickii.* The remaining members of this clade were well supported with the exception of the relationships among *H. giganteus, H. grosseserratus, H. nuttallii* spp. *nuttallii*, and *H. verticillatus* within the population and accession trees (Fig. 2.2, S2.1).

Examination of the evolution of life history/growth form using maximum likelihood ancestral state reconstruction suggest that erect perennial is most likely the ancestral state, with three independent transitions to annual life history (i.e., *H. agrestis*, *H. porteri*, and the large annual clade) and three independent transitions to basal rosette growth form (i.e, *H. heterophyllus*, *H. occidentalis*, and the clade of *H. longifolius-carnosus-radula*) (S2.2, see Supplemental Data). This result was additionally supported using the concatenation topology (data not shown).

Chloroplast, concatenation tree, and conflict measures

The plastid tree had very little resolution resulting in a large polytomy for the majority of species (Fig. 2.3). The plastid tree did support a clade consisting of *H. agrestis, H. annuus, H. argophyllus, H. cusickii, H. debilis* subspecies, *H. exilis, H. gracilentus, H. neglectus, H. niveus* subspecies, *H. petiolaris, H. porteri*, and H. *praecox* subspecies with 100 bootstrap support.

Most accessions within this clade are reciprocally monophyletic with the exception of *H. argophyllus, H. annuus, H. porteri* and *H. praecox* subspecies. There is high support for most relationships within this clade although they conflict with both the MP-EST and concatenation analyses (Fig. 2.2).

The RAxML concatenated nDNA tree is largely congruent with the MP-EST accession tree (Fig. 2.2). The tree supports the same annual clade topology and, similarly to the MP-EST tree, is unable to resolve the *H. neglectus/petiolaris* accessions; however, the concatenation tree supports all *H. praecox* ssp. *runyonii* accessions as a monophyletic group. Additionally, the concatenation tree recovered the large perennial clade with better resolution for the *H. giganteus, H. grosseserratus, H. nuttallii* spp. *nuttallii,* and *H. verticillatus* subclade. The one conflict within this clade is the placement of *H. maximiliani* as being sister to *H. salicifolius* in the concatenation tree, versus sister to the entire rest of the subclade in MP-EST accession tree (Fig. 2.2). Measures of gene tree conflict at these nodes (Fig. 2.2; A,F) show a large degree of conflict for both nodes with no dominant alternative topology. The major differences between the concatenation tree and the MP-EST accession tree appear to be at the deeper nodes within the perennial clade; specifically, nodes involving *H. agrestis*, *H. arizonensis/laciniatus* clade, *H. cusickii*, *H. gracilentus*, *H. mollis*, and *H. occidentalis.* These nodes were poorly supported or resulted in polytomies within the MP-EST accession tree. Examination of conflict at these nodes (Fig. 2.2; B-E, G-J) shows fewer than nine gene trees in agreement.

Discussion

Comparison of phylogenetic methods

The results of this study constitute the most well-resolved phylogeny of diploid *Helianthus* to date. Given known rampant hybridization and reticulate evolution in the genus

(Timme et al., 2007a; Rieseberg, 2006; Bock et al., 2014a) and the difficulties encountered by previous efforts to resolve the genus, it is encouraging that both concatenation and MP-EST analyses were largely congruent and able to resolve nearly all taxa as monophyletic (Fig. 2.2). There were few areas of conflict between the two analyses and these primarily occurred at the short, deeper internodes within the tree (i.e., *H. agrestis*, *H. arizonensis/laciniatus*, *H. cusickii*, *H. gracilentus*, *H. mollis*, *H. occidentalis*). Further examination of alternative topologies at these nodes found high levels of variable gene trees with no dominant topology. Together these results suggest retention of ancient polymorphisms resulting in high levels of incomplete lineage sorting, possibly indicating a rapid radiation and/or high levels of reticulate evolution among perennial *Helianthus* species.

These conflicts highlight the issues with resolving species-level phylogenies of problematic groups. As noted by Timme et al. (2007a), *Helianthus* is known to have high levels of hybridization likely resulting in reticulate evolution within the genus. Unfortunately, while MP-EST does not model hybridization, it is likely that hybridization among taxa has played a role in the speciation process within the genus. This scenario may explain the conflicting topologies between the nuclear phylogeny and the plastid tree. This conflict between trees is not uncommon in species-level studies and is often thought to occur through chloroplast capture resulting from hybridization among or between taxa followed by introgression (Wolfe and Elisens, 1995; McKinnon et al., 1999; Acosta and Premoli, 2010; Zhang et al., 2014; Stephens et al., 2015). The exact circumstances that promote chloroplast capture are not well known (see Bock et al., 2014b). Hypotheses range from selectively neutral events—such as incomplete lineage sorting (Comes and Abbott, 2001), asymmetric reproductive barriers (McKinnon et al., 2004), introgression during range expansions (Neiva et al., 2010), and differential allocation in

female reproduction (Tsitrone et al., 2003)—to possible local cytoplasmic adaptation (Sambatti et al., 2008; Greiner and Bock, 2013). In general, chloroplast capture should be more frequent among sympatric species with weak reproductive barriers (Acosta and Premoli, 2010). The conflicting relationships between the *Helianthus* cpDNA tree and nDNA tree are primarily among taxa that are sympatric or nearly sympatric and many are known to hybridize. For example, *H. petiolaris* and *H. annuus* have a range that almost completely overlaps (Fig. 2.4B) and are well known to hybridize with one another. Moreover, *H. annuus* and *H. argophyllus* are both sympatric with *H. praecox* ssp. *runyonii* (Fig. 2.4C). These three species (i.e. *H. petiolaris, H. annuus, H. praecox* ssp. *runyonii*) form a well-supported clade within the cpDNA tree with one accession of *H. praecox* ssp. *runyonii* grouping with *H. argophyllus* (Fig. 2.3). Interestingly, Sambatti et al. (2008) found environment-dependent selection on cytonuclear interactions between *H. petiolaris* and *H. annuus* supporting the possibility that chloroplast capture may be selected for within these species. Future work examining cytonuclear variation across the ranges of various *Helianthus* species may help elucidate not only introgression across the genus, but also the possible causes for chloroplast capture.

Relationships within the annual clade

The results of our 170-gene analyses agree reasonably well with previous attempts regarding the relationships of annual *Helianthus* species and subgroups thereof. Both the single-gene phylogeny of Timme et al. (2007a) and the multilocus phylogenies of Rieseberg (1991) and Moody and Rieseberg (2012) recovered the large monophyletic annual clade resolved here, with similar but not identical relationships among taxa within that clade. The sister species of *H. petiolaris* and *H. neglectus* were found to comprise a single polytomy by MP-EST, and *H. petiolaris* was found to be paraphyletic by concatenation (Fig. 2.2). A recent population genetic

study of these two taxa indicates that that *H. petiolaris* and *H. neglectus* are not genetically isolated from one another, and that *H. neglectus* is probably best considered an ecologically and phenotypically distinct geographic subspecies of *H. petiolaris* (Raduski et al., 2010; Fig. 2.4B, 2.4E).

Relationships within the perennial clades

The large perennial clade was also recovered as monophyletic in Timme et al. (2007a) along with the here-unplaced *H. glaucophyllus*; however, Timme et al. (2007a) had very little resolution within the clade – indeed, only accessions of *H. maximiliani* and *H. salicifolius* formed monophyletic groups in that phylogeny. Additionally, the Timme et al. (2007a) placement of *H. arizonensis* and *H. laciniatus* as sister species in alliance with the large perennial clade is also recovered in the current phylogeny, and the basal placement of *H. porteri* in the current phylogeny is similar to the placement in Timme et al. (2007a), which places *H. porteri* in a basal clade of the genus.

The organization of other clades and the placement of other species differed strongly between the present phylogeny and previous efforts. The relationships found among perennial taxa in Bock et al. (2014a) places *H. divaricatus*, *H. grosseserratus*, and *H. maximiliani* as more closely related than *H. grosseserratus* and *H. giganteus*, in contrast to present results. This difference may result from the inclusion of polyploid taxa in Bock et al. (2014a), which sought to resolve the origin of the polyploid Jerusalem artichoke (*H. tuberosus*). The single-gene phylogeny of Timme et al. (2007a) places the southeastern perennial clade in four separate clades distributed across the tree, while present results combine these species into a single monophyletic group with good support. The current phylogeny is actually quite similar to aspects of some of the first efforts at estimation of the phylogeny of *Helianthus* (Schilling and Heiser,

1981), which grouped together the southeastern perennials along with *H. occidentalis* based on morphology and crossing data. The placement of other taxa in the current phylogeny (*H. agrestis, H. cusickii, H. gracilentus, H. mollis,* and *H. occidentalis*) also differs from Timme et al. (2007a).

The phylogenetic results presented here match well with expectations based on morphology and geography, with most clades and sister taxa predicted by morphology (e.g., Heiser et al., 1969; Schilling and Heiser, 1981) and range locations (Fig. 2.4). However, the concatenation and MP-EST trees differ in three key ways. First, concatenation supports *H. cusickii* and *H. gracilentus* as sister species, as would be expected from shared growth form, leaf morphology, and proximity of ranges (Fig. 2.4E). This result has been found in multiple previous phylogenies, placing these two species in a clade with *H. pumilus* (Gentzbittel et al., 1992; Timme et al., 2007a). The inclusion of *H. pumilus* may have supported these previous findings, but unfortunately individuals of *H. pumilus* were not successfully sequenced. Introgression has been demonstrated to sometimes cause taxa to move to more basal positions in a bifurcating tree (e.g., Moody and Rieseberg, 2012), and may explain the placement of *H. gracilentus* in the MP-EST tree (Fig. 2.2). Second, concatenation supports *H. mollis* and *H. occidentalis* as sister species, which might be expected from similar leaf morphology, shared long rhizomes, large overlap in ranges (Fig. 2.4A, 2.4D), and known interfertility (Heiser et al., 1969). Heiser et al. (1969) suggested that *H. occidentalis* was perhaps most related to *H. mollis*, but noted that it would likely not be a close relationship, which may be reflected in the MP-EST result here that places these taxa ambiguously as either part of the perennial clades polytomy (Fig. 2.1) or as basal members of two separate perennial clades (Fig. 2.2, Appendix S3), with low support in both placements. Third, the conflict between concatenation and MP-EST trees on the placement

of *H. agrestis* fits with long-standing identification of this species as an outlier among *Helianthus*. This species occurs in a restricted range in peninsular Florida (Fig. 2.4F), and is considered to be morphologically distinct from all other *Helianthus*, so much so that it has been placed in its own taxonomic section with only three other sections encompassing the entire rest of the genus (Schilling and Heiser, 1981).

Only a handful of accessions were not found to be reciprocally monophyletic by either MP-EST or concatenation (Fig. 2.2). The species *H. angustifolius* and *H. floridanus* have long been known to experience introgression in the overlap between their ranges (Fig. 2.4A, 2.4D); in fact, the more aggressive *H. angustifolius* has been noted to overtake and replace populations of *H. floridanus* through hybridization, such that pure forms of *H. floridanus* are rare (Heiser et al., 1969). Contamination of the Crystal River Preserve (CRP) population of *H. angustifolius* by this process would explain the results seen here. The lack of monophyly for *H. giganteus* and *H. verticillatus* by MP-EST accession analysis may result from a complex non-bifurcating radiation and/or ongoing known hybridization in that clade, which occupies a broad geographic swath across a large proportion of the country with widely overlapping ranges (Fig. 2.4; Heiser et al., 1969).

Conclusions

There have been multiple attempts to resolve the sunflower group resulting in conflicting species relationships (e.g., Schilling and Heiser, 1981; Rieseberg et al., 1991; Spring and Schilling, 1991; Schilling et al., 1998; Timme et al., 2007a). These conflicting results highlight the difficulties in resolving the species relationships within this group given widespread hybridization and polyploidization. Here we sampled 170 genes across 103 accessions using a target enrichment approach. This sampling scheme, in combination with both coalescent and

concatenation approaches, draws a clearer picture of the phylogenetic relationships within the genus. Overall, this work provides a solid well-resolved diploid backbone phylogeny of the genus *Helianthus* upon which future work can build. For future work, the authors recommend using the resulting species tree from the coalescent approach, given that this genus has undoubtedly high levels of incomplete lineage sorting compounded by hybridization. These potential sources of gene tree discordance are best mitigated with use of the multispecies coalescent model (Degnan and Rosenberg, 2009; Edwards, 2009), which accounts for the independent evolution of individual genes. Major remaining challenges include disentangling phylogenetic relationships among the multiple polyploid complexes, better characterizing variation in the process of speciation (i.e., modeling reticulate evolution), and better understanding phenotypic trait evolution across this highly diverse and economically relevant genus.

References

ACOSTA, M. C. AND A. C. PREMOLI. 2010. Evidence of chloroplast capture in South American *Nothofagus* (subgenus *Nothofagus*, Nothofagaceae). *Molecular Phylogenetics and Evolution* 54: 235–242.

ALTSCHUL, S. F., T. L. MADDEN, A. A. SCHÄFFER, J. ZHANG, Z. ZHANG, W. MILLER, AND D. J. LIPMAN. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402.

BOCK, D. G., N. C. KANE, D. P. EBERT, AND L. H. RIESEBERG. 2014a. Genome skimming reveals the origin of the Jerusalem Artichoke tuber crop species: neither from Jerusalem nor an artichoke. *New Phytologist* 3: 1021–1030.

BOCK, D. G., R. L. ANDREW, AND L. H. RIESEBERG. 2014b. On the adaptive value of cytoplasmic genomes in plants. *Molecular Ecology* 23: 4899–4911.

BOLLBACK, J. P. 2006 Stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7**: 88.

CANTAMUTTO, M., A. PRESOTTO, M. I. FERNANDEZ, D. ALVAREZ, M. POVERENE, AND G. SEILER. 2010. High infraspecific diversity of wild sunflowers (*Helianthus annuus* L.) naturally developed in central Argentina. *Flora* 205: 306–312.

CASTRESANA, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552.

CHIKHI, R. AND P. MEDVEDEV. 2013. Informed and automated k-Mer size selection for genome assembly. *Bioinformatics* doi:10.1093/bioinformatics/btt310.

COMES, H. P. AND R. J. ABBOTT. 2001. Molecular phylogeography, reticulation, and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). *Evolution* 55: 1943–1962.

DARRIBA, D., G. L. TABOADA, R. DOALLO, AND D. POSADA. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

DEGNAN, J. H., N. A. ROSENBERG. 2009. Gene tree discordance, phylogenetic inference and multispecies coalescent. *Trends in Ecology and Evolution* 24: 332–340.

EDWARDS, S. V. 2009. Is a new and general theory of molecular systematics emerging? *Evolution* doi:10.1111/j.1558-5646.2008.00549.x

ELLIS, J. R., C. H. PASHLEY, J. BURKE, AND D. E. MCCAULEY. 2006. High diversity in a rare and endangered sunflower as compared to a common congener. *Molecular Ecology* 15: 2345–2355.

FAIRCLOTH, B. C., J. E. MCCORMACK, N. G. CRAWFORD, M. G. HARVEY, T. C. BRUMFIELD, AND T. C. GLENN. 2012. Ultraconserved elements anchor thousands of genetic markers for target enrichment spanning multiple evolutionary timescales. *Systematic Biology* 61: 717–726.

FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.

FU, L., B. NIU, Z. ZHU, S. WU, AND W. LI. 2012. CD-HIT: accelerated for clustering the next generation sequencing data. *Bioinformatics* 28: 3150–3152. doi: 10.1093/bioinformatics/bts565.

GENTZBITTEL, L., A. PERRAULT, AND P. NICOLAS. 1992. Molecular phylogeny of the *Helianthus* genus, based on nuclear restriction-fragment length polymorphism (RFLP). *Molecular Biology and Evolution* 9: 872–892.

GRABHERR, M. G., B. J. HAAS, M. YASSOUR, J. Z. LEVIN, D. A. THOMPSON, I. AMIT, X. ADICONIS, ET AL. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29: 644–652.

GREINER, S. AND R. BOCK. 2013. Tuning a ménage à trois: co-evolution and co-adaptation of nuclear and organelle genomes in plants. *BioEssays* 35: 354–365.

HEISER, C. B. JR., D. M. SMITH, S. B. CLEVENGER, AND W. C. MARTIN. 1969. The North American sunflowers (*Helianthus*). *Memoirs of the Torrey Botanical Club* 22: 1–218.

HEYDUK, K., D.W. TRAPNELL, C.F. BARRETT, J. LEEBENS-MACK. 2015. Estimating relationships within *Sabal* (Arecaceae) through multilocus analyses of sequence capture data. Biological Journal of the Linnean Society.

HUANG, X. AND A. MADAN. 1999. CAP3: A DNA Sequence Assembly Program. *Genome Research* 9: 868-877.

HUELSENBECK, J. P., R. NEILSEN, AND J. P. BOLLBACK. 2003. Stochastic mapping of morphological characters. *Systematic Biology* 52: 131-138.

KANE, N. C., J. M. BURKE, L. MAREK, G. SEILER, F. VEAR, G. BAUTE, S. J. KNAPP, ET AL. 2013. Sunflower genetic, genomic and ecological resources. *Molecular Ecology Resources* 13: 10–20.

KATOH, K. AND D. M. STANDLEY. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

LANGMEAD, B., C. TRAPNELL, M. POP, AND S. L. SALZBERG. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* doi:10.1186/gb-2009-10-3-r25.

LI, H., B. HANDSAKER, A. WYSOKER, T. FENNELL, J. RUAN, N. HOMER, G. MARTH, ET AL. 2009. The sequence alignment/map (SAM) format and SAMtools. *Bioinformatics* 25: 2078–2079.

LI, W., L. JAROSZEWSKI, AND A. GODZIK. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22: 1658–1659.

LIU, L., L. YU, AND S. V. EDWARDS. 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. *BMC Evolutionary Biology* 10: 302.

LODHI, M. A., Y. GUANG-NING, N. F. WEEDEN, AND B. I. REISCH. 1994. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis. Plant Molecular Biology Reporter* 12: 6–13.

LÖYTYNOJA, A. AND N. GOLDMAN. 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. *Science* doi:10.1126/science.1158395.

MAMANOVA, L., A. J. COFFEY, C. E. SCOTT, I. KOZAREWA, E. H. TURNER, A. KUMAR, E. HOWARD, J. SHENDURE, AND D. J. TURNER. 2010. Target-enrichment strategies for next-generation sequencing. *Nature Methods* 7: 111–118.

MANDEL, J. R., R. B. DIKOW, V. A. FUNK, R. R. MASALIA, S. E. STATON, A. KOZIK, R. W. MICHELMORE, ET AL. 2014. A target enrichment method for gathering phylogenetic information from hundreds of loci: an example from the Compositae. Applications in Plant Sciences. doi: http://dx.doi.org/10.3732/apps.1300085.

MARTINS E. P. AND T. F. HANSEN. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *The American Naturalist* 149: 646-667. doi: 10.2307/2463542

MCCORMACK J. E., M. G. HARVEY, B. C. FAIRCLOTH, N. G. CRAWFORD, T. C. GLENN, AND R. T. BRUMFIELD. 2013. A phylogeny of birds based on over 1,500 loci collected by target enrichment and high-throughput sequencng. *PLoS One*. doi: 10.1371/journal.pone.0054848

MCKINNON, G. E., D. A. STEANE, B. M. POTTS, AND R. E. VAILLANCOURT. 1999. Incongruence between chloroplast and species phylogenies in *Eucalyptus* subgenus *Monocalyptus* (Myrtaceae). *American Journal of Botany* 86: 1038–1046.

MCKINNON, G. E., R. E. VAILLANCOURT, D. A. STEANE, AND B. M. POTTS. 2004. The rare silver gum, *Eucalyptus cordata*, is leaving its trace in the organellar gene pool of *Eucalyptus globulus*. *Molecular Ecology* 13: 3751–3762.

MOODY, M. L. AND L. H. RIESEBERG. 2012. Sorting through the chaff, nDNA gene trees for phylogenetic inference and hybrid identification of annual sunflowers (*Helianthus* sect. *Helianthus*). *Molecular Phylogenetics and Evolution* 64: 145–155.

MULLER, M-H., M. LATREILLE, AND C. TOLLON. 2011. The origin and evolution of a recent agricultural weed: population genetic diversity of weedy populations of sunflower (*Helianthus annuus* L.) in Spain and France. *Evolutionary Applications* 4: 499–514.

NEIVA, J., G. PEARSON, M. VALERO, AND E. SERRAO. 2010. Surfing the wave on a borrowed board: range expansions and spread of introgressed organellar genomes in the seaweed *Fucus ceranoides* L. *Molecular Ecology* 19: 4812–4822.

NOTREDAME, C., D. G. HIGGINS, AND J. HERINGA. 2000. T-Coffee: a novel method for fast and accurate multiple sequence alignment. *Journal of Molecular Biology* 302: 205–217. doi:10.1006/jmbi.2000.4042.

PANERO, J. L. AND V. A. FUNK. 2002. Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). *Proceedings of the Biological Society of Washington* 115: 909–922.

R DEVELOPMENT CORE TEAM. 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; ISBN: 3-900051-07-0; Vienna, Austria. Available online: http://www.R-project.org (accessed on 4 December 2014).

RADUSKI, A. R., L. H. RIESEBERG, AND J. L. STRASBURG. 2010. Effective population size, gene flow, and species status in a narrow endemic sunflower, *Helianthus neglectus*, compared to its widespread sister species, *H. petiolaris*. *International Journal of Molecular Sciences* 11: 492–506.

RATAN, A. 2009. Assembly algorithms for next generation sequence data. Ph.D. dissertation, Pennsylvania State University, State College, Pennsylvania, USA.

REHOREK, V. 1997. Cultivated and escaped perennial *Helianthus* species in Europe. *Preslia* 69: 59–70.

REVELL, L. J. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3: 217-223. doi:10.1111/j.2041-210X.2011.00169.x

RIESEBERG, L. H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

RIESEBERG, L. H. 2006. Hybrid speciation in wild sunflowers. *Annals of the Missouri Botanical Garden.* 93: 34–48.

RIESEBERG, L. H., S. M. BECKSTROM-STERNBERG, A. LISTON, AND D. ARIAS. 1991. Phylogenetic and systematic inferences from chloroplast DNA and isozyme variation in *Helianthus* sect. *Helianthus* (Asteraceae). *Systematic Botany* 16: 50–76.

ROBINSON, H. 1981. A revision of the tribal and subtribal limits of the Heliantheae (Asteraceae). *Smithsonian Contributions to Botany* 51: 1–102.

SAMBATTI, J. B. M., D. ORTIZ-BARRIENTOS, E. J. BAACK, AND L. H. RIESEBERG. 2008. Ecological selection maintains cytonuclear incompatibilities in hybridization sunflowers. *Ecology Letters* 11: 1082–1091.

SCHILLING, E. E. 1983. Flavonoids of *Helianthus* series *angustifolii. Biochemical Systematics and Ecology.* 11: 341–344.

SCHILLING, E. E. 1997. Phylogenetic analysis of *Helianthus* (Asteraceae) based on chloroplast DNA restriction site data. *Theoretical and Applied Genetics* 94: 925–933.

SCHILLING, E. E. 2001. Phylogeny of *Helianthus* and related genera. *Oleagineux, Crops Gras, Lipides.* 8: 22–25.

SCHILLING, E. E. AND C. B. HEISER. 1981. Infrageneric classification of *Helianthus* (Asteraceae). *Taxon* 30: 393–403.

SCHILLING, E. E., C. R. LINDER, R. D. R. D. NOYES, AND L. H. RIESEBERG. 1998. Phylogenetic relationships in *Helianthus* (Asteraceae) based on nuclear ribosomal DNA internal transcribed spacer region sequence data. *Systematic Botany* 23: 177–187.

SCHILLING, E. E. AND J. L. PANERO. 2002. A revised classification of subtribe Helianthinae (Asteraceae: Heliantheae). I. Basal lineages. *Botanical Journal of the Linnean Society* 140: 65–76.

SEILER, G. J., T. J. GULYA, G. KONG, S. THOMPSON, AND J. MITCHELL. 2008. Collection of wild naturalized sunflowers from the land down under. Proceedings of the 30th Sunflower

Research Workshop, National Sunflower Association, January 10–11, 2008, Fargo, ND. Available from http://www.sunflowersnsa.com/uploads/research/317/Seiler_etal_DownUnder_08.pd.

SONG, S., L. LIU, S. V. EDWARDS, AND S. WU. 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. *Proceedings of the National Academy of Science* 109: 14942–14947. doi: 10.1073/pnas.1211733109

SPRING, O. AND E. E. SCHILLING. 1989. Chemosystematic investigation of the annual species of *Helianthus* (Asteraceae). *Biochemical Systematics and Ecology* 18: 139–143.

SPRING, O. AND E. E. SCHILLING. 1990. The sesquiterpene lactone chemistry of *Helianthus* sect. *Ciliares* (Asteraceae, Heliantheae). *Biochemical Systematics and Ecology* 18: 139–143.

SPRING, O. AND E. E. SCHILLING. 1991. The sesquiterpene lactone chemistry of *Helianthus* sect. *Atrorubentes* (Asteraceae, Heliantheae). *Biochemical Systematics and Ecology* 19: 59–79.

STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* doi:10.1093/bioinformatics/btl446.

STEPHENS, J. D., W. L. ROGERS, K. HEYDUK, J. M. CRUSE-SANDERS, R. O. DETERMANN, T. C. GLENN, AND R. L. MALMBERG. 2015. Resolving phylogenetic relationships for the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Molecular Phylogenetics and Evolution* 85: 76–87.

TIMME, R. E., B. B. SIMPSON, AND C. R. LINDER. 2007a. High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18S-26S ribosomal DNA external transcribed spacer. *American Journal of Botany* 94: 1837–1852.

TIMME, R. E., J. V. KUEHL, J. L. BOORE, AND R. K. JANSEN. 2007b. A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: identification of divergent regions and categorization of shared repeats. *American Journal of Botany* 94: 302–312.

TSITRONE A., M. KIRKPATRICK, AND D .A. LEVIN. 2003. A model for chloroplast capture. *Evolution* 57: 1776–1782.

UDVARDY, M. D. AND M. D. F. UDVARDY. 1975. *A classification of the biogeographical provinces of the world* (Vol. 8). Morges, Switzerland: International Union for Conservation of Nature and Natural Resources.

WEITEMIER, K., S. C. K. STRAUB, R. C. CRONN, M. FISHBEIN, R. SCHMICKL, A. MCDONNELL, AND A. LISTON. 2014. Hyb-Seq: Combining target enrichment and genome skimming for plant phylogenomics. *Applications in Plant Sciences* doi:10.3732/apps.1400042.

WOLFE, K. H. AND W. J. ELISENS. 1995. Evidence of chloroplast capture and pollen-mediated gene flow in *Penstemon* sect. *Peltanthera* (Scrophulariaceae). *Systematic Botany* 20: 395–412.

ZERBINO, D. R. AND E. BIRNEY. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research* 18: 821.

ZHANG, J. Q., S. Y. MENG, G. A. ALLEN, J. WEN, AND G. Y. RAO. 2014. Rapid radiation and dispersal out of the Qinghai-Tibetan Plateau of an alpine plant lineage Rhodiola (Crassulaceae). *Molecular Phylogenetics and Evolution* doi:10.1016/j.ympev.2014.04.013.

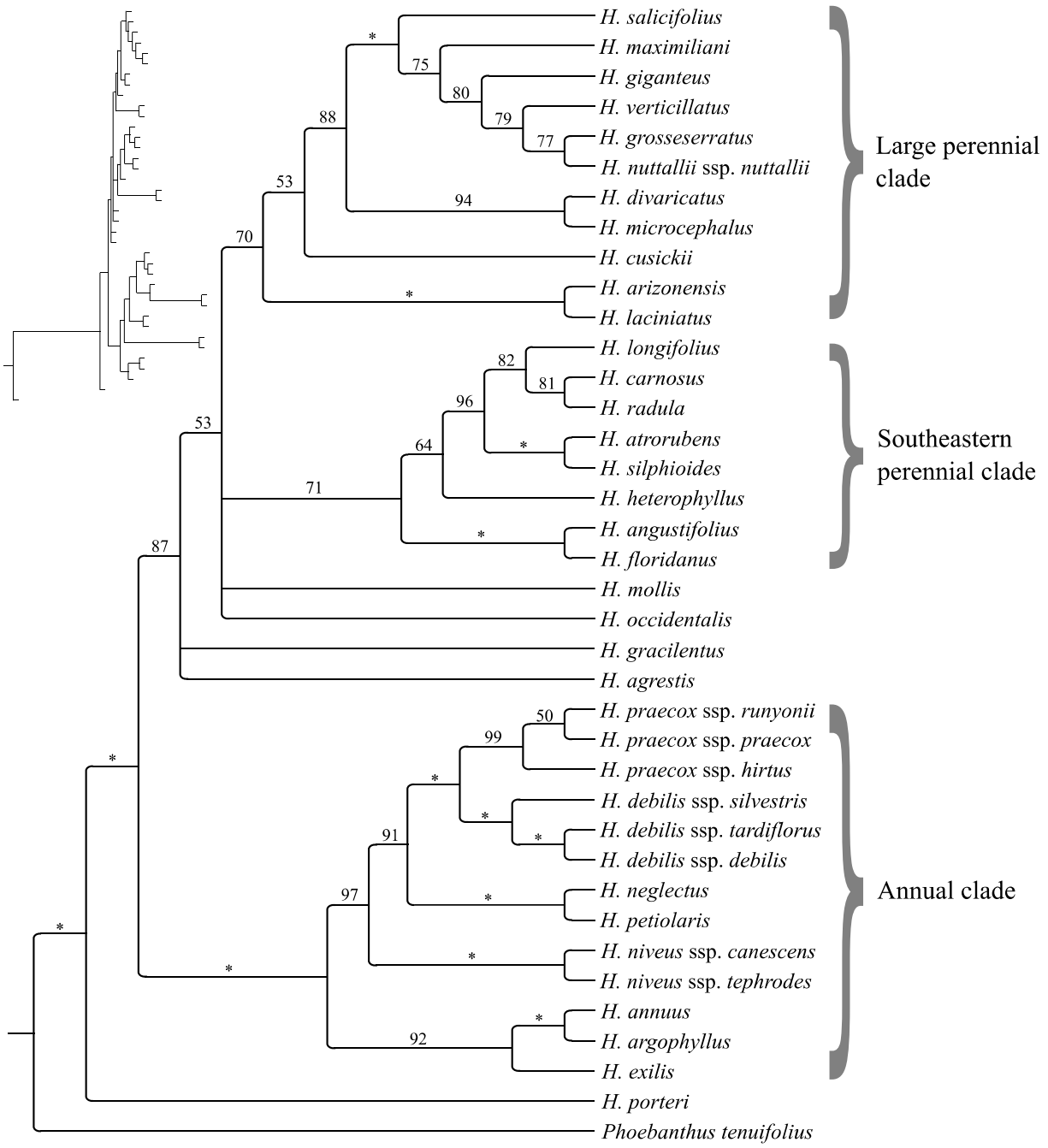

Figure 2.1- *Helianthus* MP-EST species tree based on 170 nuclear genes across 103 individuals. Nodes with <50 bootstrap support are collapsed; asterisks indicate bootstrap support of 100; all other support is listed at the nodes. The branch lengths in the MP-EST phylogram inset are represented in coalescence units ($2\tau/\theta$) with theta (θ) designating the population size estimator and tau (τ) as the parameterized branch length. Terminal tips are not estimated in MP-EST.

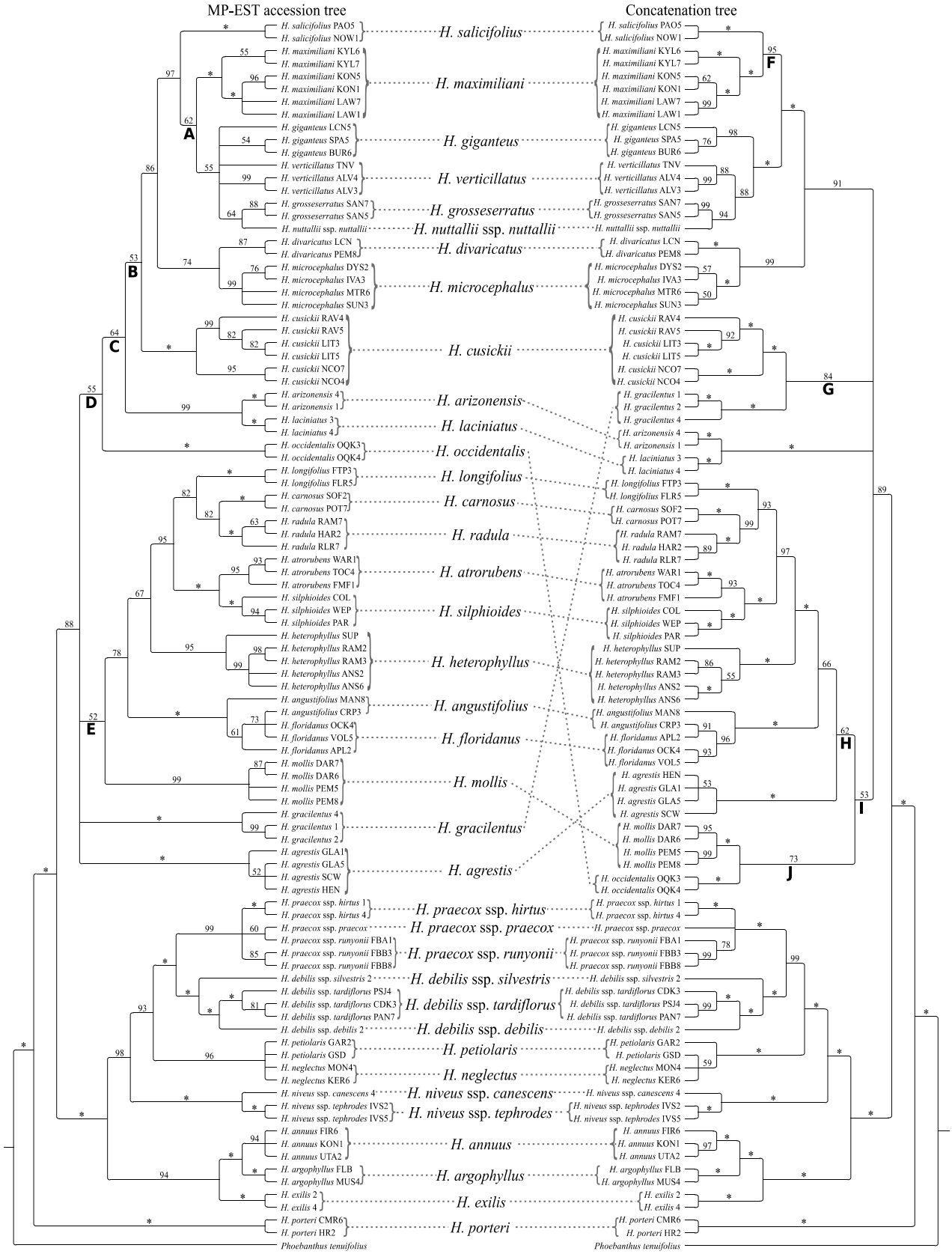
MP-EST accession tree
Concatenation tree
H. salicifolius PAO5
H. salicifolius NOW1
H. maximiliani KYL6
H. maximiliani KYL7
H. maximiliani KON5
H. maximiliani KON1
H. maximiliani LAW7
H. maximiliani LAW1
H. giganteus LCN5
H. giganteus SPA5
H. giganteus BUR6
H. verticillatus TNV
H. verticillatus ALV4
H. verticillatus ALV3
H. grosseserratus SAN7
H. grosseserratus SAN5
H. nuttallii ssp. nuttallii
H. divaricatus LCN
H. divaricatus PEM8
H. microcephalus DYS2
H. microcephalus IVA3
H. microcephalus MTR6
H. microcephalus SUN3
H. cusickii RAV4
H. cusickii RAV5
H. cusickii LIT3
H. cusickii LIT5
H. cusickii NCO7
H. cusickii NCO4
H. arizonensis 4
H. arizonensis 1
H. laciniatus 3
H. laciniatus 4
H. occidentalis OQK3
H. occidentalis OQK4
H. longifolius FTP3
H. longifolius FLR5
H. carnosus SOF2
H. carnosus POT7
H. radula RAM7
H. radula HAR2
H. radula RLR7
H. atrorubens WAR1
H. atrorubens TOC4
H. atrorubens FMF1
H. silphioides COL
H. silphioides WEP
H. silphioides PAR
H. heterophyllus SUP
H. heterophyllus RAM2
H. heterophyllus RAM3
H. heterophyllus ANS2
H. heterophyllus ANS6
H. angustifolius MAN8
H. angustifolius CRP3
H. floridanus OCK4
H. floridanus VOL5
H. floridanus APL2
H. mollis DAR7
H. mollis DAR6
H. mollis PEM5
H. mollis PEM8
H. gracilentus 4
H. gracilentus 1
H. gracilentus 2
H. agrestis GLA1
H. agrestis GLA5
H. agrestis SCW
H. agrestis HEN
H. praecox ssp. hirtus 1
H. praecox ssp. hirtus 4
H. praecox ssp. praecox
H. praecox ssp. runyonii FBA1
H. praecox ssp. runyonii FBB3
H. praecox ssp. runyonii FBB8
H. debilis ssp. silvestris 2
H. debilis ssp. tardiflorus PSJ4
H. debilis ssp. tardiflorus CDK3
H. debilis ssp. tardiflorus PAN7
H. debilis ssp. debilis 2
H. petiolaris GAR2
H. petiolaris GSD
H. neglectus MON4
H. neglectus KER6
H. niveus ssp. canescens 4
H. niveus ssp. tephrodes IVS2
H. niveus ssp. tephrodes IVS5
H. annuus FIR6
H. annuus KON1
H. annuus UTA2
H. argophyllus FLB
H. argophyllus MUS4
H. exilis 2
H. exilis 4
H. porteri CMR6
H. porteri HR2
Phoebanthus tenuifolius
H. salicifolius
H. maximiliani
H. giganteus
H. verticillatus
H. grosseserratus
H. nuttallii ssp. nuttallii
H. divaricatus
H. microcephalus
H. cusickii
H. arizonensis
H. laciniatus
H. occidentalis
H. longifolius
H. carnosus
H. radula
H. atrorubens
H. silphioides
H. heterophyllus
H. angustifolius
H. floridanus
H. mollis
H. gracilentus
H. agrestis
H. praecox ssp. hirtus
H. praecox ssp. praecox
H. praecox ssp. runyonii
H. debilis ssp. silvestris
H. debilis ssp. tardiflorus
H. debilis ssp. debilis
H. petiolaris
H. neglectus
H. niveus ssp. canescens
H. niveus ssp. tephrodes
H. annuus
H. argophyllus
H. exilis
H. porteri
Phoebanthus tenuifolius
A
B
C
D
E
F
G
H
I
J

Figure 2.2- Comparison of *Helianthus* MP-EST accession tree and concatenated nDNA accession tree (106,862bp) estimated from RAxML based on 170 nuclear genes across 103 individuals. Nodes with <50 bootstrap support are collapsed; asterisks indicate bootstrap support of 100; all other support is listed at the nodes. Letters at nodes for both MP-EST accession tree and concatenation tree indicate areas of disagreement between the two analyses. Letters correspond to gene tree discordance results listed in the text.

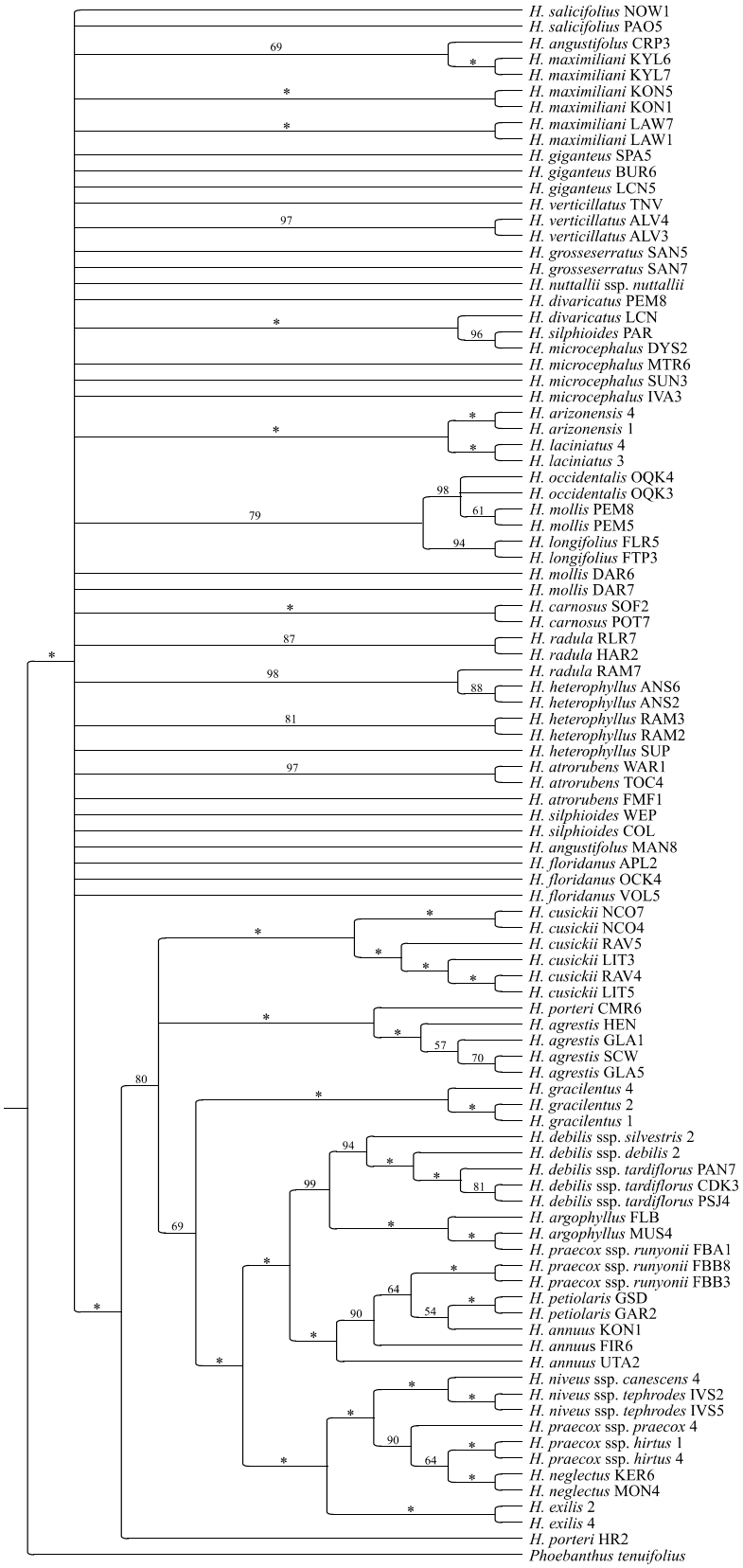
H. salicifolius NOW1
H. salicifolius PAO5
H. angustifolus CRP3
H. maximiliani KYL6
H. maximiliani KYL7
H. maximiliani KON5
H. maximiliani KON1
H. maximiliani LAW7
H. maximiliani LAW1
H. giganteus SPA5
H. giganteus BUR6
H. giganteus LCN5
H. verticillatus TNV
H. verticillatus ALV4
H. verticillatus ALV3
H. grosseserratus SAN5
H. grosseserratus SAN7
H. nuttallii ssp. *nuttallii*
H. divaricatus PEM8
H. divaricatus LCN
H. silphioides PAR
H. microcephalus DYS2
H. microcephalus MTR6
H. microcephalus SUN3
H. microcephalus IVA3
H. arizonensis 4
H. arizonensis 1
H. laciniatus 4
H. laciniatus 3
H. occidentalis OQK4
H. occidentalis OQK3
H. mollis PEM8
H. mollis PEM5
H. longifolius FLR5
H. longifolius FTP3
H. mollis DAR6
H. mollis DAR7
H. carnosus SOF2
H. carnosus POT7
H. radula RLR7
H. radula HAR2
H. radula RAM7
H. heterophyllus ANS6
H. heterophyllus ANS2
H. heterophyllus RAM3
H. heterophyllus RAM2
H. heterophyllus SUP
H. atrorubens WAR1
H. atrorubens TOC4
H. atrorubens FMF1
H. silphioides WEP
H. silphioides COL
H. angustifolus MAN8
H. floridanus APL2
H. floridanus OCK4
H. floridanus VOL5
H. cusickii NCO7
H. cusickii NCO4
H. cusickii RAV5
H. cusickii LIT3
H. cusickii RAV4
H. cusickii LIT5
H. porteri CMR6
H. agrestis HEN
H. agrestis GLA1
H. agrestis SCW
H. agrestis GLA5
H. gracilentus 4
H. gracilentus 2
H. gracilentus 1
H. debilis ssp. *silvestris* 2
H. debilis ssp. *debilis* 2
H. debilis ssp. *tardiflorus* PAN7
H. debilis ssp. *tardiflorus* CDK3
H. debilis ssp. *tardiflorus* PSJ4
H. argophyllus FLB
H. argophyllus MUS4
H. praecox ssp. *runyonii* FBA1
H. praecox ssp. *runyonii* FBB8
H. praecox ssp. *runyonii* FBB3
H. petiolaris GSD
H. petiolaris GAR2
H. annuus KON1
H. annuus FIR6
H. annuus UTA2
H. niveus ssp. *canescens* 4
H. niveus ssp. *tephrodes* IVS2
H. niveus ssp. *tephrodes* IVS5
H. praecox ssp. *praecox* 4
H. praecox ssp. *hirtus* 1
H. praecox ssp. *hirtus* 4
H. neglectus KER6
H. neglectus MON4
H. exilis 2
H. exilis 4
H. porteri HR2
Phoebanthus tenuifolius

Figure 2.3- *Helianthus* plastid accession tree (96,789bp) estimated from RAxML for 103 individuals. Nodes with <50 bootstrap support are collapsed; asterisks indicate bootstrap support of 100; all other support is listed at the nodes.

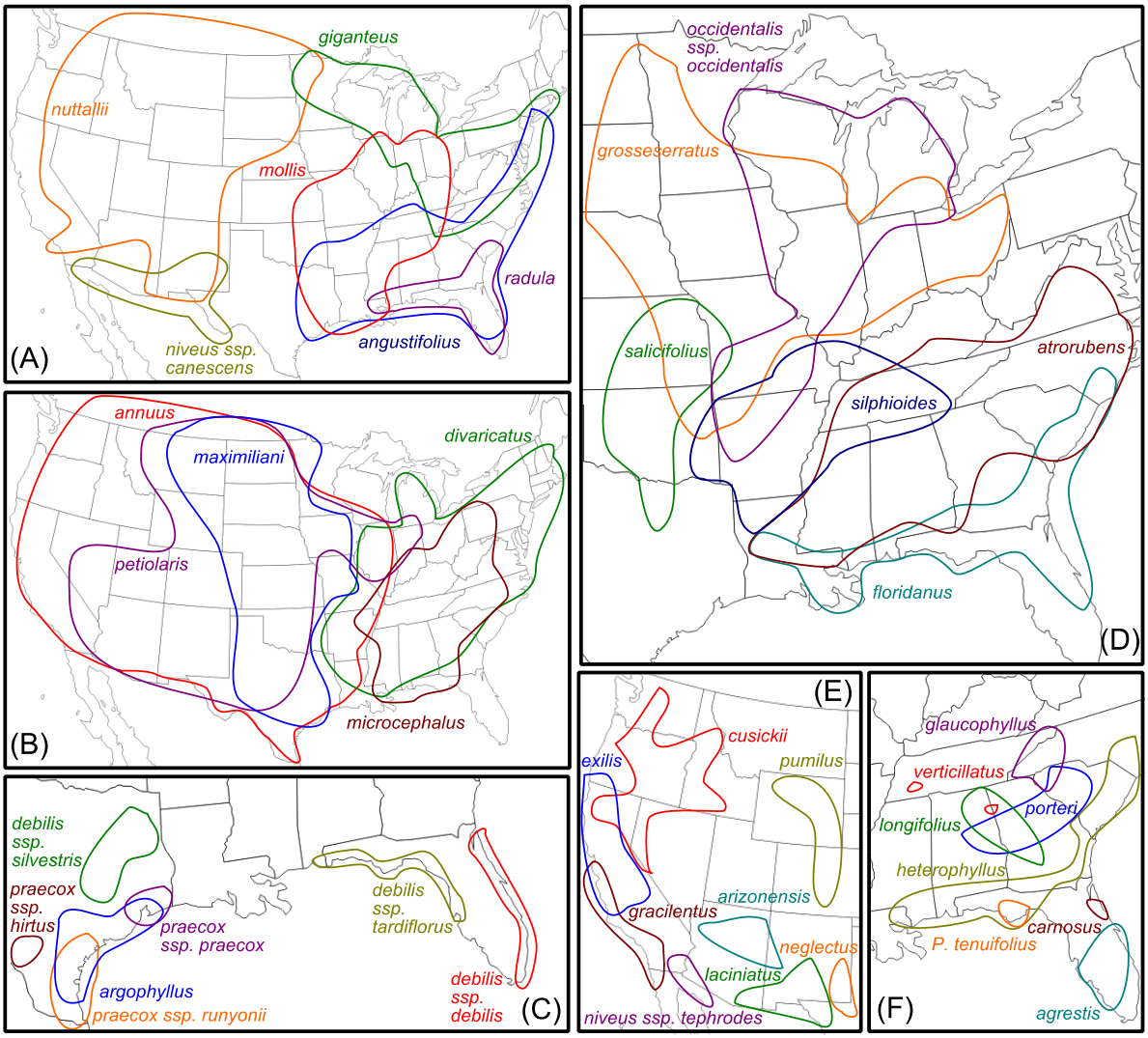

Figure 2.4- Approximate ranges of the 40 diploid species and subspecies considered in this study, based on the Biota of North America Program and Heiser et al. (1969).

CHAPTER III:

RESOLVING PHYLOGENETIC RELATIONSHIPS OF THE RECENTLY RADIATED CARNIVOROUS PLANT GENUS *SARRACENIA* USING TARGET ENRICHMENT[2]

[2]Stephens JD, Rogers WL, Heyduk K, Cruse-Sander JM, Determann RO, Glenn TC, Malmberg RL. 2015. Resolving phylogenetic relationships of the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. Molecular Phylogenetics and Evolution 85: 76–87. http://www.sciencedirect.com/science/article/pii/S1055790315000330

Abstract

The North American carnivorous pitcher plant genus *Sarracenia* (Sarraceniaceae) is a relatively young clade (<3 million years ago) displaying a wide range of morphological diversity in complex trapping structures. This recently radiated group is a promising system to examine the structural evolution and diversification of carnivorous plants; however, little is known regarding evolutionary relationships within the genus. Previous attempts at resolving the phylogeny have been unsuccessful, most likely due to few parsimony-informative sites compounded by incomplete lineage sorting. Here, we applied a target enrichment approach using multiple accessions to assess the relationships of *Sarracenia* species. This resulted in 199 nuclear genes from 75 accessions covering the putative 8-11 species and 8 subspecies/varieties. In addition, we recovered 42kb of plastome sequence from each accession to estimate a cpDNA-derived phylogeny. Unsurprisingly, the cpDNA had few parsimony-informative sites (0.5%) and provided little information on species relationships. In contrast, use of the targeted nuclear loci in concatenation and coalescent frameworks elucidated many relationships within *Sarracenia* even with high heterogeneity among gene trees. Results were largely consistent for both concatenation and coalescent approaches. The only major disagreement was with the placement of the *purpurea* complex. Moreover, results suggest an Appalachian massif biogeographic origin of the genus. Overall, this study highlights the utility of target enrichment using multiple accessions to resolve relationships in recently radiated taxa.

Introduction

The evolution of carnivory in angiosperms has long fascinated evolutionary biologists, with the most notable being Charles Darwin (Darwin, 1875). This interest partially stems from the complex trapping structures used in attraction, retention, and digestion of prey and subsequent absorption of nutrients (Albert et al., 1992; Juniper et al. 1989). These carnivorous adaptations to nutrient poor habitats have independently evolved six times in flowering plants, resulting in approximately 645 species, which often display tremendous morphological diversity at both the infrafamilial and infrageneric level (Albert et al., 1992; Ellison and Gotelli, 2009). Insight into the patterns of structural evolution and diversification across these groups requires an explicit understanding of their evolutionary relationships. Phylogenies currently exist for many carnivorous genera including *Utricularia* (bladderworts, Jobson et al., 2003; Müller and Borsch, 2005), *Drosera* (sundews, Rivadavia et al., 2003), and *Nepenthes* (Old World pitcher plants, Meimberg et al., 2001), yet the evolutionary relationships of one of the more well-studied genera, *Sarracenia* (New World pitcher plants), remain largely ambiguous.

Sarracenia is the most recently diverged group of the three extant genera within the family Sarraceniaceae (Ellison et al., 2012; Neyland and Merchant, 2006). All species within Sarraceniaceae are carnivorous with no geographical overlap among genera. The basal monotypic lineage, *Darlingtonia californica,* is restricted to serpentine seeps in Oregon and California, while the estimated 15 *Heliamphora* species are confined to the Guiana Highlands tepuis in South America (McPherson, 2007). *Sarracenia* is endemic to seepage slopes, wet pine savannas, and fens of North America, predominately the southeastern United States Coastal Plain with one subspecies, *purpurea* ssp. *purpurea*, extending into the northeastern United States and southern Canada. Unfortunately these habitats are being destroyed and estimates suggest less

than 3% of historic *Sarracenia* habitat remains (Folkerts, 1982; Folkerts and Folkerts, 1993). This continued habitat loss has resulted in the U.S. Fish and Wildlife and Convention on International Trade in Endangered Species (CITES) listing of three endangered taxa within *Sarracenia* and one taxa considered a candidate for listing (www.cites.org). Complicating protection status of other members of this genus is the disagreement among sources in the number of recognized species, subspecies, and varieties with numbers ranging between 8-11 species and as many as 41 subspecies, varieties, and forms (Ellison et al., 2014).

Previous attempts at constructing a phylogeny for *Sarracenia* from nuclear (Ellison et al., 2012; Neyland and Merchant, 2006), chloroplast (Bayer et al., 1996; Ellison et al., 2012), and mitochondrial regions (Ellison et al., 2012) have been inconsistent, typically with numerous polytomies within the genus. In addition, the relatively short branch lengths dated at roughly 0.5-3 million years ago (mya) (Ellison et al., 2012) indicate that this group may have undergone a recent, rapid diversification. Further complicating phylogenetic resolution is frequent hybridization among sympatric species (Furches et al., 2013; Mellichamp and Case, 2009). Both short branches and hybridization can have dramatic effects on species tree estimation. In particular, a recent radiation increases the chance that genes retain ancestral polymorphisms, resulting in incomplete lineage sorting (Pamilo and Nei, 1988); additionally, hybridization can lead to reticulation within gene trees (Hennig, 1966). Using multilocus data and modeling differences in gene history with use of the multispecies coalescent model can mitigate these potential sources of gene tree discordance within the species tree (Degnan and Rosenberg, 2009; Knowles, 2009; Liu et al., 2009). Increasing loci is expected to produce more accurate model parameters and therefore increase nodal support values in phylogenetic analyses (Maddison, 1997; Song et al., 2012), and use of multispecies coalescence has repeatedly outperformed

concatenation methods under simulated and empirical data (Kubatko and Degnan, 2007; McCormack et al., 2012; Song et al., 2012). Including multiple accessions per species can also decrease the variance around the effective population size parameter within the coalescent framework (Heled and Drummond, 2009).

To further our understanding of evolutionary relationships of *Sarracenia* we conducted target enrichment of nuclear genes from multiple accessions per species sequenced on an Illumina HiSeq platform. Target enrichment involves the use of oligonucleotide probes that retain selected genomic regions for sequencing while reducing non-selected DNA (Mamanova et al., 2010). Target enrichment is highly applicable for phylogenetics as it works well for non-model organisms, is cost-efficient, and allows for an increase in the number of species and individuals for phylogenetic analysis (Faircloth et al., 2012a; Lemmon and Lemmon, 2013). Here, we (1) assessed the utility of this method for a recently radiated, non-model genus, (2) compared the multispecies coalescent approach with a concatenation approach, and (3) determined the evolutionary relationships within *Sarracenia*. The resolved species level phylogeny is then discussed in regard to the current taxonomy, biogeography, and conservation status of this group. Taken together, this multilocus and multiaccessional approach represents the most robust attempt to resolve the *Sarracenia* phylogeny to date and has implications for other recently radiated groups.

Material and methods

Taxon sampling

The majority of leaf tissue was sampled from the Atlanta Botanical Garden, which maintains an extensive living collection of *Sarracenia* species from various localities for conservation and as a reference for the North American Plant Collections Consortium. The remaining samples were

collected from plant stocks maintained at the University of Georgia Plant Biology greenhouse and field collections. Current estimates list between 8 to 11 species with many varieties and subspecies being designated to the species level based on differing taxonomic schemes (Ellison et al., 2014). We sampled 71 *Sarracenia* accessions covering putative species, varieties, and subspecies. These include the eleven species recognized by Mellichamp and Case (2009) (*alabamensis, alata, flava, jonesii, leucophylla, minor, oreophila, psittacina, purpurea, rosea, rubra*) with 1-8 localities spanning the southeastern range of each species (See Table S3.1) and additional samples from Maryland, Nova Scotia, and Wisconsin for *purpurea* ssp. *purpurea*. The 71 accessions also include three subspecies/varieties from the *purpurea* complex (ssp. *purpurea*, ssp. *venosa*, ssp. *venosa* var. *montana*), two subspecies from the *rubra* complex (ssp. *gulfensis*, ssp. *wherryi*), one *minor* variety (var. *okefenokeensis*), and two *flava* varieties (var. *rugelii*, var. *rubricorpora*). These putative subspecies and varieties are based on a combination of taxonomic descriptions between Mellichamp and Case (2009) and McPherson and Schnell (2011). Taxonomic descriptions have frequently designated *alabamensis* and *jonesii* as subspecies within the *rubra* complex and *rosea* as *purpurea* ssp. *venosa* var. *burkii*. Three *Darlingtonia californica* and one *Heliamphora minor* (both within Sarraceniaceae) were used as outgroups for the genus. This coverage of varieties, subspecies, and range distribution of putative species allows for a comprehensive analysis of this genus. Voucher specimens were deposited in either the University of Georgia Herbarium (UGA) or the Texas A&M Herbarium (TAES) (Table S3.1).

Probe design

Targets for enrichment were initially identified by aligning *Sarracenia psittacina* and *S. purpurea* transcriptomes (Srivastava et al., 2011). All repeat-like regions were masked using RepeatMasker (http://www.repeatmasker.org/) prior to probe design. Targets with promising

single nucleotide polymorphisms for phylogenetic analyses and at least two independent reads from each species were selected for further processing (~1,000 contigs). Because paralogous sequences are not ideal for phylogenetic inference due to their independent evolutionary histories, potential targets were screened for paralogous signals using two methods. First, a within-species BLAST (Altshul et al., 1997) search of possible paralogous sequences was conducted with a stringent e-value cut off of $< 3 \times 10^{-20}$. A reciprocal best BLAST(blastn) hit approach was then used on the subsequent targets to determine orthologous sequences between the two species. Targets that did not meet the cut off criterion were discarded from the potential target database; this resulted in 646 genes for target sequencing. Previous work suggests that *Sarracenia* may be a partial polyploid (Srivastava et al., 2011); however, we are confident that our stringent screening of paralogs prior to probe design and additional downstream removal of duplicates adequately addresses this possible source of conflict. Approximately three 120-mer oligonucleotide probes were designed for each gene per the manufacturer's probe design specifications. These probes were commercially synthesized by Mycroarray® into a custom MYbaits kit (http://www.mycroarray.com; Ann Arbor, MI).

DNA extraction, library preparation, sequencing

All leaves (i.e. pitchers) were cut near the base of the plant, sliced open, and cleaned of any insect residue, algae, soil, and other particulates. Areas of the leaf that were senescing, discolored, or greatly impacted from decomposing insect prey were removed and discarded. The subsequent leaves were ground to powder using liquid nitrogen. Initially DNA extractions were conducted using 'option Y' from Peterson et al. (2000), but we were unable to extract high-quality DNA from older *Sarracenia* tissue and outgroups. Therefore, the majority of extractions were performed following the methods described in Lodhi et al. (1994) with slight modifications.

Specifically, we replaced the 5M sodium chloride solution with 3M sodium acetate, and used two consecutive 2mL treatments with 24 parts chloroform to 1 part octanol instead of a single 6mL purification. We also used 1 volume cold iso-propanol in the final spin at 13,000rpm to precipitate DNA. All DNA extractions were assessed for concentration and purity using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). Samples were then sheared to approximately 180-500bp lengths on a Biorupter Standard (Diagenode Cat No. UCD-200, Woburn, MA). Library construction was completed using a protocol developed by Glenn et al. (In preparation). This protocol consists of removing overhangs created from shearing, phosphorylating the 5' ends and adding a single adenosine to the 3' end, ligating unique Illumina adapters with custom 10nt indexes to DNA fragments, and finally amplification of ligated DNA fragments with universal p5 and p7 primers (Faircloth and Glenn, 2012) to create uniquely indexed Illumina TruSeqHT compatible libraries. Samples with similar NanoDrop readings were combined at equal ratios resulting in two or three indexed individuals per tube. The MYbaits protocol was followed per manufacturer's instructions. To reduce daisy chaining during the hybridization, a blocking oligonucleotide with 10 inosines at the index location was used (c.f., Faircloth et al., 2012b). After target enrichment the subsequent libraries were sequenced on an Illumina HiSeq PE100 arranged by the Georgia Genomic Facility.

Assembly and alignment

All demultiplexed pooled reads were assessed for quality using FastQC v0.10.1 (http://www.bioinformatics.babraham.ac.uk/). Sequence reads were trimmed at the 3' end with a Phred score of <20 to a minimum length of 40bp using FastX v 0.013.2 (http://hannonlab.cshl.edu/fastx_toolkit/). Finally, all Illumina adapter contaminants were removed via FAR v2.15.

We used a combination of two approaches for target enrichment assembly similar to Heyduk et al. (*in review*). First, a *de novo* assembly method was conducted using Trinity version r2013-02-25 (Grabherr et al., 2011). The Trinity assembler was designed for RNA-seq, therefore Trinity tends to make multiple isoforms within contigs to take into account alternative splicing. To remove poorly supported isoforms (<1% per-component read support) the Trinity output file was parsed using RSEM in Bowtie 1.1.0 as described in the Trinity manual (Langmead et al., 2009). The second approach was a reference method, whereby the targeted genes used for probe design were used by the Columbus extension module (Zerbino, 2010) in VELVET to aid in assembly of reads. For each library, the k-mer length was optimized via KmerGenie (Chikhi and Medvedev, 2013) and then assembled with VELVET v 1.2.08 (Zerbino and Birney, 2008).

Contigs from both assembly methods that had at least 95% identity over 20bp were merged using CAP3 v10/2011 (Huang and Madan, 1999). The resulting contigs were matched against the gene targets used for probe designs using BLAST (Altshul et al., 1997) and extracted for use via two steps. First, contigs were extracted if they had a 1:1 hit with the gene target. Second, a separate BLAST output containing instances where two contigs from an accession had best hits to the same target was created. If the two hits were non-overlapping (possibly due to intron regions) they were extracted and concatenated. The extracted contigs from each BLAST output were merged, renamed according to gene target, parsed into gene files, and aligned via Prank v100802 (Löytynoja and Goldman, 2008). The subsequent aligned gene files were filtered to remove poorly aligned regions using Gblocks v0.91b (Castresana, 2000). Finally, pairwise distances were calculated for each gene file. Alignments with an average pairwise distance of less than 0.35, at least 50% of the accessions present, showing no more than 45% missing data, and with at least one outgroup were used for subsequent downstream analyses.

Species tree analysis

Prior to species tree estimation, five genes were randomly selected to determine the model of evolution in JModelTest 2.1.1 using AIC (Darriba et al., 2012). All five genes had the same best-fitting model (GTRGAMMA) which was used for the whole dataset and implemented in RAxML v04/26/2012 (Stamatakis, 2006) for gene tree estimation using 500 bootstraps. These gene trees were then used as input data for species tree estimation. Species tree estimations were conducted using the Maximum Pseudolikelihood Estimation of the Species Tree v1.4 (MP-EST) method (Liu et al., 2010). MP-EST accounts for gene tree discordance resulting from incomplete lineage sorting and implements the triplet algorithm across gene trees to estimate the species tree topology. We used two approaches for phylogenetic analysis in MP-EST: one in which accessions were grouped into putative taxonomic designations by Mellichamp and Case (2009) and McPherson and Schell (2011) and a second approach that treats all accessions as terminal taxa. The latter method was used to examine whether accessions exhibited reciprocal monophyly within their taxonomic groupings.

To test hypotheses of hybrid species within the genus, we used the Species Tree Estimation using Maximum Likelihood with hybridization (STEM-hy) method (Kubatko, 2009). This method uses a model-selection framework to evaluate hypotheses of hybridization in the presence of incomplete lineage sorting (Kubatko, 2009). STEM-hy requires estimates of theta (θ) and rate multipliers (*ri*). Theta (θ) was estimated and averaged from five gene trees using MIGRATE-n version 3.6.4 (Beerli, 2009), which estimates population parameters using maximum likelihood estimation under a coalescent framework (Beerli and Felsenstein, 2001). Estimation of rate multiplier (*ri*) values for each gene tree were calculated as the average divergence from the outgroups (Yang, 2002). The MP-EST species tree was used as the input

species tree for STEM-hy. Tests of possible hybrid species were conducted on taxa that showed incongruence between the concatenated tree and MP-EST tree.

To assess discordance among genes, custom Perl scripts were used to query the presence and support values of nodes in consensus gene trees produced in RAxML. Five nodes of particular interest were queried based on conflict seen between the MP-EST accession tree estimation and the concatenation (see below) methodology. Nodes labeled in Figure 3.2 were checked in all gene trees against either the MP-EST tree (nodes A and B) or the concatenated tree (nodes C, D, and E). Gene trees were classified as follows: a gene tree which had the node present with bootstrap support >80 "strongly agreed"; trees with support on the queried node between 50 and 80 "weakly agreed"; trees with support between 20 and 50 "weakly conflicted"; and trees with bootstrap support of less than 20 "strongly conflicted."

Concatenation analysis of nDNA and cpDNA

To compare with the coalescent analysis, we concatenated all 199 nuclear genes into a 'supergene' of 128,110bp. This concatenated dataset was used to estimate an accession tree in RAxML with 1,000 bootstraps. In addition to the targeted nuclear genes, an average of 178,748 trimmed reads mapped back to a reference *Vitis vinifera* plastid genome (Jansen et al., 2006) using Bowtie2 v2.2.1 (Langmead and Salzberg, 2012). *Vitis vinifera* plastid genome was used as the reference due to high sequence similarity between *Vitis* and *S. psittacina/purpurea* found in Srivastava et al. (2011); furthermore, the placement of the Ericales is contested (Soltis and Soltis, 2004), though high sequence similarity between *Vitis* and *Sarracenia* species indicates it may be closer to rosids than asterids. These mapped reads were extracted from the trimmed reads for each accession using SAMtools (Li et al., 2009). Assembly of the chloroplast reads was conducted in the reference-based assembler YASRA (Ratan, 2009) with the *Vitis vinifera* plastid

genome as a reference. Contigs were then BLASTed back to the reference, corrected for strandedness, and finally concatenated for each accession. The resulting concatenated sequence for each accession was aligned with the reference using MAFFT v7.029-e (Katoh and Standley, 2013) and poorly aligned regions were filtered using default setting with Gblocks v0.91b (Castresana, 2000) followed by visual inspection in Geneious v7.0.6 (http://www.geneious.com/). An accession chloroplast tree was estimated in RAxML v04/26/2012 (Stamatakis, 2006) under a GTRGAMMA model with 1,000 bootstraps.

Results

Assembly and gene trees

Each accession had roughly 3.5 million trimmed reads which resulted in an average of 7,124 contigs after Trinity assembly and 67,894 contigs from the Velvet assembly (Table S3.1). These assemblies were subsequently merged into an average of 5,608 contigs per accession with 546 contigs matching the 646 gene targets. The contigs on target had an average N50 of 503bp with approximately 11x coverage.

In total 199 genes were used for subsequent phylogenetic analyses after poor alignments, genes with >50% missing data, and those missing an outgroup were discarded. Sixty-three (32%) of the 199 gene trees had all putative ingroup species represented, while 76 (38%) gene trees had one missing species, 44 (22%) had two missing species, 14 (7%) had three missing species, and 2 (1%) had four missing species. Among the 199 genes, an average of 56 accessions were present with a length of 642bp per gene. This totaled 128,110bp used for nuclear phylogenetic analyses. Of the 128,110bp, 11,202bp (8.7%) were variable with only 5,066bp (4%) being parsimony-informative within the ingroups. From the trimmed sequencing reads, we were able to recover 42,031bp of the plastome, which contained 783 variable sites (~1.9%) and 216

parsimony-informative sites (~0.5%) within the ingroups. The 42,031bp recovered consisted of intron and exon regions within the Long Single Copy and Short Single Copy segment of the chloroplast. Resulting gene trees, gene alignments, and species trees have been deposited in Dryad repository http://dx.doi.org/10.5061/dryad.nn153.

MP-EST species and accession tree

The MP-EST analyses supported the monophyly of the *Sarracenia* clade with *Darlingtonia californica* as basal to *Heliamphora* and *Sarracenia* (Fig. 3.1,3.2), which is consistent with the concatenated nDNA tree and plastid tree (Fig. 3.2,3.3). Additionally, MP-EST resolved many of the phylogenetic relationships within *Sarracenia* with high bootstrap support (Fig. 3.1,3.2). Specifically, both MP-EST analyses support an "*oreophila* clade" consisting of *oreophila* as sister to the *alata*, *leucophylla*, and the *rubra* complex (i.e. *alabamensis, jonesii,* ssp. *wherryi,* ssp. *gulfensis*) and another clade comprising *flava, minor*, *psittacina*, and the *purpurea* complex (ssp. *venosa* var. *montana*, ssp. *venosa*, ssp. *purpurea*, and *rosea*). Within the *oreophila* clade there was high bootstrap support for *alabamensis* sharing a more recent common ancestor with *leucophylla* (Fig. 3.1). This result suggests that the *rubra* complex is a polyphyletic group. It should be noted that there is low bootstrap support (<65) for relationships between *leucophylla, alata* and the other members of the *rubra* complex. In addition, the MP-EST accession tree (Fig. 3.2) shows a polytomy among *alabamensis, alata, leucophylla,* and the *rubra* complex with the exception of *rubra* as sister to *jonesii.* All *jonesii* accessions formed a monophyletic clade with 80 bootstrap support. The relationships between *alata* accessions were unresolved as well as their relationships to members of *rubra* complex.

The other subclade recovered within *Sarracenia* consists of *flava*, *minor*, *psittacina*, and the *purpurea* complex with 89 bootstrap support (Fig. 3.1). All members of the *purpurea*

complex were monophyletic with *purpurea* ssp. *venosa* var. *montana* sister to all other *purpurea* subspecies (bootstrap value = 100; Fig. 3.1). In addition, infraspecific relationships within the *purpurea* clade suggest that the *venosa* subspecies are paraphyletic. Moreover, *rosea* (*purpurea* ssp. *venosa* var. *burkii*) is placed within the MP-EST *purpurea* clade (Fig. 3.1). All *purpurea* accession relationships were unresolved, with the exception of *purpurea* ssp. *venosa* var. *montana* and *purpurea* ssp. *purpurea* accessions (bootstrap = 50, 81, respectively; Fig. 3.2). Conversely, all accessions for *flava*, *psittacina*, and *minor* had 100 bootstrap support, however varieties within species were not monophyletic. The species and accession tree analyses supports the placement of the *purpurea* complex as sister to the *flava*, *psittacina*, and *minor* clade.

Concatenated nDNA and plastid tree

The RAxML concatenated nDNA accession tree has a similar overall topology to the MP-EST accession tree (Fig. 3.2). The tree shows strong support for *oreophila* as sister to *alata*, *leucophylla*, and the *rubra* complex (bootstrap value = 100). Additionally, it supports a polyphyletic *rubra* complex with *alata* as being a part of the *rubra* polytomy. Many relationships within the *rubra* complex are unresolved with the exception of *rubra* and *jonesii*, though two accessions of *rubra* are placed closer to the *jonesii* clade (Fig. 3.2). Unlike the MP-EST accession tree, concatenation supported the placement of *leucophylla* as sister to a polytomy of *alata* and the *rubra* complex, albeit with low support (bootstrap value = 58).

The concatenation tree supports the *purpurea* complex as sister to the *oreophila* clade with high support (bootstrap value = 91). Moreover, it places *psittacina* as sharing a more recent common ancestor with *minor*, although this relationship is poorly supported (bootstrap value = 55; Fig. 3.2). Accessions within these clades were monophyletic at 100 bootstrap support. Similar to the MP-EST accession analysis both *purpurea* ssp. *venosa* var. *montana* and *purpurea*

ssp. *purpurea* are supported as monophyletic taxa, however monophyly is not supported for *purpurea* ssp. *venosa* and *rosea* (*purpurea* ssp. *venosa* var. *burkii*).

In comparison to both nuclear analyses, the plastid tree had very low resolution with polyphyletic relationships across most species and in both the *purpurea* and *rubra* complexes (Fig. 3.3). *Sarracenia jonesii* (*rubra* ssp. *jonesii*) and *purpurea* ssp. *venosa* var. *montana* comprised one clade within the plastid accession tree with a 100 percent bootstrap support (Fig. 3.3). This result may indicate introgression of the chloroplast between these two species as these species have overlapping distributions (Fig. 3.4a,c).

Gene tree discordance

For all nodes queried (Fig. 3.2, A-E), the majority of gene trees showed some degree of conflict; no node had more than 3 gene trees that agreed. Conflict at nodes A-E (Fig. 3.2) may be the result of factors other than incomplete lineage sorting, such as gene reticulation resulting from hybridization. The hypothesis that *minor*, *psittacina* and the *purpurea* complex may be the result of hybridization between sister taxa was not supported in STEM-hy.

Discussion

Target enrichment with recently radiated taxa

There have been three attempts at resolving the relationships within *Sarracenia* with little resolution or agreement in species relationships (Bayer et al., 1996; Ellison et al., 2012; Neyland and Merchant 2006). Incongruence among previous attempts that used few genes highlights the difficulties of inferring phylogenies of recently radiated groups. These groups often have not accumulated enough polymorphisms to overcome the signals left by incomplete lineage sorting. To circumvent this issue, we used 199 genes for phylogenetic analyses. This is a 28-fold increase of loci for analysis when compared to previous phylogenetic studies of this group (Ellison et al.,

2012), and garnered additional parsimony-informative sites (~5,000) for a more robust resolution of relationships within *Sarracenia*. This study emphasizes the utility of target enrichment for discerning relationships among recently diverged taxa.

Comparison of phylogenetic approaches

The use of next generation data requires that the methods adequately model the complexities inherent in multilocus datasets (Lemmon and Lemmon, 2013). The multispecies coalescent has been shown through simulations and theory to handle incomplete lineage sorting and produce accurate species trees when compared to concatenation methods (Degnan and Rosenberg, 2009; Edwards, 2009; Liu et al., 2008; Liu et al., 2009; Liu et al., 2010). Our analysis shows few conflicts between the MP-EST accession tree and the concatenation tree. This result may be due to robust taxon sampling in the genus (and possibly the use of multiple accessions) as previous simulations have shown that concatenation often gives incorrect topology as the number of missing taxa increases (Song et al., 2012). Additionally, 70% of the gene trees had either all taxa or just one missing taxon. This combination of conservative filtering of genes with complete taxon sampling may have contributed to the overall congruence between methods. Another possible reason for congruence between analyses could be due to lack of an "anomaly zone" (i.e., a highly probable gene topology that conflicts with the species tree) (Degnan and Rosenberg, 2006). Concatenation analyses are particularly susceptible to anomalous gene trees as this approach estimates the species tree based on the commonly observed gene tree (Degnan and Rosenberg, 2006; Liu and Edwards, 2009). Examination of gene trees in our study show high levels of gene tree heterogeneity with no dominant topology, possibly decreasing the likelihood of anomalous gene trees.

To further compare the concatenation tree and MP-EST accession tree, we examined the two major nodes that conflicted between the methods: the placement of the *purpurea* complex and *psittacina*. Alternative topologies were counted at these nodes to see if the conflicting topology was more frequent. Due to high levels of variable gene trees we were unable to discern a common, alternative topology for all nodes queried. These topological conflicts, which notably occur at short internodes, most likely resulted from retention of ancient polymorphisms resulting in high levels of incomplete lineage sorting. In addition to examining gene tree discordance, we tested hypotheses of speciation for *minor*, *psittacina* and the *purpurea* complex resulting from hybridization between sister taxa. These hypotheses were rejected from STEM-hy; however, speciation within this genus may be the result of hybridization among numerous taxa for which we were unable to test within STEM-hy. Current hybridization has been well documented for *Sarracenia* with nineteen known hybrids occurring in wild populations and these are not limited to hybridization between sister taxa (Mellichamp, 2009). While care was taken to select accessions that exhibited no phenotypic signs of hybridization, ancient hybridization among species has most likely influenced speciation within this genus and contributed to incongruent topologies among gene trees.

In addition, the conflicting topologies between the nuclear phylogeny and the plastid tree further support the role of hybridization and incomplete lineage sorting within this genus. For example, the cpDNA tree supports a monophyletic clade consisting of all *jonesii* (*rubra* ssp. *jonesii*) and *purpurea* ssp. *venosa* var. *montana* accessions, a result contradicted by both coalescent and concatenated nuclear trees. This suggests possible introgression of the maternally-inherited plastome. These two species are largely isolated in mountain bogs in Georgia, North Carolina, and western South Carolina and are known to hybridize where they occur in sympatry

(Fig. 3.4a,c; Mellichamp and Case, 2009). In contrast, Ellison et al. (2012) found signals of introgression between *purpurea* ssp. *venosa* var. *montana* and *oreophila* within the chloroplast. While we did not recover this result, many *oreophila* individuals were found to be sister to the *purpurea* ssp. *venosa* var. *montana* and *jonesii* clade possibly resulting from the proposed introgression.

Further complicating resolution using cpDNA is the lack of informative sites. In general, all accessions are not reciprocally monophyletic and there is little resolution across the tree. This is not unexpected as the chloroplast genome is more slowly evolving than the nuclear genome (Wolfe et al., 1987). Given the estimated radiation of this genus at 0.5-3 mya (Ellison et al., 2012) there has been little time for the chloroplast to accumulate enough informative polymorphisms. This lack of resolution is similar to a previous attempt to resolve these relationships using chloroplast data (Ellison et al., 2012). In addition, recently radiated taxa are often known to have conflicting cpDNA or mtDNA trees compared to nDNA trees (Sanders et al., 2013; Shaw, 2002; Zhang et al., 2014).

Evolutionary relationships within Sarracenia

Sarracenia oreophila clade

Similar to previous attempts, *oreophila, alata, leucophylla,* and the *rubra* complex share a close affinity with each other (Ellison et al., 2012; Neyland and Merchant, 2006), with *oreophila* as sister to the rest of the clade. In addition, there are a number of ambiguous relationships within the clade, specifically involving members of the *rubra* complex. This is not surprising, as the relationships and numbers of species/subspecies within the *rubra* complex have been highly debated due to considerable phenotypic variation maintained across disjunct populations within the range of *rubra* (Bell, 1949; Case and Case, 1976; McDaniel, 1971;

Schnell, 1977, 1978). However, MP-EST and concatenation analyses did show strong support for the relationship between *rubra* and *jonesii* (*rubra* ssp. *jonesii*). These two members of the complex are closer geographically than the other subspecies within this complex (Fig. 3.4a).

Similar to *jonesii, alabamensis* (*rubra* ssp. *alabamensis*) is found in isolated populations (Fig. 3.4a) and is phenotypically different from other members of the r*ubra* complex (Schnell, 1977). In addition, *alabamensis* grows phyllodia (i.e. non carnivorous leaves), a trait which is absent in other *rubra* complex members and *alata* and present in *oreophila* (Ainsworth and Ainsworth, 1996). This character supports the MP-EST species tree placement of *alabamensis* as more closely related to *oreophila* suggesting a polyphyletic *rubra* complex. However, in both accession trees *alabamensis* is in a polytomy with *alata, leucophylla,* and other members of the *rubra* complex. The inclusion of additional accessions of *alabamensis* may have supported monophyly within the species, but unfortunately were not successfully sequenced.

The other subspecies (*rubra* ssp. *gulfensis*, *rubra* ssp. *wherryi*) relationships within the *rubra* complex remain unresolved in the accession trees and have low bootstrap support in the MP-EST species tree. Both are found in the Gulf Coastal Plain where they are sympatric with numerous *Sarracenia* species (Fig. 3.4). Interestingly, *rubra* ssp. *gulfensis* and *alata* form a polytomy in the species tree, and both subspecies are in a polytomy with *rubra* ssp. *wherryi* in the accession tree. This result suggests a very close affinity between *alata* and the *rubra* complex, which has been suggested by previous taxonomic descriptions of this group based on similar pitcher morphology, petal shape, size of flowers, and degree of reflexion in pitcher lid (Case and Case, 1976; McDaniel, 1966; Schnell, 1976, 1978; Sheridan, 1991). Sister to the clade containing *alata* and the *rubra* complex is *leucophylla,* which is morphologically distinct from all other members within this clade (i.e. it is the only *Sarracenia* species with white coloration in

leaves). Even with its distinct morphology, there was low bootstrap support for its placement within the MP-EST species tree. Additionally, the *leucophylla* accession clade fell within a polytomy with the clade containing *alata* and the *rubra* complex in the MP-EST accession tree, but was supported as sister to those species in the concatenation tree. Locally abundant hybrids among *leucophylla, rubra,* and *alata* with complex backcrosses are common where species are in sympatry (McPherson and Schnell, 2011) and show signs of genetic admixture when in sympatry (Furches et al., 2013).

We did not find any phylogenetic structure for populations of *alata* sampled from either side of the Mississippi (these populations are separated by roughly 300 km with the western populations being allopatric) in the MP-EST analyses, but accessions west of the Mississippi were grouped together in the concatenation analysis. Combined with the unresolved relationships with members of the *rubra* complex, our analyses suggest that *alata* could be considered a subspecies within the *rubra* complex. The potential for *alata* to be a subspecies is in contrast to the result from the species delimitation approach conducted by Carstens and Satler (2013), suggesting that *alata* consists of two cryptic species on either side of the Mississippi River. In either case there is phenotypic variation among the *rubra* complex with geographic isolation of numerous members suggesting that this group may be in the midst of speciation.

Sarracenia purpurea complex

Sarracenia purpurea is the most widespread species within the genus, extending from the Gulf Coastal Plain into Newfoundland and across to British Columbia (Fig. 3.4c; Fernald, 1937). The infraspecific designations within the *purpurea* complex are the product of discontinuity in the distribution of this species. Both the Gulf Coastal Plain *purpurea* (*rosea*/*purpurea* ssp. *venosa* var. *burkii*) and *purpurea* ssp. *venosa* var. *montana* are geographically disjunct from the

other portions of the range. The latter variety is found in isolated seep bogs in northern Georgia and the western Carolinas (Schnell and Determann, 1997). The more contiguous portion of the range consists of two named subspecies delineated near Maryland; *purpurea* ssp. *venosa* in the south and *purpurea* ssp. *purpurea* north of Maryland and across Canada. There is not a complete geographic break between the two subspecies and these species form a hybrid zone at the delineation point (Ainsworth and Ainsworth, 1996). Similar to Ellison et al. (2012), we found *rosea* (*purpurea* ssp. *venosa* var. *burkii*) as sister to *purpurea* ssp. *venosa* and *purpurea* ssp. *purpurea*. However, unlike previous results suggesting that the *purpurea* complex is sister to all other *Sarracenia* species (Ellison et al., 2012; Neyland and Merchant, 2006), our results suggest that the *purpurea* complex is sister to the clade containing *minor*, *psittacina,* and *flava* (MP-EST) or *oreophila* clade (concatenation). Examination of the accession trees did show reciprocal monophyly for *purpurea* ssp. *venosa* var. *montana* and *purpurea* ssp. *purpurea*, but not for *purpurea* ssp. *venosa* or *rosea* (*purpurea* ssp. *venosa* var. *burkii*). The latter result, more specifically *rosea* (*purpurea* ssp. *venosa* var. *burkii*), is in contrast to population level analyses of subspecies/varieties found in Godt and Hamrick (1999) and Sheridan (2010). Overall, the geographic isolation, phenotypic differences, and population genetics of the subspecies/varieties within the *purpurea* complex suggest this group may be diversifying, similar to the *rubra* complex.

Sarracenia minor-psittacina-flava clade

Both concatenation and MP-EST analyses strongly support the relationships between *minor*, *psittacina,* and *flava* described previously (Bayer et al., 1996; Neyland and Merchant, 2006). In addition, the MP-EST relationships of *psittacina* and *flava* as sister taxa were strongly supported by Ellison et al. (2012). Morphologically these two species are remarkably different;

psittacina is the smallest species within the genus and has decumbent pitchers, while *flava* pitchers can reach 65 centimeters in height. This relationship highlights the extreme range of morphological variation in trapping structures across sister species in this recently radiated group. Lastly, the relationships of *flava* varieties within the species tree are well supported, however are not reciprocally monophyletic in the accession trees. Varieties within *flava* are generally designated by anthocyanin presence. For example, *flava* var. *rubricorpora* has an almost completely red pitcher, while *flava* var. *rugelii* is characterized by the red coloration at the throat of the pitcher. Anthocyanin presence can be highly variable, responding dramatically to ecological conditions and therefore, the designation of variety based on coloration may be unwarranted.

Biogeographic hypotheses for Sarracenia

Recent estimates suggest the majority of *Sarracenia* diversification occurred less than 3 million years ago during the Pleistocene epoch (Ellison et al., 2012). The Pleistocene has been documented as having a large influence on the distribution and diversification of many southeastern United States species, most likely caused by interglacial activities (see Avise, 1996; Soltis et al., 2006). This constant climatic oscillation likely influenced *Sarracenia* speciation. Interestingly, two out of the three *Sarracenia* species found on ancient Appalachian soils (i.e. *oreophila* and *purpurea* ssp. *venosa* var. *montana*; Fig. 3.4a,c) are basal to other species within their respective clades. This suggests the common ancestor of *Sarracenia* may have originated from the southern Appalachian massif, which is a known area of antiquity and endemism. Resulting diversification and speciation may have occurred through two possibilities. In the first scenario ancestors to the two *Sarracenia* subclades (Fig. 3.1) migrated from the Appalachian massif by drainages into the Gulf and Atlantic (Godt and Hamrick, 1998) as *Sarracenia* seeds are

primarily water dispersed (Schnell, 1976). Under this scenario, the ancestor of the *purpurea*/*minor* clade may have migrated along drainages leading to the Atlantic Coastal Plain while the *oreophila* ancestor may have followed the Apalachicola-Chattahoochee-Flint (ACF) River drainage to the Gulf Coastal Plain with secondary contact between the two clades occurring at the Apalachicola region. Current species ranges seem to support this hypothesis: species within the *oreophila* clade primarily occur along the Gulf Coastal Plain with the exception of more recently diverged species (*rubra* and *jonesii*) shifting along the Fall Line into Georgia and South Carolina (Fig. 3.4a). In addition, the MP-EST species tree indicates that *oreophila* shares a most recent common ancestor with *alabamensis* (restricted to central Alabama), possibly as a result of dispersal via drainages. Moreover, the ranges of *flava*, *minor*, and *purpurea* mostly occur along the Atlantic Coastal Plain with the exception of *psittacina* and *purpurea* ssp. *venosa* var. *burkii* (Fig. 3.4b,c). In the latter case, the *purpurea* complex may have covered a larger continuous range with *purpurea* ssp. *venosa* var. *montana* becoming disjunct followed by isolation of *purpurea* ssp. *venosa* var. *burkii* from *purpurea* ssp. *venosa.* In addition, the geography of the *flava* clade also suggests dispersal to the Atlantic Coastal Plain, as the basal *flava* occupies the northern limits of the its range, while the more recently diverged varieties occur at the southern limits, with *flava* var. *rubricorpora* restricted to the Florida panhandle. This biogeography hypothesis of speciation within this group is supported by numerous phylogeography studies of this region (Soltis et al., 2006; Pauly et al., 2007). Worth noting is that the *Sarracenia* obligate symbiont *Exyra semicrocea* (pitcher plant moth) exhibits a similar genetic break in this region (Stephens et al., 2011), while *E. fax* and *E. ridingsii* are restricted to the Atlantic Coastal Plain due to their specialization on *purpurea* and *flava*, respectively.

In contrast, speciation and diversification of *Sarracenia* could have centralized around the Apalachicola region, which is a known biodiversity hotspot and has the highest overlap of *Sarracenia* species. Under this scenario, the ancestor of *Sarracenia* migrated along the Gulf Coast drainages from the Appalachian massif with successive interglacial activity fragmenting populations (J.L. Hamrick, personal communication). Subsequent speciation may have occurred through movement east and west of this area, which is supported by the current ranges of the two subclades of *Sarracenia* (Fig. 3.4). Future phylogeographic work on various *Sarracenia* species may help to elucidate the biogeographic history of these species and provide further insights into *Sarracenia* speciation.

Conservation implications

Sarracenia species are generally restricted to open wet pine savannas in the Coastal Plain that are maintained through frequent fires. Unfortunately, less than 3% of suitable habitat currently remains, as a cumulative result of fire restrictions, urbanization, forestry, and agriculture (Folkerts, 1982; Folkerts and Folkerts, 1993). This has lead to numerous *Sarracenia* species listed as state threatened or endangered with three (*oreophila*, *rubra* ssp. *jonesii*, and *rubra* ssp. *alabamensis*) listed as federally endangered and one (*purpurea* ssp. *venosa* var. *montana*) is a candidate for protection at the federal level (Department of the Interior, 2014). All species are listed in the Convention on International Trade in Endangered Species, Appendix II (www.cites.org) due to collection pressures. Confusion in nomenclature designations for *Sarracenia* confounds conservation practices. For example, Mellichamp and Case (2009) recognize 11 species with six subspecies, while McPherson and Schnell (2011) identify 8 species and 41 infraspecific designations. As pointed out by Ellison et al. (2014), this confusion can have serious consequences for the protection status of species within this genus. In lieu of a more well

resolved phylogeny, we suggest a complete reevaluation of nomenclature across the genus. Ellison et al. (2014) recommends abandoning the use of "variety" in plant systematics so that "subspecies" is the only infraspecific designation below the rank of species. Following these recommendations, *minor* var. *okefenokeensis* and *flava* varieties will need to be reevaluated. Additionally, all taxonomic designations within the *purpurea* and *rubra* complexes should be reassessed. Given our results, *alata* should be included in the taxonomic revision of the *rubra* complex as well as a more thorough description of *jonesii* (*rubra* ssp. *jonesii*) and *alabamensis* (*rubra* ssp. *alabamensis*). Overall, a taxonomic reevaluation of this group is warranted and will hopefully lead to less confusion for management and conservation officials in charge of protecting these rare and endangered species.

Conclusions

We demonstrate the utility of using target enrichment and coalescent-based approaches for phylogenetic resolution of recently diverged taxa. Using target enrichment, we were able to successfully use 199 loci across 75 individuals to elucidate relationships within this genus. In addition, we were able to pull out 42kb of cpDNA-derived sequences for a plastid tree analysis, however the plastid analysis was unable to resolve relationships. Overall, this study has resolved numerous relationships within this genus, which has important implications on the protection status of these species. Understanding the evolutionary history of *Sarracenia* lays the foundation for examining questions pertaining to evolution and speciation in this group.

References

Ainsworth, J., Ainsworth, J., 1996. *Sarracenia*: North American Pitcher Plants, The National Council for the Conservation of Plants and Gardens, UK.

Albert, V.A., Williams, S.E., Chase, M.W., 1992. Carnivorous plants: phylogeny and structural evolution. Science 257, 1491–1495.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389–3402.

Avise, J.C., 1996. Towards a regional conservation genetic perspective: Phylogeography of faunas in the southeastern United States. In: Avise, J.C., Hamrick, J.L., eds. Conservation Genetics; Case Histories from Nature. New York: Chapman & Hall. Pp 431–470.

Bayer, R.J., Hufford, L., Soltis, D.E., 1996. Phylogenetic relationships in Sarraceniaceae based on rbcL and ITS sequences. Systematic Botany 21,121–134

Beerli, P., 2009. How to use migrate or why are markov chain monte carlo programs difficult to use?, in: Bertorelle, G., Bruford, M.W., Hauffe, H.C., Rizzoli, A., Vernesi, C. (Eds.) Population Genetics for Animal Conservation, volume 17 of Conservation Biology, Cambridge Press, Cambridge UK, pp. 42-79.

Beerli, P., Felsenstein, J., 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Science of the USA 98, 4563–4568.

Bell, C.R., 1949. A cytotaxonomic study of the Sarraceniaceae of North America. Journal of Elisha Mitchell Scientific Society 65, 137–166.

Case, F.W., Case, R.B., 1976. The *Sarracenia rubra* complex. Rhodora 78, 270–325.

Carstens, B.C., Satler, J.D., 2013. The carnivorous plant described as *Sarracenia alata* contains two cryptic species. Biological Journal of the Linnean Society 109, 737–746.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17, 540–552.

Chikhi, R., Medvedev, P., 2013. Informed and automated k-Mer size selection for genome assembly. Bioinformatics doi:10.1093/bioinformatics/btt310.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772.

Darwin, C., 1875. Insectivorous Plants. John Murray, London.

Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. PLoS Genetics doi:10.1371/journal.pgen.0020068.

Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends in Ecology and Evolution 24, 332–340.

Department of Interior, 2014. U.S. Fish & Wildlife Service: Endangered Species Program. Available online: http://www.fws.gov/endangered/. Last accessed 14 September 2014.

Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? Evolution doi:10.1111/j.1558-5646.2008.00549.x

Ellison, A.M., Gotelli, N.J., 2009. Energetics and the evolution of carnivorous plants-Darwin's "most wonderful plants in the world". Journal of Experimental Botany 60, 19–42.

Ellison, A.M., Butler, E.D., Hicks, E.J., Naczi, R.F.C., Calie, P.J., Bell, C.D., Davis, C.C., 2012. Phylogeny and biogeography of the carnivorous plant family Sarraceniaceae. PLoS ONE doi:10.1371/journal.pone.0039291.

Ellison, A.M., Davis, C.C., Calie, P.J., Naczi, R.F.C., 2014. Pitcher plants *(Sarracenia)* provide a 21st –century perspective on infraspecific ranks and interspecific hybrids: a modest proposal for appropriate recognition and usage. Systematic Botany 39, 939–949.

Faircloth, B.C., Glenn, T.C., 2012. Not all sequence tags are created equal: Designing and validating sequence identification tags robust to indels. PLoS ONE 7,e42543.

Faircloth, B.C., Glenn, T.C., McCormack, J.E., Harvey, M.G., Sorsenson, L., Alfaro, M., White, N., 2012a. Target enrichment of Illumina libraries. Available at http://ultraconserved.org. Accessed 3 October 2014.

Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, T.C., Glenn, T.C., 2012b. Ultraconserved elements anchor thousands of genetic markers for target enrichment spanning multiple evolutionary timescales. Systematic Biology 61, 717–726.

Fernald, M.L., 1937. Plants of the Inner Coastal Plain of Virginia. Rhodora 39, 386–387.

Folkerts, G.W., 1982. The Gulf Coast pitcher plant bogs. American Scientist 70, 260–267.

Folkerts, G.W., Folkerts, D.R., 1993. Southeastern pitcher plant bogs: A natural history sketch. Tipularia 8, 10–16.

Furches, M.S., Small, R.L., Furches, A., 2013. Hybridization leads to interspecific gene flow in *Sarracenia* (Sarraceniaceae). American Journal of Botany 100, 2085–2091.

Glenn, T.C., Faircloth, B.C., Nilsen, R., Kieran, T.J., Finger Jr., J.W., Pierson T.W., García-De-Leon, F.J., del Rio Portilla, M.A., Reed K., Anderson, J.L., Meece, J.K., Alabady, M., Belanger, M. Adapterama I: Universal stubs and primers for thousands of dual-indexed Illumina Nextera and TruSeqHT compatible libraries (iNext & iTru). Submitting to Molecular Ecology Resources.

Godt, M.J.W., Hamrick, J.L., 1999. Genetic divergence among infraspecific taxa of *Sarracenia purpurea*. Systematic Botany 23, 427–438.

Grabherr, M.G., et al., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology 29, 644–652.

Heled, J., Drummond, A.J., 2010. Bayesian inference of species tree from multlocus data. Molecular Biology and Evolution 27, 570–580.

Hennig, W., 1966. Phylogenetic Systematics. Illinois Press, Urbana-Champaign (IL).

Heyduk, K., Trapnell, D.W., Barrett, C.F., Leebens-Mack, J., In Review. Estimating relationships within *Sabal* (Arecaceae) through multilocus analyses of sequence capture data.

Huang, X., Madan, A., 1999. CAP3: A DNA Sequence Assembly Program. Genome Research 9, 868-877.

Jansen, R.K., Kaittanis, C., Saski, C., Lee, S.B., Tomkins, J., Alverson, A.J., Daniell, H., Phylogenetic analyses of *Vitis* (Vitaceae) based on complete chloroplast genome sequences: effects of taxon sampling and phylogenetic methods on resolving relationships among rosids. BMC Evolutionary Biology doi:10.1186/1471-2148-6-32.

Jobson, R.W., Playford, J., Cameron, K.M., Albert, V.A., 2003. Molecular phylogenetics of Lentibulariaceae inferred from plastid *rps*16 intron and *trn*L-F DNA sequences: implications for character evolution and biogeography. Systematic Botany 28, 157–171.

Juniper, B.E., Robins, R.J., Joel, D.M., 1989. The Carnivorous Plants. Academic Press, London.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772–780.

Knowles, L.L., 2009. Estimating species trees: Methods of phylogenetic analysis when there is incongruence across genes. Systematic Biology 58, 463–467.

Kubatko, L.S., Degnan, J.H., 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Systematic Biology 56, 17–24.

Kubatko, L.S., 2009. Identifying hybridization events in the presence of coalescence via model selection. Systematic Biology 58, 478–488.

Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biology doi:10.1186/gb-2009-10-3-r25.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9, 357–359.

Lemmon, E.M., Lemmon, A.R., 2013. High-throughput genomic data in systematics and phylogenetics. Annual Review of Evolutionary Systematics 44, 99–121.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 1000 Genome Project Data Processing Subgroup, 2009. The sequence alignment/map (SAM) format and SAMtools. Bioinformatics 25, 2078–2079.

Liu, L., Pearl, D.K., Brumfield, R.T., Edwards, S.V., 2008. Estimating species trees using multiple-allele DNA sequence data. Evolution 62, 2080–2091.

Liu, L., Edwards, S.V., 2009. Phylogenetic analysis in the anomaly zone. Systematic Biology doi:10.1093/sysbio/syp034.

Liu, L., Yu, L., Pearl, D.K., Edwards, S.V., 2009. Estimating species phylogenies using coalescence times among sequences. Systematic Biology 58, 468–477.

Liu, L., Yu, L., Edwards, S.V., 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. BMC Evolutionary Biology 10, 302.

Lodhi, M.A., Guang-Ning, Y., Weeden, N.F., Reisch, B.I., 1994. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis.* Plant Molecular Biology Reporter 12, 6–13.

Löytynoja, A., Goldman, N., 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. Science doi:10.1126/science.1158395.

Maddison, W.P., 1997. Gene trees in species trees. Systematic Biology 46, 523–536.

Maddison, W.P., Knowles, L.L., 2006. Inferring phylogeny despite incomplete lineage sorting. Systematic Biology 55, 21–30.

Mamanova, L., Coffey, A.J., Scott, C.E., Kozarewa, I., Turner, E.H., Kumar, A., Howard, E., Shendure, J., Turner, D.J., 2010. Target-enrichment strategies for next-generation sequencing. Nature Methods 7, 111–118.

McCormack, J.E., Faircloth, B.C., Crawford, N.G., Gowaty, P.A., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species tree analyses. Genome Research 22, 746–754.

McDaniel, S., 1966. Distribution of *S. purpurea* in Canada, in: A taxonomic revision of *Sarracenia* (Sarraceniaceae), Ph.D. dissertation, The Florida State University, Tallahassee, Florida, USA.

McDaniel, S.T., 1971. The genus *Sarracenia* (Sarraceniaceae). Bulletin of Tall Timbers Research Station 9, 1–36.

McPherson, S., 2007. Pitcher Plants of the Americas. The McDonald & Woodward Publishing Company, Blacksburg, VA, USA.

McPherson, S., Schnell, D., 2011. Sarraceniaceae of North America. Redfern Natural History Productions Ltd., Poole, Dorset, England.

Meimberg, H., Wistuba, A., Dittrich, P., Heubl, G.R., 2001. Molecular phylogeny of Nepenthaceae based on cladistic analysis of plastid trnK intron sequences. Plant Biology 3, 164–175.

Mellichamp, T.L., Case, F.W., 2009. Sarraceniaceae. Flora of North America 8, 348–363.

Müller, K., Borsch, T., 2005. Phylogenetics of *Utricularia* (Lentibulariaceae) and molecular evolution of the *trn*K intron in a lineage with high substitutional rates. Plant Systematics and Evolution 250, 39–67.

Neyland, R., Merchant, M., 2006. Systematic relationships of Sarraceniaceae inferred from nuclear ribosomal DNA sequences. Madroño 53, 223–232.

Pamilo, P., Nei, M., 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution 5, 568–583.

Pauly, G.B., Piskurek, O., Shafer, H.B., 2007. Phylogeographic concordance in the southeastern United States: the flatwoods salamander, *Ambystoma cingulatum*, as a test case. Molecular Ecology 16, 415–429.

Peterson, D.G., Tomkins, J.P., Frisch, D.A., Wing, R.A., Paterson, A.H., 2000. Construction of plant bacterial artificial chromosom (BAC) libraries: An illustrated guide. Journal of Agricultural Genomics 5, www.ncgr.org/research/jag.

Ratan, A., 2009. Assembly algorithms for next generation sequence data. Ph.D. dissertation, Pennsylvania State University, State College, Pennsylvania, USA.

Rivadavia, F., Kondo, K., Kato, M., Hasebe, M., 2003. Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast and nuclear 18S ribosomal DNA sequences. American Journal of Botany 90, 123–130.

Sanders, K.L., Lee, M.S.Y., Mumpuni, Bertozzi, T., Rasmussen, A.R., 2013. Multilocus phylogeny and recent rapid radiation of the viviparous sea snakes (Elapidae: Hydrophiinae). Molecular Phylogenetics and Evolution doi: 10.1016/j.ympev.2012.09.021

Schnell, D.E., 1976. Carnivorous Plants of the United States and Canada, John F. Blair, Publisher, Winston-Salem, NC.

Schnell, D.E., 1977. Infarspecific variation in Sarracenia rubra Walt.: some observations. Castanea 42, 149–170.

Schnell, D.E., 1978. Systematic flower studies of *Sarracenia* L. Castanea 43, 211–220.

Schnell, D.E., Determann, R.O., 1997. *Sarracenia purpurea* L. subspecies *venosa* (Raf.) Wherry variety *montana* Schnell & Determann (Sarraceniaceae): a new variety. Castanea 62, 60–62.

Sheridan, P.M., 1991. What is the identity of the west Gulf coast pitcher plant, *Sarracenia alata* Wood? Carnivorous Plant Newsletter 20, 102–110.

Sheridan, P.M., 2010. Ecological and genetic status of the purple pitcher plant, *Sarracenia purpurea* L., in Maryland and Virginia. Ph.D. dissertation, Old Dominion University, Virginia.

Shaw, K.L., 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proceedings of the National Academy of Science 99, 16122–16127.

Soltis, P.S., Soltis, D.E., 2004. The origin and diversification of angiosperms. American Journal of Botany 91, 1614–1626.

Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S., Soltis, P.S., 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 14, 671–688.

Song, S., Liu, L., Edwards, S.V., Wu, S., 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proceedings of the National Academy of Science doi:10.1073/pnas.1211733109.

Srivastava, A., Rogers, W.L., Breton, C.M., Cai, L., Malmberg, R.L., 2011. Transcriptome analysis of *Sarracenia*, an insectivorous plant. DNA Research doi:10.1093/dnares/dsr014.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics doi:10.1093/bioinformatics/btl446.

Stephens, J.D., Santos, S.R., Folkerts, D.R., 2011. Genetic differentiation, structure, and a transition zone among populations of the pitcher plant moth *Exyra semicrocea*: Implications for conservation. PLoS ONE 6:e22658.

Wolfe, K.H., Li, W.H., Sharp, P.M., 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proceedings of the National Academy of Science 84, 9054–9058.

Yang, Z., 2002. Likelihood and Bayes estimation of ancestral population sizes in hominoids using data from multiple loci. Genetics 162, 1822–1823.

Zerbino, D.R., Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Research 18, 821.

Zerbino, D.R. 2010. Using the Columbus extension to Velvet. http://bioweb2.pasteur.fr/docs/modules/velvet/1.1.02/Columbus_manual.pdf. Accessed 12 June 2014.

Zhang, J.Q., Meng, S.Y., Allen, G.A., Wen, J., Rao, G.Y., 2014. Rapid radiation and dispersal out of the Qinghai-Tibetan Plateau of an alpine plant lineage Rhodiola (Crassulaceae). Molecular Phylogenetics and Evolution doi:10.1016/j.ympev.2014.04.013.

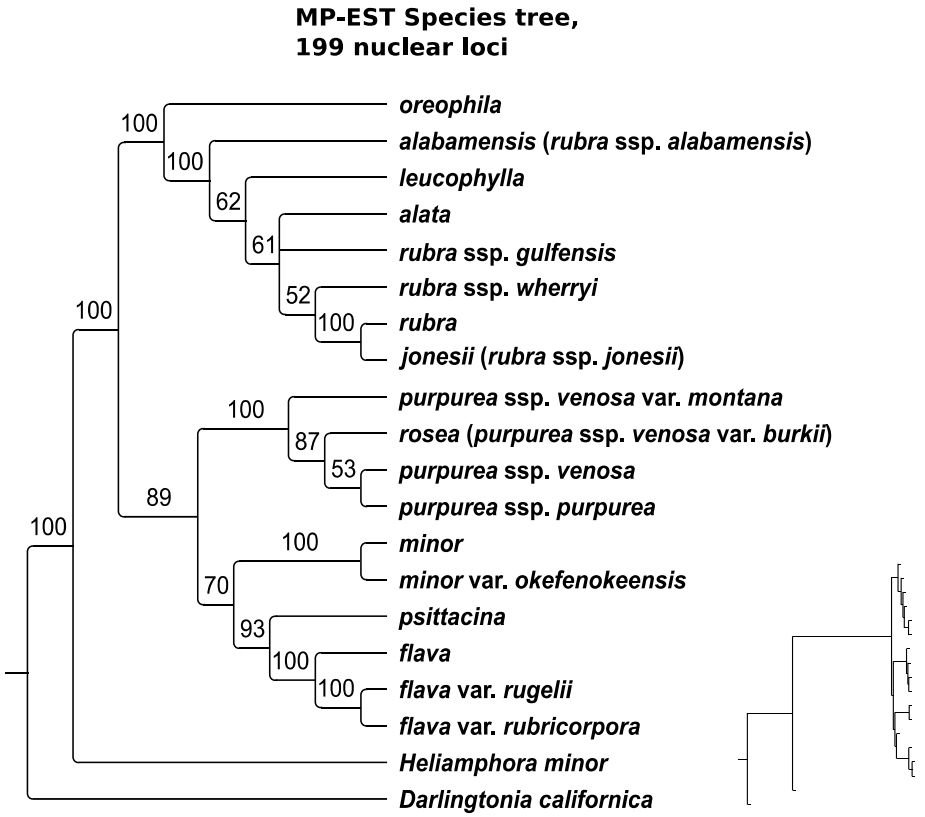

Fig. 3.1 *Sarracenia* MP-EST species tree based on 199 nuclear genes. Bootstrap support values are listed above respective branches. Nodes with <50 bootstrap support are collapsed. MP-EST phylogram representing branch lengths in coalescence units ($2\tau/\theta$), whereby theta (θ) is the population size estimator and tau (τ) is the parameterized branch length. Branch lengths at the terminal tips are not estimated.

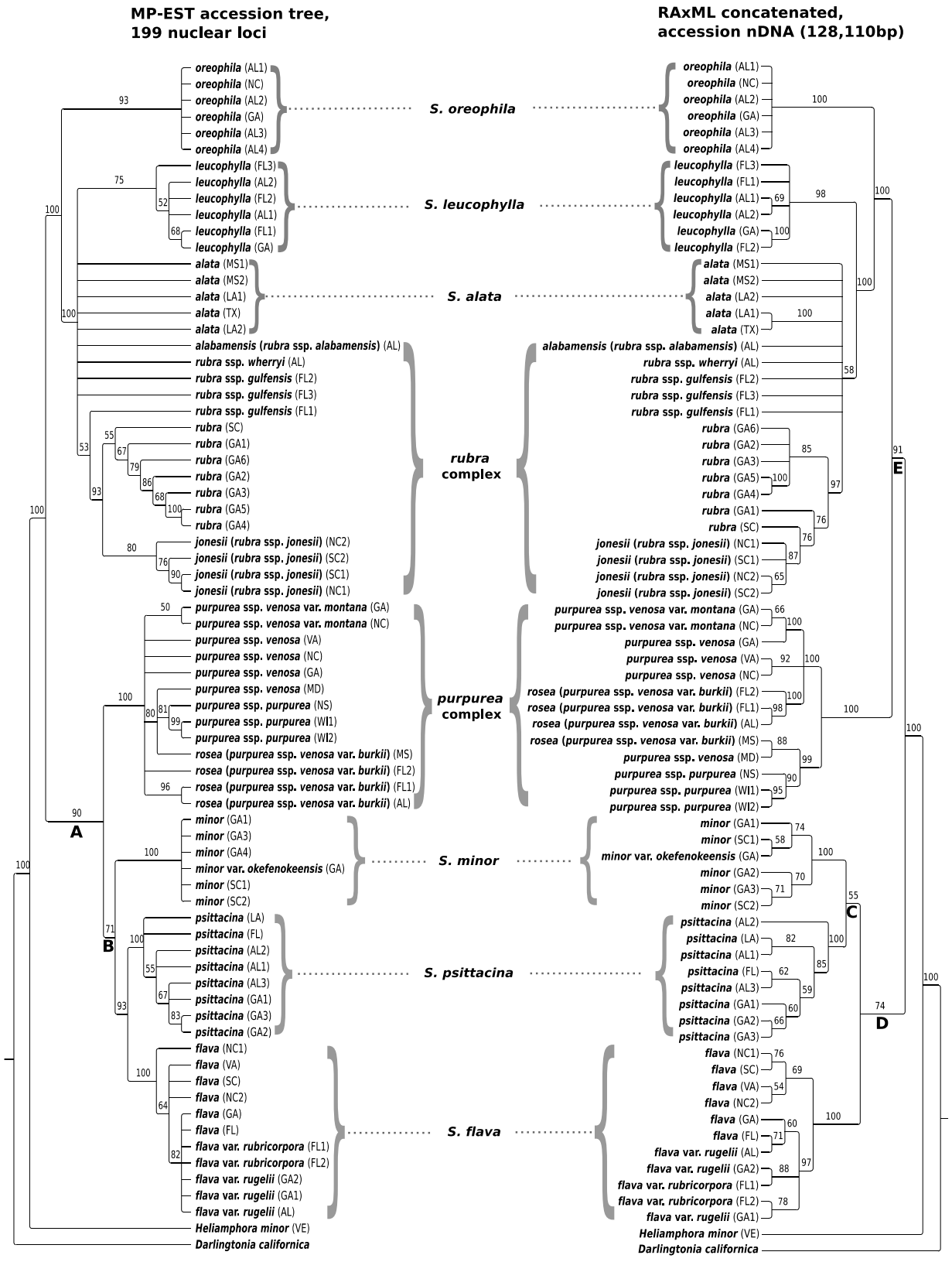
MP-EST accession tree,
199 nuclear loci
RAxML concatenated,
accession nDNA (128,110bp)
S. oreophila
S. leucophylla
S. alata
rubra complex
purpurea complex
S. minor
S. psittacina
S. flava
A
B
C
D
E
oreophila (AL1)
oreophila (NC)
oreophila (AL2)
oreophila (GA)
oreophila (AL3)
oreophila (AL4)
leucophylla (FL3)
leucophylla (AL2)
leucophylla (FL2)
leucophylla (AL1)
leucophylla (FL1)
leucophylla (GA)
alata (MS1)
alata (MS2)
alata (LA1)
alata (TX)
alata (LA2)
alabamensis (rubra ssp. alabamensis) (AL)
rubra ssp. wherryi (AL)
rubra ssp. gulfensis (FL2)
rubra ssp. gulfensis (FL3)
rubra ssp. gulfensis (FL1)
rubra (SC)
rubra (GA1)
rubra (GA6)
rubra (GA2)
rubra (GA3)
rubra (GA5)
rubra (GA4)
jonesii (rubra ssp. jonesii) (NC2)
jonesii (rubra ssp. jonesii) (SC2)
jonesii (rubra ssp. jonesii) (SC1)
jonesii (rubra ssp. jonesii) (NC1)
purpurea ssp. venosa var. montana (GA)
purpurea ssp. venosa var. montana (NC)
purpurea ssp. venosa (VA)
purpurea ssp. venosa (NC)
purpurea ssp. venosa (GA)
purpurea ssp. venosa (MD)
purpurea ssp. purpurea (NS)
purpurea ssp. purpurea (WI1)
purpurea ssp. purpurea (WI2)
rosea (purpurea ssp. venosa var. burkii) (MS)
rosea (purpurea ssp. venosa var. burkii) (FL2)
rosea (purpurea ssp. venosa var. burkii) (FL1)
rosea (purpurea ssp. venosa var. burkii) (AL)
minor (GA1)
minor (GA3)
minor (GA4)
minor var. okefenokeensis (GA)
minor (SC1)
minor (SC2)
psittacina (LA)
psittacina (FL)
psittacina (AL2)
psittacina (AL1)
psittacina (AL3)
psittacina (GA1)
psittacina (GA3)
psittacina (GA2)
flava (NC1)
flava (VA)
flava (SC)
flava (NC2)
flava (GA)
flava (FL)
flava var. rubricorpora (FL1)
flava var. rubricorpora (FL2)
flava var. rugelii (GA2)
flava var. rugelii (GA1)
flava var. rugelii (AL)
Heliamphora minor (VE)
Darlingtonia californica

Fig. 3.2. *Sarracenia* MP-EST accession tree based on 199 nuclear genes and concatenated nDNA accession tree (128,110bp) estimated from RAxML. Bootstrap support is indicated at the nodes; <50 bootstrap support nodes collapsed. Letters indicate areas of disagreement between the two analyses. Gene tree discordance was assessed at these nodes and letters correspond to results listed in the text.

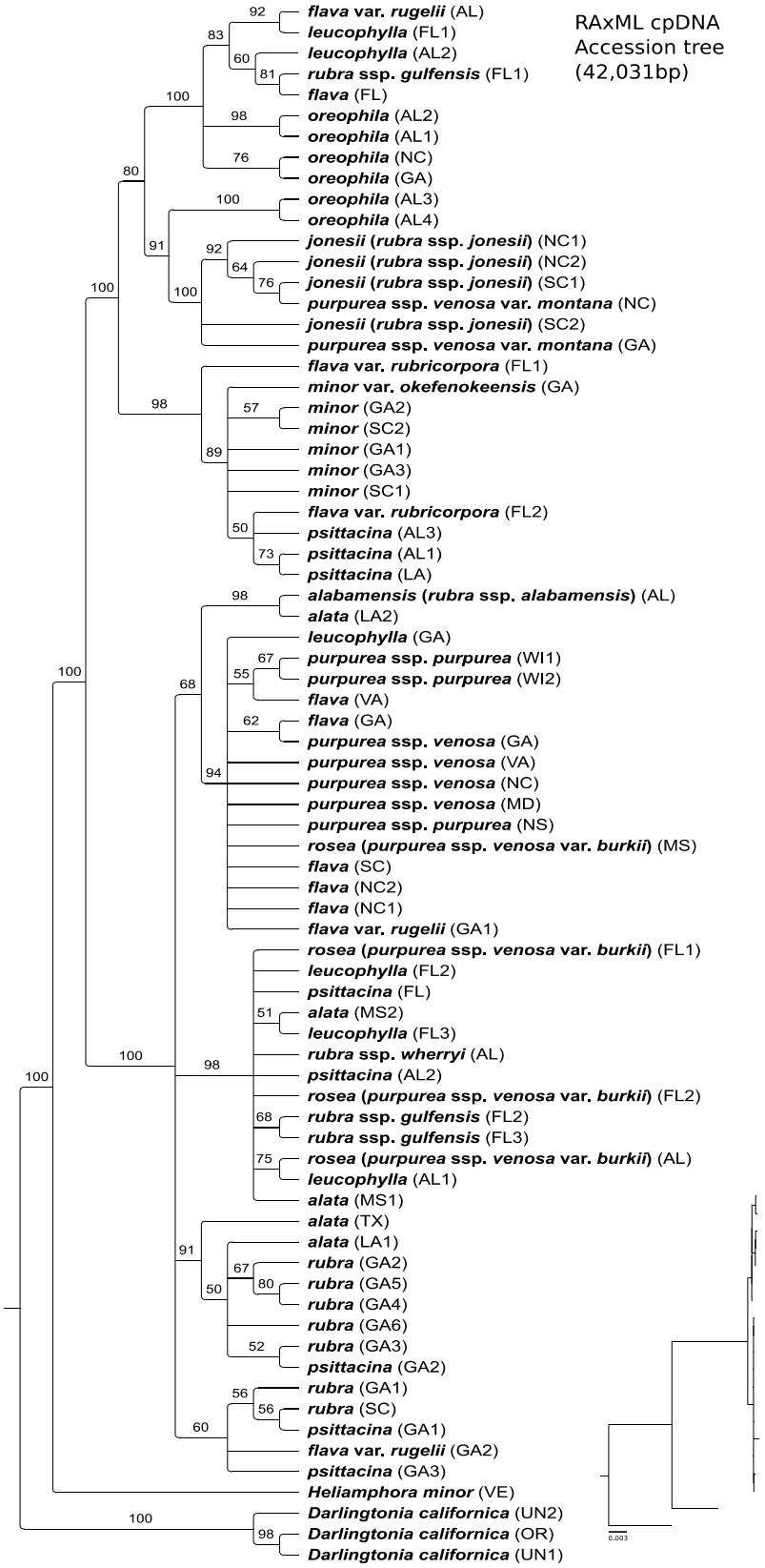

Fig. 3.3. *Sarracenia* plastid accession tree (42,031bp) estimated from RAxML. Bootstrap support is indicated on the cladogram at the nodes; <50 bootstrap support nodes collapsed. Phylogram representing nucleotide substitutions per site.

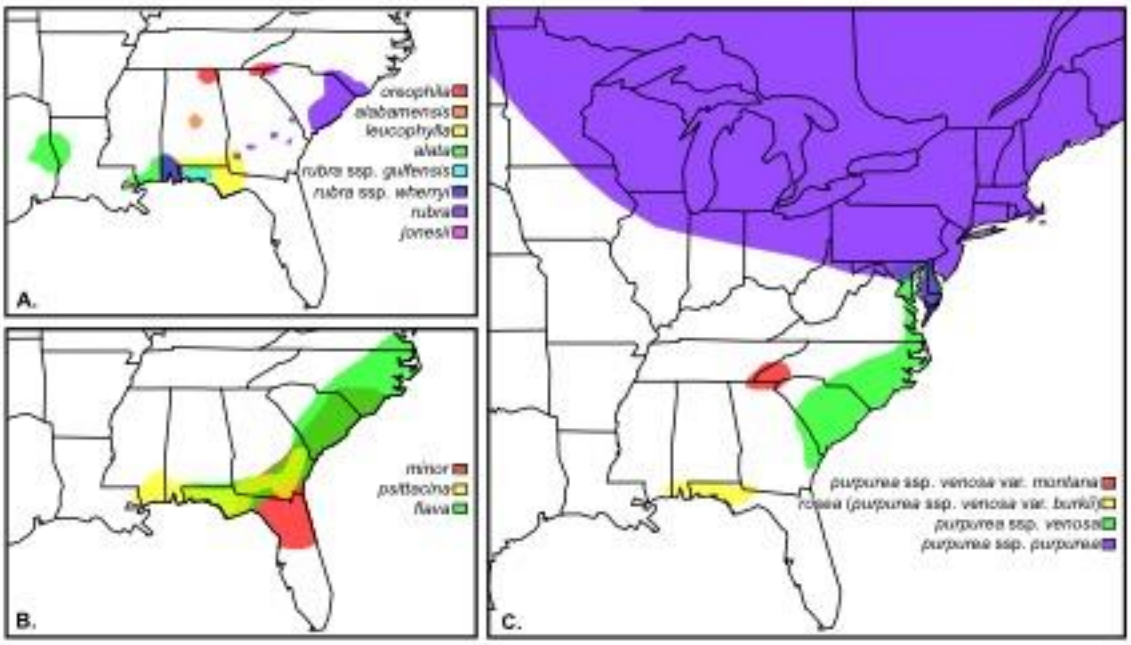

Fig. 3.4. Range maps for *Sarracenia* species. (A.) Species and subspecies ranges from the *oreophila* clade in the MP-EST species tree. (B.) Ranges for the *flava-minor-psittacina* clade; varieties are not shown. (C.) Ranges of the subspecies within the *purpurea* complex.

CHAPTER IV:

CARNIVOROUS PLANT SYNDROMES: SUITES OF PHENOTYPIC TRAITS ASSOCIATE WITH PREY CAPTURED ACROSS THE GENUS *SARRACENIA*

Stephens JD, Determann RO, Folkerts DR, Malmberg RL. To be submitted to New Phytologist.

Abstract

Carnivorous plants display a tremendous amount of morphological variation in trapping structures that have been hypothesized to be the result of selective pressures to specialization in prey capture. While there have been studies to quantify prey types across carnivorous plant species, few studies have directly measured both trapping traits and prey capture. Here, we identified captured prey and measured nine trapping traits thought to influence prey capture across the New World pitcher plant genus *Sarracenia.* After controlling for phylogeny, we found significant covariation in trapping traits so that height was positively correlated with width of the pitcher mouth (peristome) and trichome density under the hood of the pitcher. Height was negatively correlated with trichome density along the exterior of the pitcher and the ala width. Furthermore, we found that these covarying suites of traits were highly correlated with the type of prey they captured. Smaller pitcher plants caught more ground crawling prey (e.g., ants, millipedes, snails) and taller species caught more flying prey types (e.g., bees, moths, butterflies). These results support the hypothesis that carnivorous plants have evolved suites of covarying traits/carnivorous syndromes for prey specialization and that this specialization may be responsible for the large variation in trapping morphology within *Sarracenia*.

Introduction

Biologists have long recognized that ecological interactions among organisms can be significant drivers of adaptive evolution and diversification of species (Darwin, 1859; Ehrlich & Raven, 1964). The interactions between plants and insects are thought to be a major contributor to diversification, in particular through herbivory (Futuyma & Agrawal, 2009; Basset *et al*., 2012) and pollination (Sargent, 2004). These interactions may have resulted in the extensive repertoire, and potentially coadapted complexes, of plant defensive traits (Agrawal & Fishbein, 2006) and floral phenotypes (Fenster *et al.*, 2004). Whereas both these interaction types and their influence on plant evolution have been extensively studied, many other plant interactions may additionally be responsible for plant diversification.

Carnivorous plants are predated by herbivores and often rely on insect pollinators, but they also interact with insects as prey to obtain vital nutrients (Folkerts, 1999). This unique interaction, resulting from inhabiting high-light, nutrient-poor, wet locations, has made carnivorous plants highly dependent on insects (Givnish *et al.*, 1984), so much so that prey capture can directly affect seed production (Thum, 1988; Eckstein & Karlsson, 2001; Ne'eman *et al.*, 2006) and plant growth (Ellison, 2006; Adamec, 2008). This dependence on prey is predicted to cause strong selection pressure on traits related to prey attraction, capture frequency, and retention of prey (Darwin, 1875; Ellison & Gotelli, 2009; Bauer & Federle, 2009), while intense competition among sympatric species may additionally amplify selection towards prey partitioning (Gibson *et al*., 1991; Folkerts *et al*., 1999; Moran *et al*., 1999; Ellison & Gotelli, 2001; Stephens *et al*., 2015a). Further supporting the adaptive significance of trapping morphology is the tremendous morphological diversity in trapping structures within families and genera (Bauer *et al.*, 2011; Givnish, 2015).

Moreover, comparisons of prey captured at a broad scale of trap types (e.g., sticky traps, bear traps, pitcher traps) highlight the potential for these species to specialize on prey (Ellison & Gotelli, 2009) and there have been reports of pitcher plant species that exclusively feed on particular taxa (*Nepenthes*, Kato *et al*., 1993; *Sarracenia minor*, Fish 1976; Folkerts, 1992; Stephens *et al*., 2015a) or even tree shrew or bat excrement (*Nepenthes lowii*, Clarke *et al*., 2009; *N. hemsleyana*, Schöner *et al*., 2015). Together these studies highlight the role functional diversity may play in prey capture. However, larger comparative approaches comparing trapping traits with prey can better inform broad scale patterns of how selective forces may have driven trap diversification and the subsequent ability to capture particular prey or prey functional groups. In this macroevolutionary scenario, we should predict that species that represent convergent evolution of covarying traits should result in the attraction and capturing of similar prey types.

The genus *Sarracenia* (Sarraceniaceae; the New World pitcher plants) is an excellent system to examine the potential for carnivorous syndromes. Even though the genus evolved fairly recently (< 3mya) (Ellison *et al*., 2012), it displays a considerable amount of intra-generic diversity of trapping structures (Figure 4.1; Lloyd 1942; Juniper *et al*., 1989). In addition, numerous studies have noted the diversity of prey capture across *Sarracenia* species (Folkerts, 1992; Ellison & Gotelli, 2009; Stephens *et al*., 2015a). Lastly, many species were historically sympatric increasing the likelihood of interspecific competition leading to prey specialization. Here, we investigate (1) whether species within the genus *Sarracenia* exhibit suites of covarying traits; and (2) whether these suites of traits are associated with type of prey captured lending support to carnivorous syndromes.

Materials and Methods

Sampling methods

Eight mature and fully developed pitchers per species were haphazardly collected in June and September of 2014 and 2015 from an outdoor common garden in Gainesville, GA. Species available for sampling include *S. alabamensis, S. alata, S. flava* var. *rubricorpora, S. flava* var. *rugelii, S. leucophylla, S. minor, S. minor* var. *okefenokeensis, S. oreophila, S. psittacina, S. purpurea* ssp. *venosa, S. purpurea* ssp. *burkii, S. purpurea* ssp. *venosa* var. *montana, S. rubra* ssp. *jonesii,* and *S. rubra* ssp. *wherryi.* An additional species, *S. rubra* ssp. *gulfensis,* was collected in the 2015 samples. All prey content was removed from each sample and preserved in 70% ethanol. Prey items were often partially digested and dismembered, therefore, to avoid biasing prey amounts only arthropod heads were counted. All prey were identified to order and in some cases lower taxonomic levels (Table S4.1). All downstream analyses used relative proportion of prey for each pitcher or *Sarracenia* species.

Quantification of the following nine leaf traits thought to influence prey capture were conducted on the same samples selected for prey estimation (Table S4.2): (1) *Total height*, the total length from the base of the pitcher to the top of the hood. (2) *Height to peristome,* the height from the pitcher base to the lip of the pitcher. (3) *Peristome* (*mouth) width*, the width at the widest point of the pitcher orifice. (4) *Ala width*, the width of laminar wing along the ventral surface at the midpoint on the pitcher. (5) *Bottom trichome density*, exterior trichome density at the base of the pitcher determined by using an ocular grid sampling a 1.5-mm^2 patch. (6) *Middle trichome density*, trichome density at the mid-point of the exterior surface of the pitcher. (7) *Lip trichome density*, trichome density at the lip of the pitcher. (8) *Top hood trichome density*, trichome density on the top of the hood of the pitcher. (9) *Bottom hood trichome density*,

trichome density under the hood of the pitcher. These traits were selected as trap size is often cited as a determinate of prey types captured (Darwin, 1875; Gibson, 1991; Ellison & Gotelli, 2009) and upward pointing trichomes along the exterior of the pitcher are hypothesized to function to direct insect prey towards the pitcher mouth (Studnička, 2013; Stephens *et al.*, 2015a). Likewise, ala size may act as an additional mechanism to direct prey towards the mouth, acting as a drift net in more decumbent species (Folkerts, 1999). To test for both phenotypic and prey differences within species between seasons and years a permutation multivariate analysis of variance (perMANOVA) was conducted in the *vegan* package under the "adonis" function at 1000 permutations (Oksanen *et al.*, 2017) in R v3.3.2 (R Core Team, 2016).

Phylogenetic inference

We used the most recent phylogenetic tree for all comparative analyses (Stephens *et al.*, 2015b); however branch lengths for the tree are in coalescent units with the terminal tips set at a default of 9. Therefore, we used an approach similar to Stephens *et al.*, (2015c) to estimate branch lengths in substitution units. This approach uses a fixed tree topology determined from Stephens *et al.* (2015b) and the total sequence alignments to generate 500 bootstrap replicates to estimate branch lengths in RAxML v04262012 (Stamatakis, 2006). The resulting tree was converted to an ultrametric tree via penalized likelihood (Sanderson, 2002) using the *ape* package (Paradis *et al.* 2004) in R. Taxa that were not in the common garden were pruned from the tree (i.e., *S. purpurea* ssp. *purpurea*, *S. flava*, *S. rubra*). This resulted in a tree with 14 and 15 taxa (2014 and 2015 sampling respectively) for comparative analyses.

The accuracy of parameter estimates for comparative analyses can be affected by the size of the phylogeny being tested. More specifically, having phylogenies containing 20 species or less increases the likelihood of type II errors in phylogenetic signal (Blomberg *et al.*, 2003;

Münkemüller *et al.*, 2012). Unfortunately, *Sarracenia* is comprised of approximately 11 species with numerous subspecies and varieties, therefore we made an effort to measure traits and prey from at least one member (i.e., variety or subspecies) of the 11 supported *Sarracenia* species and also as many other subspecies and varieties as possible. While the phylogeny contains less than 20 species, we are confident that the species selected represent all of the existing range of trait variation within this genus. In addition, we collected data from eight replicates per species to determine measurement error for resulting analyses, which has been shown to greatly reduce biases in parameter estimates (Ives *et al.*, 2007).

Phylogenetic signal

Estimation of statistical non-independence among species, or phylogenetic signal, in traits was conducted using Blomberg's *K* statistic (Blomberg *et al.*, 2003) and Pagel's λ (Pagel 1999; Freckleton *et al.*, 2002) using the "phylosig" function in the R package *phytools* at 1000 bootstraps (Revell, 2012). We incorporated standard error, calculated from the eight replicate pitchers within species, into statistical estimations when possible for both statistics (Ives *et al.*, 2007). Both *K* and λ assume a Brownian motion model of evolution with a significant departure from the null model (values close to one) indicating phylogenetic non-independence of traits (Münkemüller *et al.*, 2012). In addition to the univariate Blomberg's *K* and Pagel's λ, analyses of all measured traits were analyzed using the multivariate version of Blomberg's *K* statistic (K_{mult}; Adams, 2014a) implemented in the program *geomorph* at 1000 iterations (Adams & Otarola-Castillo, 2013). Lastly, phylogenetic signal of traits was estimated separately by year and season, given that phenotypic measures varied between season and years for many species and would increase standard error around the mean (Table S4.3).

Testing for suites of correlated traits

To examine whether sampled traits form covarying suites of traits, possibly resulting in carnivorous syndromes, we used two methods. First, we estimated pairwise correlation coefficients (*r*) between all traits using both nonparametric Spearman rank correlations and phylogenetic independent contrasts (Felsenstein, 1985) using the "pic" function in *ape* (Paradis *et al.*, 2004). Secondly, we followed the approach of recent studies of plant defense syndromes and performed a hierarchical clustering analysis (Kusar & Coley, 2003; Agrawal & Fishbein, 2006; Johnson *et al.*, 2014) using the "hclust" function in R. This method creates a distance matrix that is calculated as 1-(Pearson correlation coefficient) and clusters traits using the 'average' method. The resulting hierarchical clusters support was calculated at 10,000 bootstrap probabilities in the program *pvclust* (Suzuki & Shimodaira, 2006). These analyses were conducted using all individuals regardless of sampling period given that covarying traits should scale with each other.

Correlated evolution of prey and trap traits

All tests of correlation between suites of traits and prey types were conducted using a Brownian motion model. We took this conservative approach given the mixed results with tests of phylogenetic signal and likelihood of type II errors associated with < 20 species. In addition, phylogenetic models, even when model assumptions are violated, have been shown to outperform nonphylogenetic models in simulations (Martins *et al.*, 2002).

Historically, statistical tools for analyzing correlation of traits in a phylogenetic context have been modeled on univariate characters, limiting the analysis of multivariate trait correlations for researchers. Common approaches to these limitations are to analyzing each trait independently, implement distance matrix approaches (i.e. partial Mantel tests) or reducing

dimensionality of traits into a univariate framework (e.g., PCA, Uyeda *et al.*, 2015). Each of these approaches has various tradeoffs and potential negative consequences. For example, analyzing each trait individually using explicit models of evolution can influence type I errors associated with multiple tests and disregards the interdependence of traits. Partial Mantel tests, while accounting for multivariate traits by creating distance matrices can result in high levels of type I and II errors, when used incorrectly (Harmon & Glor, 2010). Finally, collapsing highly correlated traits into principal component (PC) axes to create a 'univariate' trait can result in a biased sampling of multivariate patterns (Uyeda *et al.*, 2015) and ignores nonindependence among observations for species (Revell, 2009). To mitigate these issues, phylogenetic PCA has been developed to correct for species relationships in reduction of multivariate traits (Revell, 2009).

Here, we used multiple approaches to examine whether the suites of trap traits are correlated with prey captured. First, we implemented a partial Mantel test (Smouse *et al.*, 1986) in the vegan package at 1000 permutations by converting all prey and trait data into two distance matrices, respectively, and creating a third matrix of phylogenetic distance. The prey data was also collapsed into functional group (i.e., crawling and flying prey, see Table S4.1) and likewise used to make a distance matrix for analysis. Secondly, we used a phylogenetic PCA to collapse the multivariate trap traits and used the first two axes (pPC1, pPC2) and compared these two crawling and flying proportion types in a phylogenetic independent contrasts framework. Finally, we used a more recent distance based multivariate test *D*-PGLS, which can handle high-dimensional trait data (Adams, 2014b; Adams & Collyer, 2015) to examine the relationship between leaf traits and prey functional group type. This analysis is robust to multivariate trait

data. The *D*-PGLS analysis was performed in the *geomorph* package at 1000 iterations to test for significance (Adams & Otarola-Castillo, 2013).

Results

Previous attempts at examining prey partitioning among species of carnivorous plants have been limited by few species occurring in syntopy (Folkerts 1992; Stephens *et al.*, 2015a; Chin *et al.*, 2014) and potential prey abundance and taxonomic diversity differences among sample sites (see Ellison & Gotelli, 2009; Chin *et al.*, 2014). Therefore, we sampled prey and leaf traits across *Sarracenia* species using an experimental outdoor common garden approach. This ensured plants were all grown under similar environmental conditions therefore minimizing the influences of environmental effects on phenotypes related to prey capture. Moreover, this common garden scenario ensures that all sampled species are exposed to similar levels of prey abundance and diversity.

Prey capture

In total, 8151 prey items were identified from 435 pitchers (excluding pitchers that did not have prey) spanning 6 classes and 15 orders (Table S4.1). The majority of prey (~94%) was Coleoptera, Diptera, Hymenoptera, Lepidoptera, Formicidae, and Hemiptera (Figures 4.2,4.3). Prey composition varied substantially by season and year with most species having large shifts in prey composition across season (Figures 4.2,4.3; Table S4.4). Total prey captured increased by roughly 1000 in the fall sampling for both years (2014: 1608 vs. 2440; 2015: 1422 vs. 2681).

Phylogenetic signal of trap traits

Three traits (height to peristome, lip trichomes, and top hood trichomes) exhibited non-significant phylogenetic signal for both measures (Table 4.1). Total height and middle trichomes showed overall weak to medium phylogenetic signal with significant phylogenetic signal for the

fall 2014 sampling period. Furthermore, traits sampled in the spring and fall of 2014 appeared to have significant deviations from the null model of phylogenetic independence (Table 4.1). Phylogenetic PC2 displayed significant values of Blomberg's *K* and Pagel's λ for most sampling periods, while pPC1 was only significant for the fall 2014 *K* value. Generally, the first principal component (pPC1) summarized variation in total height and height to peristome across all sampling time points, as well as summarizing ala width variation in the spring samples (Figure 4.4). Top hood trichome density loaded on pPC1 for spring and fall 2014 and peristome width on fall 2014 and spring 2015. Lip trichome and bottom hood trichome density were mostly explained by the pPC2 axis, with all other traits being split between the two axes. Finally, phylogenetic signal was significant when all traits were considered when estimated with K_{mult}, with the exception of spring 2014. Overall, these results suggest many traits thought to aid in attracting and trapping prey in *Sarracenia* have low phylogenetic signal with some sampling periods showing stronger phylogenetic signal among traits than others (i.e., fall 2014). However, it should be noted that type II errors are more common with phylogenies under 20 species (Blomberg *et al.*, 2003; Münkemüller *et al.*, 2012), so interpretation of these results should be taken with caution.

Testing for suites of correlated traits

Many traits were both negatively and positively correlated regardless of controlling for phylogeny (Table 4.2). Height was significantly positively correlated with height to peristome, peristome width, lip trichomes and bottom hood trichomes, however the latter two were not supported with the phylogenetic analyses. Height was also significantly negatively correlated with ala width, and bottom and middle trichomes in both analyses. Height to mouth and peristome width exhibited similar trends as height and trichomes generally were positively

correlated with each other with the exception of bottom hood trichomes. Taken together, this suggests taller pitcher plants have fewer trichomes along the exterior of the pitcher, a wider peristome, smaller ala width, and a higher density of trichomes under the hood.

Similar to the pairwise correlations of traits, the hierarchical clustering identified two main clusters/syndromes of measured traits that may have evolved together to form suites of traits (Figure 4.5a). The first syndrome (cluster i) consists of smaller species with increased trichome density along the outer portion of the trap. The second syndrome type (cluster ii) consists of tall species with large peristomes and low outer trichome density with the exception of trichomes below the hood of the trap. Both clusters are supported at a 99% approximately unbiased bootstrap support. Ala width did not cluster with either group (Figure 4.5a), yet after removing *S. psittacina* (which is a decumbent lobster trap with a large ala and no trichomes along the exterior of the trap, Table 4.2) this trait strongly correlates with cluster i (Figure 4.5b).

Correlated evolution of prey and trap traits

The partial Mantel test comparing a prey composition distance matrix and a trait distance matrix, while controlling for phylogenetic distance found a significant relationship across all seasons and years (Table 4.3). Correlation values (r) were all greater than 0.74. The relationship between traits and prey functional groups was also significant across all sampling periods (Table 4.3). Phylogenetic independent contrasts of pPC1 scores for each sampling period compared to crawling prey proportions also resulted in significant values with correlations above 0.51 (Table 4.4, Figure 4.6, Supplemental Figure 4.1). This relationship was maintained with flying prey with the exception of fall 2014. Phylogenetic PC1 axis accounted for over 72% of the trait variation for each sampling period (Figure 4.4). Phylogenetic independent contrasts of pPC2 scores were not significantly related to prey functional groups with the exception of fall 2015

crawling prey. This axis accounted for <22% of the trait variation. Lastly, results from the *D*-PGLS analysis also found a significant correlation in prey functional groups and leaf traits with the exception of spring 2015 (Table 4.5). Taken together, these results suggest the suites of trapping traits are highly correlated with prey captured.

Discussion

The morphological diversity in carnivorous plants has long prompted hypotheses surrounding the role of plant-prey interactions in diversification of this unique mechanism of nutrient acquisition (Darwin, 1875; Moran *et al.*, 1999; Folkerts, 1999; Ellison & Gotelli, 2001). Previous investigations of carnivorous plant traits have largely focused on intraspecific trait variation in traits or prey (Cresswell, 1993; Newell & Nastase, 1998; Bennett & Ellison, 2009) with few interspecific comparisons (Karlsson *et al.*, 1987; Thum, 1986; Folkerts, 1992; Stephens *et al*., 2015a). Here, we took a macroevolutionary approach to examine large-scale patterns of potentially covarying traits thought to influence trapping and whether these traits were associated with subsequent prey capture. Our results support the hypothesis that many traits thought to influence prey capture form suites of covarying traits and further these syndromes of traits were correlated with types of prey captured.

In *Sarracenia*, height was positively correlated with peristome width and high trichome density under the hood, while it was negatively correlated with few, if any, trichomes along the exterior of the pitcher and ala width. Leaf trichomes have been previously found to be useful in cooling or herbivore resistance (Levin, 1973), however downward pointing interior trichomes in *Sarracenia* are a mechanism to capture and prevent prey from escaping (Lloyd, 1942). Upward pointing exterior trichomes are predicted to have a similar function by facilitating the movement of crawling prey towards the mouth of the pitcher (Studnička, 2013; Stephens *et al.*, 2015a).

Furthermore, ala size is predicted to act as a drift net for crawling prey, again to direct prey towards the mouth (Folkerts, 1999). The results from this study support the prediction that smaller pitcher plant species with trichomes along the exterior of the trap and a larger ala tend to capture more crawling prey. The majority of prey captured by these species (i.e., *purpurea* and *psittacina*) consisting of primarily Formicidae, Diplopoda, and Gastropoda. This is supported by previous work describing the diet of *Sarraenia purpurea* (Newell & Nastase, 1998; Folkerts, 1992). It should be noted, that while *psittacina* had a large ala and is the most decumbent species, it had no trichomes along the exterior of the pitcher. This species is often considered to act as a lobster trap and is a morphological anomaly when compared to the pitfall trap type of all other *Sarracenia* species. In natural habitats, this species tends to prefer very wet bogs, often being submerged, and are reported to capture small aquatic invertebrates (Schnell, 1976; Slack, 1979). This microhabitat preference may explain the low capture rate of this species in the common garden (47 total prey items, ~3 prey items per individual); however, the morphology of *psittacina* outside of an aquatic habitat still selected for crawling prey types.

The 'unnatural' setting of a common garden in a single location, while containing all orders of prey generally found in *Sarracenia* habitats, may explain the large variation in prey types for *Sarracenia* species that are considered to be intermediate in trait syndromes. For example, species that captured medium proportions of crawling prey, such as *minor, oreophila,* and *rubra* ssp. *wherryi,* all exhibited intermediate levels of exterior trichomes and height; the one exception is of *rubra* ssp. *gulfensis* which was the only tall species that captured higher levels of crawling prey. In this study, *Sarracenia minor* and *rubra* ssp. *wherryi* had a diet that consisted of higher levels of flying prey with a range of crawling prey that was much lower than what has been previously documented in field studies (Fish, 1976; Rymal & Folkerts, 1982; Givnish,

1989; Stephens *et al*., 2015a). We implemented species comparisons in an outdoor common garden to control for the influence of environmental heterogeneity, as it is known to strongly influence prey availability for carnivorous plants (Zamora, 1995; Alcalá & Domínguez, 2003; Ellison & Gotelli, 2009; Chin *et al.*, 2014). While this method allowed us to examine broad scale patterns of traits and prey capture that were not previously possible, we acknowledge that some of our patterns may not be indicative of natural settings nor can the experimental garden elucidate the selective forces that may have contributed to these patterns or trait syndromes. For example, future work examining ecological character displacement can test the role that interspecific competition has on trapping traits and prey capture. Furthermore, local adaptation to prey availability may further test selective pressures on traits. This is especially interesting to examine for these intermediate species, as well as *psittacina* and its unusual morphology.

Those species that were larger (i.e., *alabamensis*, *flava* varieties, *leucophylla*, *rubra* var. *jonesii*) captured fewer crawling prey across all sampling periods with other tall species (*minor var. okefenokeensis, alata*) having slightly more crawling prey during spring sample periods. Furthermore, these species caught high proportions of Lepidoptera, flying Hymenoptera, and Diptera species when compared to other *Sarracenia* species. These results support similar assessments of prey captured for *minor var. okefenokeensis* (Stephens *et al.*, 2015a), *leucophylla* (Folkerts, 1992), and *flava* (Goodnight, 1940), but contradicted previous investigations in *alata* (Folkerts, 1992; Green & Horner, 2007; Bhattarai & Horner, 2009). Of the flying prey, these pitcher plant species caught many families that are known to be anthophilous groups. For example, we found many Halticidaes, *Bombus* species, and *Scolia dubia* individuals all known to be regular flower visitors. While we did not consider Coleoptera in the functional group analyses, as members of this order can be either crawling or flying, it should be noted that

Coleoptera comprised a large proportion of the prey in taller species in comparison with other *Sarracenia* species. Of the Coleoptera that we identified at lower taxonomic levels, we found many nectar and pollen-feeding beetle species (Cantharidae, Coccinellidae, Curculionidae, Elateridae) were captured. These families were more represented of the diet in *flava*, which was also found in previous studies (Folkerts, 1992). It may be that taller species, in addition to measured traits, are using other traits to mimic flowers in order to attract these prey types.

We measured nine traits thought to be involved in prey capture, but there are most likely other traits that could play a role in this process. Traits such as nectar, volatiles and colors have also been suggested to be traits that are involved in prey attraction and capture (Joel, 1986; Juniper *et al*., 1989). However, there has been conflicting evidence of the role that color and nectar plays in attraction (Zamora, 1995; Newell & Nastase, 1998; Green & Horner, 2007; Stephens *et al*., 2015a). For example, Newell & Nastase (1998) found *purpurea* leaves with red veination captured more prey, yet this result was not supported in an experimental approach examining color variation in the same species (Bennett & Ellison, 2009). Color also appears to have a negligible role in prey capture for both *alata* (Green & Horner, 2007; Bhattarai & Horner, 2009) and *minor* (Stephens *et al*., 2015a). Alternatively, nectar was found to have significant role in prey attraction in *purpurea* (Bennett & Ellison 2009), but not *alata* (Green & Horner, 2007; Bhattarai & Horner, 2009). Little is known about the variation in nectar concentration or location of extra-floral nectaries across *Sarraenia* species. To our knowledge nectar is primarily focused along the throat and peristome of the pitchers. Another trait that most likely influences prey attraction is pitcher volatiles. Recent work comparing volatile emissions across carnivorous plant species highlights the tremendous variation in chemical attractants across species (Jürgens *et al*., 2009). Interestingly, volatile analyses of *leucophylla* and *flava* have described traps from these

species as resembling the scent of flowers, while *minor* and *purpurea* had little to no scent profile. This would support the prediction that these taller species are using added attractants to mimic flowers. Future work examining the role of these traits and their potential correlation with traits measured in this study would further our understanding of carnivorous syndromes. In this study, we focused on prey capture, but prey attraction, particularly with visual and olfactory cues, may additionally add insight into carnivorous syndromes. For example, many insects may be attracted to traps and actually feed on nectar at the throat of the pitcher, but are not captured. In the field, hawk moths are common to see at dusk feeding on the pitcher throats, but because they never interact with the surface of the pitcher they are not captured (personal observation).

Conclusions

Similar to previous work examining the roles that herbivores and pollinators have had on resulting diversification of plants (Fenster *et al*., 2004; Agrawal & Fishbein, 2006), we conclude that insects interacting as prey have in large part played a role in carnivorous plant diversification. Our results support this hypothesis as measured traits tend to form covarying suites of traits, in what we are terming carnivorous syndromes. Within *Sarracenia* we find that in a common garden approach these syndromes are strongly associated with the type of prey captured; taller species with larger peristomes and few exterior trichomes capture more flying prey than their more decumbent counterparts. This result supports previous work examining pitcher size (i.e., height and peristome width) and its influence on prey capture (Gibson, 1991; Cresswell, 1993). Furthermore, it supports the findings that more erect carnivorous species tend to act as aerial traps capturing more flying prey than decumbent traps (*Drosera*, Thum, 1986; *Pinguicula*, Antor & García, 1994). Given the morphological diversity across other genera of carnivorous plants (Moran *et al*., 1999), we predict that similar carnivorous syndromes exist

across these groups that correlate with prey capture. Large-scale comparative studies of traits and prey capture may better inform possible convergent evolutionary patterns.

References

Adamec L. 2008. The influence of prey capture on photosynthetic rate in two aquatic carnivorous plant species. *Aquatic Botany* 89: 66–70.

Adams DC. 2014a. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematic Biology* 63: 685–697.

Adams DC. 2014b. A method for assessing phylogenetic least squares models for shape and other high-dimensional multivariate data. *Evolution* 68: 2675–2688.

Adams DC, Collyer ML. 2015. Permutation tests for phylogenetic comparative analyses of high-dimensional shape data: what you shuffle matters. *Evolution* 69: 823–829.

Adams DC, Otarola-Castillo E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4: 393–399.

Agrawal AA, Fishbein M. 2006. Plant defense syndromes. *Ecology* 87: S132–S149.

Alcalá RE, Domínguez CA. 2003. Patterns of prey capture and prey availability among populations of the carnivorous plant *Pinguicula moranensis* (Lentibulariaceae) along an environmental gradient. *American Journal of Botany* 90: 1341–1348.

Antor RJ, García MB. 1994. Prey capture by a carnivorous plant with hanging adhesive traps: *Pinguicula longifolia*. *The American Midland Naturalist* 131: 128–135.

Basset Y, Cizek L, Cuénoud P, Didham RK, et al. 2012. Arthropod diversity in a tropical forest. *Science* 338:1481–1484.

Bauer U, Federle W. 2009. The insect-trapping rim of *Nepenthes* pitchers. *Plant Signaling & Behavior* 4: 1019–1023.

Bauer U, Clemente CJ, Renner T, Federle W. 2011. Form follows function: morphological diversification and alternative trapping strategies in carnivorous *Nepenthes* pitcher plants. *Journal of Evolutionary Biology* doi: 10.1111/j.1420-9101.2011.02406.x

Bennett KT, Ellison AM. 2009. Nectar, not colour, may lure insects to their death. *Biology Letters* 5: 469–472.

Bhattarai GP, Horner JD. 2009. The importance of pitcher size in prey capture in the carnivorous plant, *Sarracenia alata* Wood (Sarraceniaceae). *American Midland Naturalist* 161: 264–272.

Blomberg SP, Garland T Jr, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745.

Chin L, Chung AYC, Clarke C. 2014. Interspecific variation in prey capture behavior by co-occurring *Nepenthes* pitcher plants. *Plant Signaling & Behavior* 9:1, e27930.

Clarke CM, Bauer U, Lee CC, Tuen AA, Rembold K, Moran JA. 2009. Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant. *Biology Letters* 5: 632–635.

Cresswell JE. 1993. The morphological correlates of prey capture and resource parasitism in pitchers of the carnivorous plant *Sarracenia purpurea. The American Midland Naturalist* 129: 35–41.

Darwin C. 1859. *On the Origins of Species by Means of Natural Selection.* John Murray, London.

Darwin C. 1875. *Insectivorous plants*. New York: D. Appleton and Company.

Eckstein RL, Karlsson PS. 2001. The effect of reproduction on nitrogen use-efficiency of three species of the carnivorous genus *Pinguicula*. *Journal of Ecology* 89: 798–806.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586–608.

Ellison AM. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* 8:740–747.

Ellison AM, Gotelli NJ. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology & Evolution* 16: 623–629.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants–Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* 60: 19–42.

Ellison AM, Butler ED, Hicks EJ, Naczi RFC, Calie PJ, Bell CD, Davis CC. 2012. Phylogeny and biogeography of the carnivorous plant family Sarraceniaceae. *PLoS ONE* 7(6): e39291. doi:10.1371/journal.pone.0039291.

Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist* 125: 1–15.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375–403.

Fish D. 1976. Insect-plant relationships of the insectivorous pitcher plant *Sarracenia minor*. *Florida Entomologist* 59: 199–203.

Freckleton RP, Harvey PH, Pagel M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist* 160: 712–726.

Folkerts DR. 1992. Interactions of pitcher plants (*Sarracenia*: Sarraceniaceae) with their arthropod prey in the southeastern United States. PhD Dissertation. University of Georgia, Athens, GA.

Folkerts DR. 1999. Pitcher plant wetlands of the southeastern United States: Arthropod associates. Pp. 247–275, *In* Batzer DP, Rader RB, Wissinger SA (eds.). *Invertebrates in Freshwater Wetlands of North America*: *Ecology and Management.* John Wiley and Sons, New York, NY.

Futuyma DJ, Agrawal AA. 2009. Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences, USA* 106:18054–18061.

Gibson TC. 1991. Differential escape of insects from carnivorous plant traps. *The American Midland Naturalist* 125: 55–62.

Givnish TJ, Burkhardt EL, Happel RE, Weintraub JW. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist* 124: 479–497.

Givnish TJ. 1989. Ecology and evolution of carnivorous plants. *In* Abrahamson WG (ed.), *Plant-animal interactions*, pg. 243–290. McGraw-Hill, New York, NY.

Givnish TJ. 2015. New evidence on the origin of carnivorous plants. *Proceedings of the National Academy of Sciences, USA* 112: 10–11.

Goodnight CJ. 1940. Insects taken by the southern pitcher plant. *Transactions of the Illinois Academy of Science* 33: 213.

Green ML, Horner JD. 2007. The relationship between prey capture and characteristics of the carnivorous pitcher plant, *Sarracenia alata* Wood. *American Midland Naturalist* 158: 424–431.

Harmon LJ, Glor RE. 2010. Poor statistical performance of the Mantel test in phylogenetic comparative analyses. *Evolution* 64: 2173–2178.

Harms S. 1999. Prey selection in three species of the carnivorous aquatic plant *Utricularia* (bladderwort). *Archiv für Hydrobiologie* 146: 449–470.

Ives AR, Midford PE, Garland Jr. T. 2007. Within-species variation and measurement error in phylogenetic comparative methods. *Systematic Biology* 56: 252–270.

Joel DM. 1986. Glandular structure in carnivorous plants: Their role in mutual exploitation of insects. pp. 219–234. *In:* Juniper BE, Southwood TRE (eds.). *Insects and Plant Surfaces*. Edward Arnold, London, UK.

Johnson MTJ, Ives AR, Ahern J, Salminen J-P. 2014. Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist* 203: 267–279.

Juniper BE, Robins RJ, Joel DM. 1989. *The Carnivorous Plants*. Academic Press, New York, NY. 353 pp.

Jürgens A, El-Sayad AM, Suckling DM. 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology* 23: 875–887.

Karlsson PS, Nordell KO, Eirefelt S, Svensson A. 1987. Trapping efficiency of three carnivorous *Pinguicula* species. *Oecologia* 73: 518–521.

Kato M, Hotta M, Tamin R, Itino T. 1993. Inter- and intra-specific variation in prey assemblages and inhabitant communities in *Nepenthes* pitchers in Sumatra. *Tropical Zoology* 6: 11–25.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.

Kusar TA, Coley PD. 2003. Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology* 31: 929–949.

Levin DA. 1973. The role of trichomes in plant defense. *The Quarterly Review of Biology* 48: 3–15.

Lloyd FE. 1942. *The Carnivorous Plants*. Ronald Press, New York, NY 723 pp.

Martins EP, Diniz-Filho JA, Housworth EA. 2002. Adaptive constraints and the phylogenetic comparative method: a computer simulation test. *Evolution* 56: 1–13.

Mauricio R, Rausher MD. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* 51:1435–1444.

Moran JA, Booth WE, Charles JK. 1999. Aspects of pitcher morphology and spectral characteristics of six Bornean *Nepenthes* pitcher plant species: Implications for prey capture. *Annals of Botany* 83: 521–528.

Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffers K, Thuiller W. 2012. How to measure and test phylogenetic signal. *Methods in Ecology and Evolution* 3: 743–756.

Ne'eman G, Ne'eman R, Ellison AM. 2006. Limits to reproductive success of *Sarracenia purpurea* (Sarraceniaceae). *American Journal of Botany* 93: 1660–1666.

Newell SJ, Nastase AJ. 1998. Efficiency of insect capture by *Sarracenia purpurea* (Sarraceniaceae), the northern pitcher plant. *American Journal of Botany* 85: 88–91.

Oksanen J, Blanchet F, Kindt R, Legendre P, O'Hare RB, et al. 2017. Vegan: Community ecology package, version 2.4-2, https://cran.r-project.org/web/packages/vegan/index.html Accessed 17 January 2017.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses and phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Revell LJ. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* 63: 3258–3268.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (an other things). *Methods Ecology and Evolution*. 3: 217–223.

Rymal DE, Folkerts GW. 1982. Insects associated with pitcher plants (*Sarracenia*: Sarraceniaceae), and their relationship to pitcher plant conservation: a review. *Journal of the Alabama Academy of Science* 53: 131–151.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101–109.

Sargent RD. 2004. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society of London B* 271:603–608.

Schöner CR, Schöner MG, Kerth G, Suhaini SN, Grafe TU. 2015. Low costs reinforce the mutualism between bats and pitcher plants. *Zoologischer Anzeiger – A Journal of Comparative Zoology* 258: 1–5.

Schnell DE. 1976. *Carnivorous Plants of the United States and Canada.* John F. Blair, Winston-Salem, North Carolina, USA.

Slack A. 1979. *Carnivorous plants.* MIT Press, Cambridge, MA.

Smouse PE, Long JC, Sokal RR. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology* 35: 627–632.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* doi:10.1093/bioinformatics/btl446.

Stephens JD, Godwin RL, Folkerts DR. 2015a. Distinctions in pitcher morphology and prey capture of the Okefenokee variety within the carnivorous plant species *Sarracenia minor*. *Southeastern Naturalist* 14: 254–266.

Stephens JD, Rogers WL, Heyduk K, Cruse-Sanders JM, Determann RO, Glenn TC, Malmberg RL. 2015b. Resolving phylogenetic relationships for the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Molecular Phylogenetics and Evolution* 85: 76–87.

Stephens JD, Rogers WL, Mason CM, Donovan LA, Malmberg RL. 2015c. Species tree estimation of diploid *Helianthus* (Asteraceae) using target enrichment. *American Journal of Botany*.

Studnička M. 2013. Different wing in pitchers of the myrmecophagous species *Sarracenia minor* and *S. rubra*. *Journal of the International Carnivorous Plant Society* 42: 19–23.

Suzuki R, Shimodaria H. 2006. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* 22: 1540–1542.

Thum M. 1986. Segregation of habitat and prey in two sympatric carnivorous plant species, *Drosera rotundifolia* and *Drosera intermedia*. *Oecologia* 70: 601–605.

Thum M. 1988. The significance of carnivory for the fitness of *Drosera* in its natural habitat. 1. The reactions of *Drosera intermedia* and *D. rotundifolia* to supplementary feeding. *Oecologia* 75: 472–480.

Uyeda JC, Caetano DS, Pennell MW. 2015. Comparative analysis of principal components can be misleading. *Systematic Biology* 64: 677–68.

Zamora R. 1995. The trapping success of a carnivorous plant, *Pinguicula vallisneriifolia*: The cumulative effects of availability, attraction, retention, and robbery of prey. *Oikos* 73: 309–322.

Table 4.1. Phylogenetic signal in plant traits.

	Spring 2014		Fall 2014		Spring 2015		Fall 2015	
Trait	*K*	λ	*K*	λ	*K*	λ	*K*	λ
Total height	0.21	0.37	**0.42**	0.91	0.23	0.59	0.33	0.69
Height to peristome	0.20	0.36	0.40	0.90	0.25	0.62	0.30	0.68
Peristome width	**0.43**	0.00	**0.40**	0.00	0.30	0.00	0.46	0.00
Ala width	**0.47**	0.89	**0.57**	0.72	0.42	0.78	**0.87**	**0.78**
Bottom trichomes	**0.65**	**1.00**	**0.85**	**1.00**	0.13	0.00	0.08	0.40
Middle trichomes	0.29	0.69	0.40	**0.86**	0.15	0.11	0.06	0.00
Lip trichomes	0.12	0.00	0.25	0.10	0.27	0.00	0.08	0.00
Top hood trichomes	0.09	0.00	0.34	0.00	0.15	0.00	0.15	0.00
Bottom hood trichomes	**0.54**	**1.00**	**0.53**	0.99	**0.60**	**1.00**	**0.66**	**1.00**
pPC1	0.20	0.40	**0.36**	0.92	0.24	0.70	0.21	0.62
pPC2	**0.45**	0.33	**0.44**	0.00	0.52	**0.98**	**0.56**	**1.00**
All traits (K_{mult})	0.23	*n/a*	**0.39**	*n/a*	**0.30**	*n/a*	**0.27**	*n/a*

pPC1 and pPC2 are the first and second phylogenetic principal components, respectively.

Table 4.2. Pairwise correlation coefficients (*r*) for measured traits across sampling times measured using both nonphylogenetic (lower left triangle) and phylogenetic (upper right triangle) methods. All bold and underline values are significant at the $p < 0.05$ level.

	Height	Height to peristome	Peristome width	Ala width	Bottom trichomes	Middle trichomes	Lip trichomes	Top hood trichomes	Bottom hood tri.
Height	-	**1.00**	**0.75**	**-0.49**	**-0.74**	**-0.78**	-0.37	0.24	0.32
Height to peristome	**1.00**	-	**0.73**	**-0.49**	**-0.76**	**-0.79**	-0.39	0.21	0.30
Peristome width	**0.57**	**0.55**	-	-0.30	-0.25	-0.24	0.15	**0.60**	**0.59**
Ala width	**-0.41**	**-0.40**	**-0.34**	-	0.32	0.44	0.04	-0.20	-0.20
Bottom trichomes	**-0.45**	**-0.45**	-0.05	0.01	-	**0.93**	**0.66**	0.24	0.00
Middle trichomes	**-0.39**	**-0.41**	**0.10**	0.06	**0.71**	-	**0.75**	0.22	0.11
Lip trichomes	**0.20**	**0.20**	**0.21**	**-0.24**	**0.20**	**0.36**	-	**0.51**	**0.51**
Top hood trichomes	0.08	0.07	**0.36**	-0.06	**0.36**	**0.45**	**0.50**	-	0.17
Bottom hood tri.	**0.58**	**0.57**	**0.21**	**-0.33**	**-0.28**	**-0.17**	**0.34**	-0.07	-

Table 4.3. Results from the partial Mantel test examining correlation between suites of trap traps and prey captured.

Sampling period	All prey data	Prey functional groups
Spring 2014	$r = 0.82$, $p < 0.01$	$r = 0.82$, $p < 0.01$
Fall 2014	$r = 0.74$, $p < 0.01$	$r = 0.77$, $p < 0.01$
Spring 2015	$r = 0.75$, $p < 0.01$	$r = 0.70$, $p < 0.01$
Fall 2015	$r = 0.82$, $p < 0.01$	$r = 0.81$, $p < 0.01$

Table 4.4. Outcome of phylogenetic independent contrasts comparing phylogenetic PCA and prey functional groups.

	pPC1		pPC2	
Sampling period	Crawling prey	Flying prey	Crawling prey	Flying prey
Spring 2014	$F_{1,12} = 24.4$, $p < 0.01$, $r = 0.81$	$F_{1,12} = 11.05$, $p < 0.01$, $r = -0.68$	$F_{1,12} = 1.25$, $p = 0.29$, $r = -0.32$	$F_{1,12} = 1.75$, $p = 0.21$, $r = 0.37$
Fall 2014	$F_{1,12} = 5.13$, $p = 0.04$, $r = 0.51$	$F_{1,12} = 2.80$, $p = 0.12$, $r = -0.43$	$F_{1,12} = 2.50$, $p = 0.14$, $r = -0.44$	$F_{1,12} = 1.74$, $p = 0.21$, $r = 0.36$
Spring 2015	$F_{1,13} = 96.45$, $p < 0.01$, $r = 0.94$	$F_{1,13} = 28.30$, $p < 0.01$, $r = -0.82$	$F_{1,13} = 0.57$, $p = 0.47$, $r = 0.25$	$F_{1,13} = 2.05$, $p = 0.18$, $r = -0.44$
Fall 2015	$F_{1,13} = 8.96$, $p = 0.01$, $r = 0.62$	$F_{1,13} = 78.43$, $p < 0.01$, $r = -0.92$	$F_{1,13} = 7.43$, $p = 0.02$, $r = 0.62$	$F_{1,13} = 0.02$, $p = 0.88$, $r = -0.03$

Table 4.5. Results from the *D*-PGLS analysis of traits and functional group type.

Sampling period	Crawling prey	Flying prey
Spring 2014	$F_{1,12} = 16.10$, $p < 0.01$	$F_{1,12} = 8.74$, $p = 0.01$
Fall 2014	$F_{1,12} = 4.67$, $p < 0.01$	$F_{1,12} = 2.64$, $p < 0.01$
Spring 2015	$F_{1,13} = 25.52$, $p = 0.14$	$F_{1,13} = 14.45$, $p = 0.36$
Fall 2015	$F_{1,13} = 8.25$, $p < 0.01$	$F_{1,13} = 33.20$, $p = 0.02$

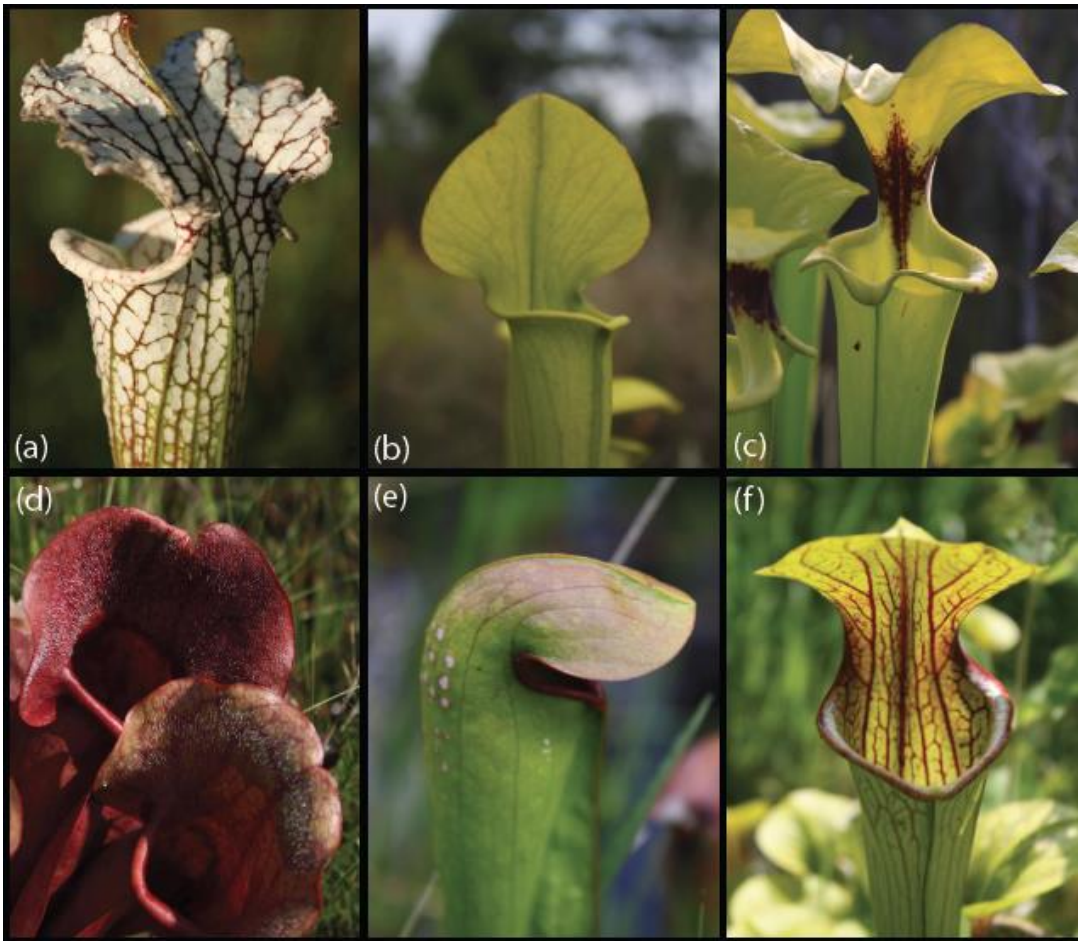

Figure 4.1. Examples of trap morphology across six *Sarracenia* species. (a) *S. leucophylla* (b) *S. alata* (c) *S. flava* (d) *S. purpurea* ssp. *venosa* var. *burkii* (e) *S. minor* (f) *S. oreophila*. All photos taken by JDS.

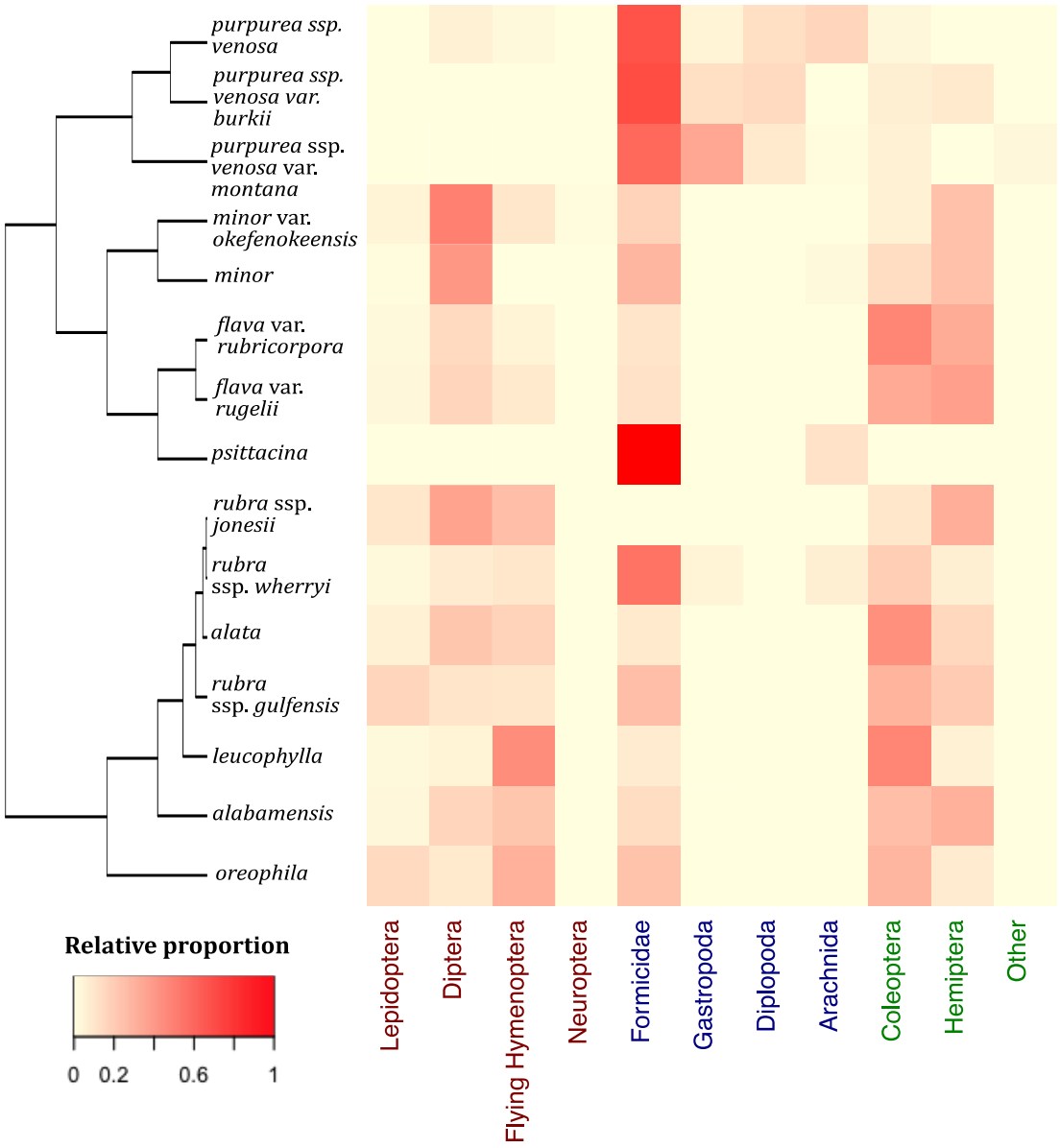

Figure 4.2. Prey composition by Order for the spring 2015 collection. The *Sarracenia* phylogeny is depicted on the left (Stephens *et al*., 2015b). Colors of prey orders designate functional groups (red = flying, blue = crawling, green = other).

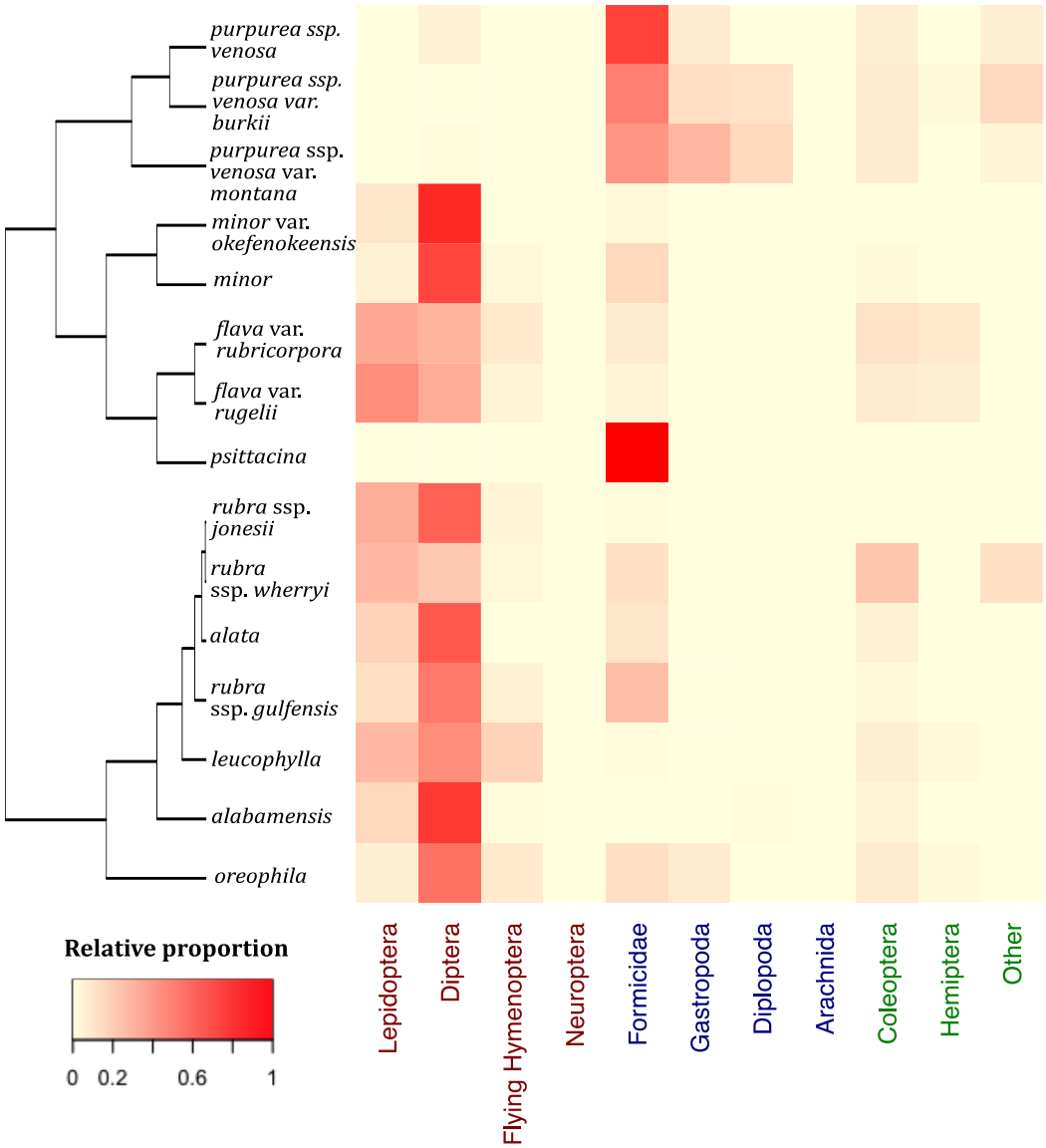

Figure 4.3. Prey composition by Order for the fall 2015 collection. The *Sarracenia* phylogeny is depicted on the left (Stephens *et al*., 2015b). Colors of prey orders designate functional groups (red = flying, blue = crawling, green = other).

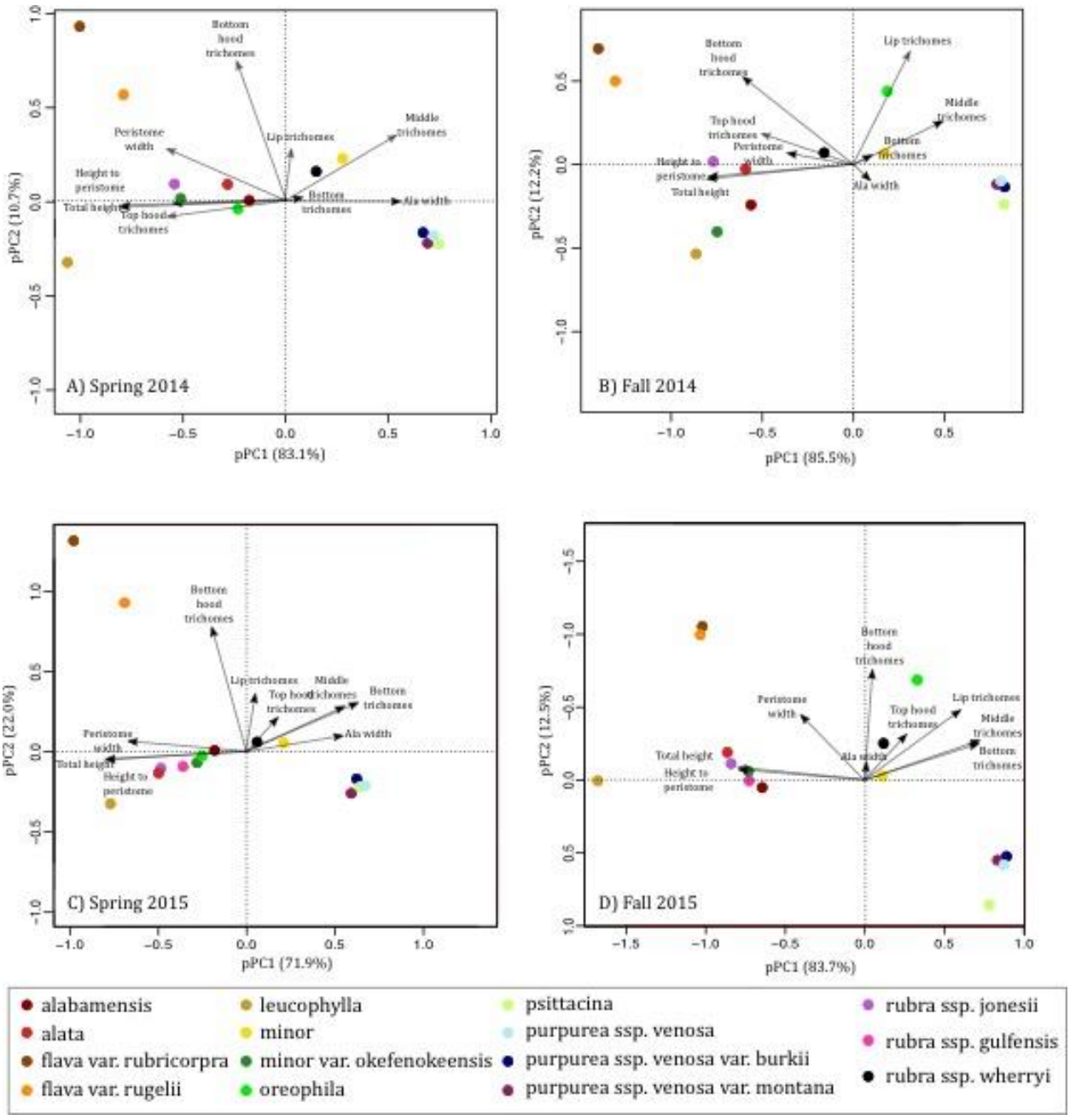

Figure 4.4. Phylogenetic corrected PCA (pPCA) of measured leaf traits for each species mean. Percent variation in traits explained by the axes are labeled on their respective axes.

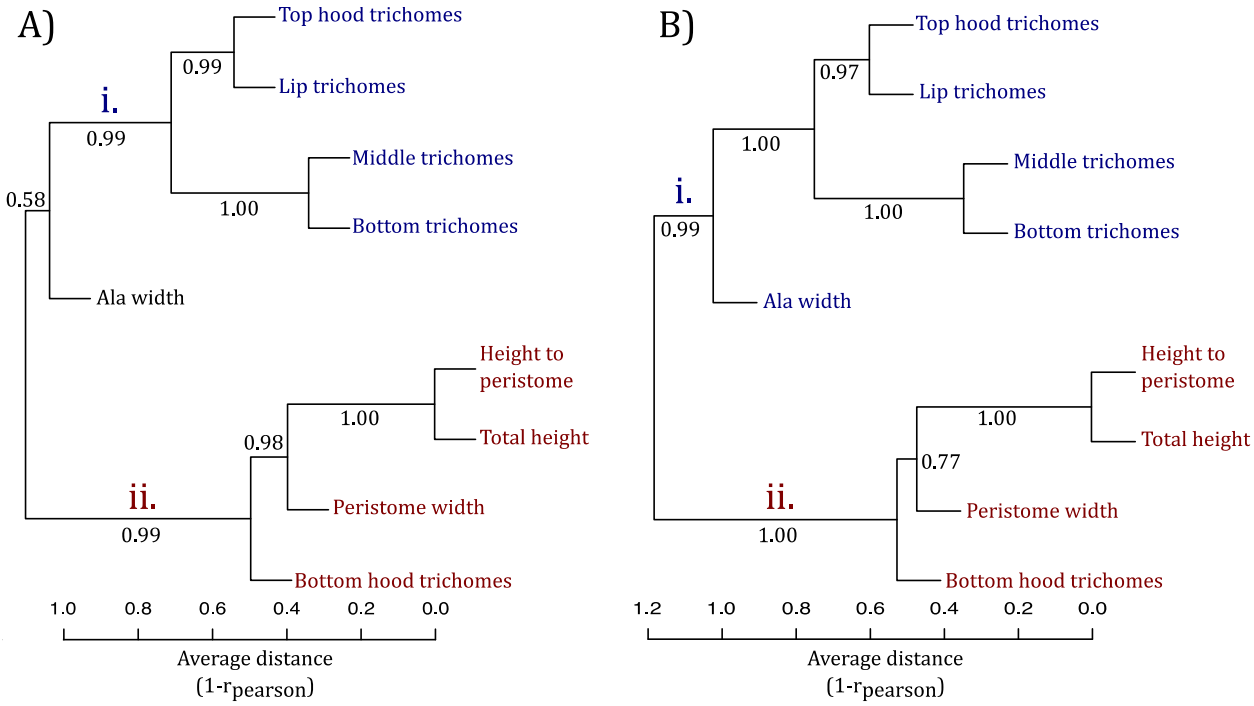

Figure 4.5. Dendrograms of hierarchical clustering of trait variation measured across all sampling periods (A) with and (B) without *S. psittacina*. Small distances correspond to traits that are strongly positively correlated, while distance values > 1 represent negative correlations among traits. Approximately unbiased bootstrap support at 10,000 iterations is represented by proportion values at each node. Colors and roman numerals represent clusters of positively correlated traits over a bootstrap support of 0.95.

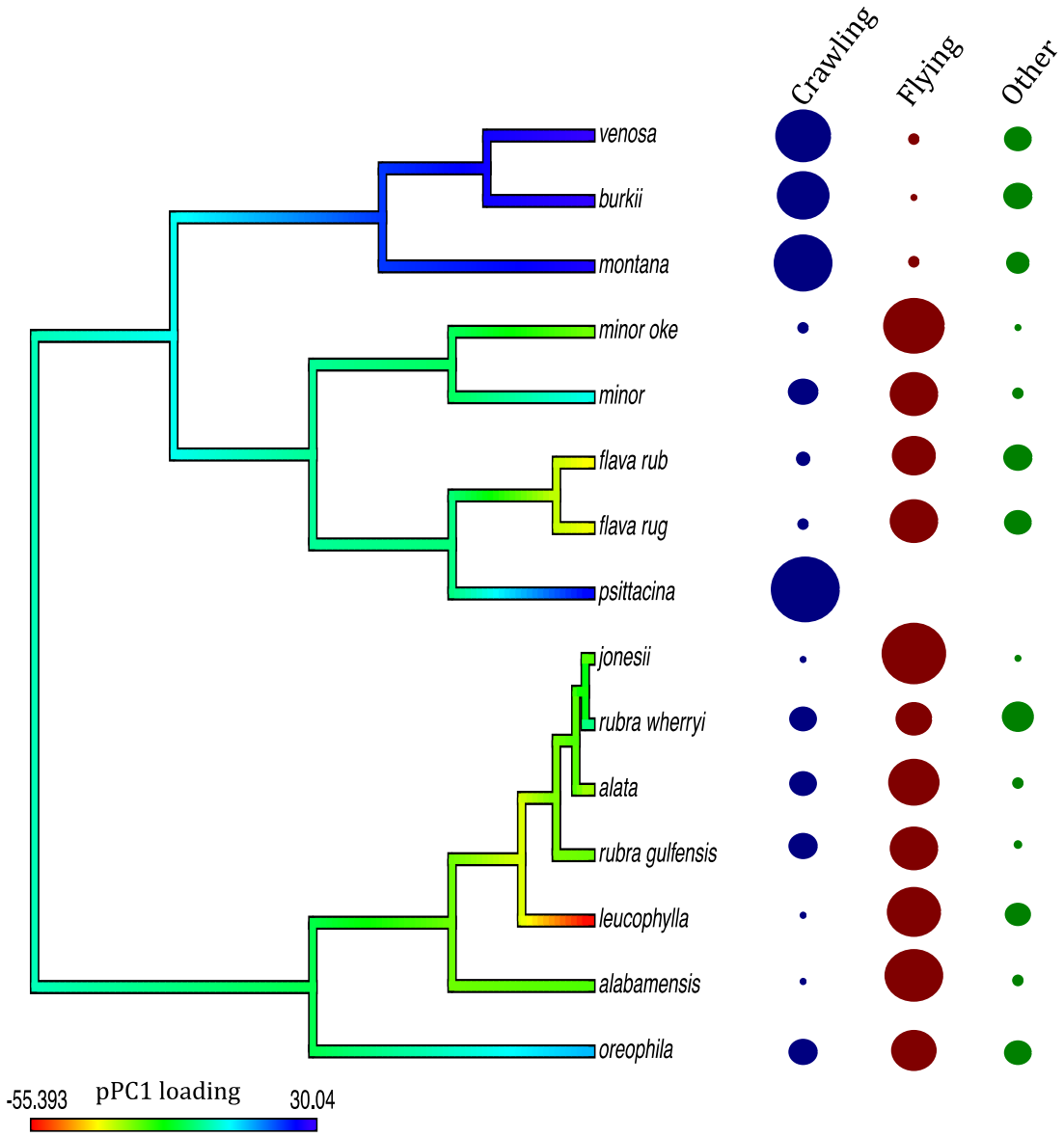

Figure 4.6. Visual representation of traits and prey. pPC1 scores for fall 2015 from Fig 4.4 (left side of figure) compared to proportion of prey functional groups captured.

CHAPTER V:

HOST SPECIES SPECIFIC AND TEMPORAL DIVERSITY IN MICROBIAL COMMUNITIES ACROSS THE CARNIVOROUS NEW WORLD PITCHER PLANT GENUS *SARRACENIA*

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Abstract

Much of our understanding of nutrient acquisition through the host-microbiome relationship has been gained using animal systems. In plants, nutrient acquisition is often facilitated by the rhizosphere microbiome often described as the extended phenotype. However, carnivorous plants have evolved alternative strategies to acquiring nutrients as they grow in nutrient poor soils. These plants attract, capture, and digest prey; the mechanisms by which this digestion occurs are not fully understood, but are thought to result from a synergistic relationship between microbes and plant enzymes. The degree of synergy is predicted to vary across different carnivorous plant species based on factors such as diet, trap type, location, and species relationships. *Sarracenia* (New World pitcher plants) capture insects via pitfall traps and absorb nutrients that result from prey decomposition. Here, we conducted a comparative approach to examine how the pitcher plant microbiome is structured across the entire genus in an outdoor common garden. Contents of leaves (i.e., pitchers) from 15 pitcher plant species were collected over the course of two years in spring and fall. Using 16S rRNA and 18S rRNA primers we amplified DNA, and sequenced it to characterize the microbes present. We found significant microbiome community structure with most of the variation explained by host species. Furthermore, communities were structured by season and year depending on host species. These patterns suggest that the host species is interacting with the microbiome so that individuals from within the same species have more similar microbiomes. Results from this study provide further insight into the patterns and possible processes involved in carnivorous plant-microbiota community assembly and symbiotic relationships.

Introduction

The last decade has seen a dramatic increase in our understanding of the complex interactions and interdependence between multicellular eukaryote hosts and their associated microbiota. These interactions can influence many phenotypic capabilities of a host, such as behavior (Collins *et al*., 2012), development (Shin *et al*., 2011), and stress resistance (Yang *et al.*, 2009; Mendes *et al.*, 2011). Together these effects have the potential to be a significant driver of eukaryote and microbial evolution; they are predicted to be stronger when a host species relies on microbes to perform essential functions (Ochman *et al*., 2010; McFall-Ngai *et al.*, 2013). Microbes associated with processing and facilitation of nutrients for eukaryotes can have a large influence on the health of the host (Claesson *et al.*, 2012; Tremaroli and Bäckhed, 2011) and, on evolutionary time-scales, host speciation (Brucker and Bordenstein, 2013). These communities can harbor enormous diversity, and assemblages may be regulated by factors such as host ecology, host genetics, and spatial geography.

Much of our understanding of the host-microbiome relationship, as it relates to nutrient acquisition, has been examined using animal systems; yet similar to animal gut microbiota, plants require assistance in nutrient uptake most often through the rhizosphere microbiome (Berendsen *et al.*, 2012; Turner *et al.*, 2013). Recent studies examining the diversity and microbial structure of the rhizosphere microbiome highlight the complex ways in which plants use microbes for nutrient acquisition (see Berendsen *et al.*, 2012; Hacquard *et al.*, 2015). Many plants living in nutrient poor habitats have evolved alternative strategies to acquiring nutrients. These plants have evolved complex structures to attract, capture, and digest prey to obtain phosphorous and nitrogen. Similar to animals, carnivorous plants may rely heavily on their microbiota to digest prey. The mechanisms by which digestion and absorption occurs in

carnivorous plants are not fully understood, but it are thought to result from a synergistic relationship between microorganisms and plant enzymes to breakdown complex organic compounds and take-in essential nutrients (Takeuchi *et al*., 2011). Furthermore, nutrient acquisition in most carnivorous plants is occurring not in the rhizosphere, but through highly modified leaves (i.e. phyllosphere). This unique adaptation is predicted to harbor a distinct and functionally different microbiota assemblage relative to previous rhizosphere/phyllosphere plant microbial studies, possibly mirroring those of animal gut microbiota.

Carnivory in plants has evolved independently nine times resulting in 583 species worldwide with a wide range of trapping structures (e.g., bear traps, sticky traps) (Givnish 2015). The species found in the genera *Nepenthes* and *Sarracenia* have been the most studied carnivorous plant-microbe system, most likely due to their trap structure. These two evolutionary independent genera capture insects via passive pitfall traps that slowly digest prey over time in a usually aqueous solution. While *Sarracenia* has been hypothesized to rely on assemblages of microbes to aid in digestion of prey (Butler *et al*., 2008), *Nepenthes* use of microbes has been debated (Buch *et al.*, 2012; Sickel *et al*., 2016). To date, most carnivorous plant-microbiota studies examining diversity and structure of microbial communities in these two genera have focused on one to two host species. These studies indicate that pitchers are completely sterile upon development (Peterson *et al.*, 2008) and are quickly colonized by a diverse assemblage of distinctive microbes (Koopman *et al.*, 2010). Furthermore, these microbial assemblages can vary between species (Sickel *et al.*, 2016), have temporal variation (Koopman *et al.*, 2010), and can have phylogeographic structure (Koopman and Carstens, 2011). However, the possible drivers of these differences in community assemblages are unknown.

To further our understanding regarding patterns of carnivorous plant microbiota

assemblages we conducted a comparative approach examining the microbiota of 15 host species/subspecies in the genus *Sarracenia* over a two-year period sampling in both the spring and fall in an outdoor common garden. While species level studies can elucidate fine scale patterns, larger comparative approaches can better inform broad scale patterns of how host evolutionary relationships and host ecology may influence the associated microbiota. Additionally, sampling the host microbiota over time (i.e., season, year) can resolve whether these patterns are maintained within species. Here, we (1) examined whether microbiota display temporal variation across *Sarracenia* species, (2) compared differences in microbiota among pitcher plant species within seasons, and (3) examined the above questions across years.

Materials and Methods

Sampling methods

Microbial samples were collected from an outdoor common garden maintained by the Atlanta Botanical Garden in Gainesville, Georgia, USA. The restricted access research site contains 15 *Sarracenia* species/subspecies from multiple populations across the southeastern United States. In this way the common garden controls for the effect of spatial geography on microbe assemblages by ensuring that all *Sarracenia* species are exposed to similar available prey and environmental variables. Eight pitchers per species were collected in June and October 2014 and 2015. The sampling method was destructive and therefore bacteria samples and prey were not collected from the same pitchers throughout the study. Pitchers were collected from the following species: *S. alata, S. flava* var. *rubricorpora, S. flava* var. *rugelii, S. leucophylla, S. minor, S. minor* var. *okefenokeensis, S. oreophila, S. psittacina, S. purpurea* ssp. *rosea, S. purpurea* ssp. *venosa, S. purpurea* ssp. *venosa* var. *montana*, S. *rubra* ssp. *alabamensis, S. rubra* ssp. *wherryi*, and *S. rubra* ssp. *jonesii*, with *S. rubra* ssp. *gulfensis* collected in the 2015 samples.

Pitchers selected were mature; they were fully developed (~30 days old), selected from plants that were >3 years old, and showed no signs of senescing. Upon collection any liquid was removed from pitchers and placed in sterile Falcon tubes. Pitchers and fluids were transferred on ice and stored in a cold room. A sterile razor blade was used to cut the pitcher to the base of the trap. Any remaining liquid was pipetted and added to previously collected liquid. After extraction of liquid from pitchers all prey items were removed with sterile equipment. Finally, deionized water was used to rinse pitchers, remaining contents were drained into the same tube as prey. This final liquid was then removed and added to 2mL tubes with the previously extracted liquid from the pitcher. Prey contents were then preserved in 70% ethanol and identified to order and further categorized by functional groups (i.e. flying vs. crawling prey). All equipment was sterilized between samples to avoid contamination and all samples were processed within five days of collection.

Extraction and sequencing of microbes

The resulting pitcher fluid was centrifuged at 14 000rpm for 15 minutes to precipitate the cells in solution. The supernatant was carefully removed and discarded without disturbing the cell pellet. DNA extractions were conducted following the protocol described in Lodhi *et al.* (1994). Due to the high amount of humic acids present in the samples two separate chloroform:iso-Amyl alcohol cleanings were conducted. Each sample was treated with RNAase A (Sigma Aldrich #R4642-10MG, St. Louis, Missouri, USA) and cleaned a final time with chloroform:iso-Amyl alcohol. Sample concentrations and purity were assessed using a Nanodrop 2000 (Thermo Scientific, Wilmington, Delaware, USA).

A ~250bp fragment of the V4 hypervariable region was targeted for the bacterial 16S rRNA gene using region specific bacterial/archaeal primers 515F and 806R developed by

Caporaso *et al.* (2012). For eukaryotes, a ~200bp fragment of the V9 hypervariable region on the 18S rRNA gene was targeted using a eukaryotic-specific primer pair [Euk1391F: 5'-GTACACACCGCCCGTC-3' (Stoeck *et al*., 2010) and EukB: 5'-TGATCCTTCTGCAGGTTCACCTAC-3' (Medlin *et al*., 1988)]. The eukaryote V9 hypervariable region was chosen as it is the ideal length for sequencing on the Illumina MiSeq platform and is found to target a broad range of diversity (Amaral-Zettler *et al*., 2009). The target fragments were amplified using the KAPA LTP library preparation kits (#KK8232, KAPA Biosystems, Wilmington, Massachusetts, USA) following manufacture's instructions. The PCR conditions consisted of a denaturing step of 96°C for 3min; followed by 28 cycles of 96°C for 30s, 55°C for bacteria and 58°C for eukaroytes for 30s, 72°C for 1min; then finally 1 cycle of 72°C for 1min. Sera-Mag SpeedBeads (#65152105050250, GE Healthcare Life Sciences GE, Pittsburgh, Pennsylvania, USA) were used to remove smaller, unwanted fragments (<200bp). Subsequent samples were re-hydrated in 25µl of dilute TE and aliquots of 5µl were run out on a 1% test gel to confirm fragment size and quantified using a Nanodrop. In order to pool samples for sequencing, each sample received unique dual-index oligonucleotide barcodes designed and manufactured by Integrated DNA Technologies (Coralville, Iowa, USA) using the above amplification procedure with a reduction from 28 cycles to 15-17 cycles. These uniquely barcoded samples were then pooled according to their concentrations. A Savant™ SpeedVac™ (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) was used to reduce the volume for the pooled samples and 100µl of each pooled library was run out on a "long" 1.5% agarose gel to excise the desired fragment. Finally, the excised fragment was cleaned per manufacture's instructions using a Zymoclean Gel DNA Recovery Kit™ (Zymo Research, Irvine, California, USA) and subsequently submitted for sequencing on an Illumina MiSeq PE250 (Illumina, San

Diego, California, USA) at the Georgia Genomics Facility, University of Georgia, Athens, Georgia, USA.

Bioinformatic pipeline

All demultiplexed raw 16S rRNA and 18S rRNA amplicon sequences were quality-filtered according to default settings and assembled using PEAR version 0.9.6 (Zhang *et al*., 2014). Subsequent assembled reads were processed through Quantitative Insights Into Microbial Ecology (QIIME) open source software version 1.7.0 (Caporaso *et al*., 2010a). All 16S rRNA sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity threshold using the UCLUST algorithm (Edgar, 2010) and assigned to taxonomy using the Ribosomal Database Project (RDP) classifier against the May 2013 release of the Greengenes reference database (McDonald *et al*., 2012). Additionally, 18S rRNA sequences were clustered into OTUs at a 90% sequence similarity threshold and assigned taxonomy using the BLAST algorithm against the July 2015 release of the SILVA 18S rRNA 97% OTU reference database (Quast *et al*., 2013). BLAST was used with the 18S dataset, as the RDP classifier was unable to assign the majority of our sequences, similar to Bittleston *et al.* (2016). To remove "low abundance" taxa that are likely due to sequencing error and chimera formation all OTU tables were filtered at a minimum fraction of 0.0005. To decrease biases arising from variable sequencing depth, 16S samples were rarefied to 2500 reads and 18S samples to 4500 reads. Any samples with less than those respective amounts were filtered from the resulting OTU table for further downstream analyses. These read number cutoffs were chosen based on rarefaction curves approaching an asymptote in OTUs (see Supplemental Figures S5.2 and S5.5) and to maximize number of samples. Representative sequences of all 16S rRNA OTUs were aligned to the Greengenes core reference alignment using PyNAST (Caporaso *et al*., 2010b). Alignments

were subsequently filtered using the Greengenes Lane mask (Lane, 1991) and construction of a phylogenetic tree was done using FastTree (Price *et al*., 2010). The resulting tree was used to calculate pairwise UniFrac distance matrices for each individual microbiome sample. This analysis was likewise conducted on the 18S dataset using the SILVA reference tree with tips not corresponding to the OTU tables being removed.

The number of OTUs and the phylogenetic whole tree metric (Faith, 1992) were calculated to assess α-diversity for both 16S rRNA and 18S rRNA for each sample. The phylogenetic whole tree metric takes into account branch lengths when assessing diversity so that OTUs that are more distantly related will contribute more to the diversity estimate. Pairwise unweighted and weighted UniFrac values were calculated for all pairs of samples. The β-diversity and subsequent distance matrixes were calculated using both the unweighted and weighted UniFrac (Lozupone and Knight, 2005; Lozupone *et al*., 2011). Weighted measures analyze the relative change in taxon abundance, while unweighted measures are qualitative and represent the presence or absence of particular taxa. The eukaryote dataset is most likely biased in quantitative measures of diversity due to organism multicellularity, therefore weighted measures were not included in 18S analyses. All analyses on the eukaryote dataset used the 'majority_taxonomy_7_levels.txt' taxonomy list in the SILVA database.

Assessment of species specific and temporal variation in microbial communities

Host species specific differences within season were conducted using the "adonis" function at 1000 permutations in the v*egan* package (Oksanen *et al*., 2015) implemented in QIIME. Adonis was also implemented to examine seasonal differences by including an interaction term of season and species at 1000 permutations for each year. We were interested in whether seasons varied by year, which we examined by comparing seasons across year and accounting for species by year

interactions in Adonis. The seasons across year bacteria analysis for 2014 was conducted without *psittacina* as bacteria from this species were missing in the spring sampling period. All analyses were run on both the unweighted and weighted UniFrac bacteria distance matrices and the unweighted UniFrac eukaryote datasets for each sampling period. All results were visualized using a non-metric multidimensional scaling (NMDS) plot with two axes. Stress values below 0.20 are considered an ordination good fit (Quinn and Keough, 2002).

Results

Composition of bacterial communities

In total, 129 OTUs were identified from the 428 leaf samples belonging to eight phyla: Actinobacteria, Bacteroidetes, Chlamyidae, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria, and Verrucomicrobia (Figure 5.1A, Supplemental Figure S5.1A). Bacteroidetes, Firmicutes, and Proteobacteria were present in all species, with the exception of Firmicutes in the fall 2014 *purpurea* ssp. *venosa*. Cyanobacteria were more common in the *purpurea* subspecies and in the spring sampling periods overall. OTUs from the orders Flavobacteriales and Sphingobacteriales made up the largest proportion of the phylum Bacteroidetes (~ 55% and ~41%, respectively; Supplemental Figure S5.2). The majority of OTUs from Firmicutes were from the Lactobacillales and Clostridiales (~47% and ~27%, respectively; Supplemental Figure S5.2). Enterobacteriales, Pseudomonadales, and Xanthomonadales comprised a large portion of the OTUs for most of *Sarracenia*, with the exception of the *purpurea* complex and *psittacina*. The *purpurea* complex had more OTUs from the Alphaproteobacteria (e.g., Rhizobiales, Rhodospirillales, Sphingomonadales) and Betaproteobacteria (e.g., Burkholderiales, Neisseriales, Rhodocyclales) relative to the other *Sarracenia* species. The number of OTUs and phylogenetic diversity varied widely across species and sampling periods (Figure 5.2, Figure 5.3). Fall

samples for both years had more variation in OTU numbers ranging from < 10 to 100 at the 2500 sequence cut-off. Spring samples ranged from 20 to 80 OTUs per individual. Faith's phylogenetic diversity index ranged from ~2 to 15 with no distinguishable pattern between seasons or species (Figure 5.3).

Composition of eukaryote communities

The eukaryote community composition consisted of anywhere between 8 and ~50 OTUs (Figure 5.4 with the most found in the spring 2014 samples. *Sarracenia flava* var. r*ugelii* had the most OTUs in three of the four sampling periods. Phylogenetic diversity ranged from 4 to 10 with the spring 2014 samples having the largest range in diversity among species (Figure 5.5). Eukaryote taxa varied substantially among samples (Figure 5.6A, Supplemental 5.3A), with *purpurea* subspecies and fall samples of *psittacina* individuals having more Euglenozoa, Rotifera, Ciliophora, Rhizaria, Protalveolata, and Stamenopile sequences than the other *Sarracenia* species. Fungal sequences made up the majority (> 90%) for most *Sarracenia* species and were more prevalent in spring samples. Nematodes were more common in the 2014 samples than they were in the 2015 samples.

Species specific and temporal variation of microbial communities

All unweighted UniFrac host species specific comparisons were significant across sampling periods for bacteria and eukaryote communities (Figures 5.1B, 5.6B, Supplemental Figures S5.1B, S5.3B). Host species specific comparisons explained a large portion of the variance in bacterial communities (R^2 range: 31-42%) and eukaryote communities (R^2 range: 32-56%). This suggests that microbiome communities have more similar community members within individuals of the same host species than among individuals from a different species. Furthermore, weighted UniFrac distance matrices for bacteria communities across sampling

periods also indicated significant structure within species, explaining 27-47% of the microbiome variation (Table Supplemental Figure S5.4). This result indicates that not only are individuals within the same species more likely to have similar bacteria community members, but they are also more likely to have similar relative abundances of community members when compared to other host species.

Microbe communities were significantly different across season for certain host species indicated by a significant interaction between species and season for both sampling years (Tables 5.1, 5.2; Supplemental Figure S5.5). This species by season interaction accounted for 9-13% of the variation in microbe communities. Together these results suggest that the relative proportions and members of bacteria communities and the members of the eukaryote communities shift across seasons for some *Sarracenia* species. Furthermore, there is a significant interaction between species and year within seasons for all datasets (Table 5.3, 5.4, Supplemental Figure S5.6). Similar to between season differences, this interaction suggests that some *Sarracenia* species have differences in community members and abundance between years based on season. This interaction explains 8-11% of the variation in microbe communities.

Discussion

Microbiome diversity across Sarracenia

Carnivorous plants have evolved to use highly modified leaves to attract, capture, and digest prey to obtain nutrients. This unique adaption, in some ways resembling the animal gut, is predicted to contain distinct and functionally different bacteria assemblages in comparison to previous rhizosphere/phyllosphere studies. Similar to Koopman *et al*., (2010), we found the dominant phyla across *Sarracenia* to consist of Bacteriodetes, Firmicutes, Cyanobacteria, and Proteobacteria. These phyla have also been found in pitcher microbiomes for *Nepenthes* species

as well (Kanokratana *et al*., 2016; Sickel *et al*., 2016). Many of these phyla have been found in non-carnivorous plant microbiomes (see Turner et al., 2013; Copeland *et al.,* 2015), however, at a finer scale taxonomic scale Bacteroidales, Clostridiales, and Lactobacillales are more common in animal gut microbiomes (see Hacquard *et al*., 2015). These fine-scale results support previous findings of bacteria identified in *Sarracenia purpurea* (Whitman *et al*., 2005; Peterson *et al*., 2008; Krieger and Kourtev, 2012; Gray *et al*., 2012), *alata* (Koopman *et al.,* 2010), *minor* (Siragusa *et al*., 2007), and *Nepenthes* species (Sickel *et al*., 2016). OTUs corresponding to known nitrogen-fixing bacteria were also prevalent in pitchers, in particular the *purpurea* complex. Members of known nitrogen-fixers have also been found in other carnivorous plants and are hypothesized to be another way in which these plants may acquire essential nutrients (Harvey and Miller, 1996; Sickel *et al*., 2016).

There have been few studies to date that have examined 18S rRNA eukaryote communities within carnivorous plants (Bittleston *et al*., 2016). The majority of the literature in *Sarracenia* comes from microorganisms identified in *Sarracenia purpurea* (Miller *et al*., 1994; Gotelli and Ellison, 2006), but no research has been conducted using 18S rRNA in *Sarracenia* communities. Our results point to a diverse community, with the *purpurea* complex having many known aquatic organisms. In particular, the *purpurea* complex and *psittacina* contained many Rotifera, Ciliophora, and Euglenozoa species known to favor these more aqueous environments (Miller *et al*., 1994; Bledzki and Ellison, 2003; Gray, 2012). Amplicon sequencing of three *Nepenthes* species also found similar eukaryotes, yet these similarities disappeared at a finer taxonomic scale (Bittleston *et al*., 2016). Other *Sarracenia* species had representatives from most of the eukaroyte taxa, but the majority of sequences were derived from fungal species. This result is not surprising, as *Sarracenia* is known to associate with a high diversity of fungal

endophytes (Glenn and Bodri, 2012).

Host specific communities across Sarracenia

Given that carnivorous plants rely heavily on insects to obtain essential nutrients, with in some cases upward of 80% of the plant's nitrogen coming this way (Karagatzides *et al*., 2009), there should be strong selection on host-microbe relationship. Here, we found that *Sarracenia* host species accounted for the majority of microbiome variation so that individuals from the same host species had similar microbiomes. These results suggest that the host species may be exerting some selection pressure on community members. While there have been no other comparative common garden studies in *Sarracenia,* this result supports microbiome work in *Sarracenia alata* and *purpurea* that found bacteria communities were more similar among individuals in each sampled month (Koopman *et al*., 2010; Gray, 2012) and when compared to microbes in surrounding environments (Koopman *et al*., 2010). Similarly, Alcaraz *et al*. (2016) found *Utricularia gibba* bladder traps have significant differences in microbiome assemblages relative to their surrounding environment suggesting possible host selection. Recent 18S work across three *Nepenthes* species did find significant differences among host eukaryote communities across three sampling locations, suggesting host selection in this genus (Bittleston *et al*., 2016). However, a comparative study of seven *Nepenthes* species in a common area found variability among bacteria communities within species and that pitchers with more similar pH levels had similar bacteria communities (Kanokratana *et al*., 2016).

The way in which the host may be exerting selection pressure on the microbe community is unknown, but more recent work examining *Nepenthes* alludes to the ability by carnivorous plants to secrete enzymes (Takeuchi *et al*., 2011; Hatano and Hamada, 2012; Buch *et al*., 2013). Buch *et al.*, (2013) found numerous antimicrobial and antifungal proteins in the pitcher fluid

possibly as a way to alleviate competition with microbes over prey. However, Takeuchi *et al*. (2011) highlighted the potential positive role of bacteria in releasing enzymes to further decomposition of prey. The role that secreted enzymes play in digestion of prey in *Sarracenia* is not well established with little investigation into proteomic activity in S*arracenia* (Gallie and Chang, 1997). There is evidence, however, that the relationship between *Sarracenia* and their microbes is most likely mutualistic (Mouquet *et al*., 2008; Buckley *et al*., 2010), suggesting that *Sarracenia* species may have different interactions at a proteomic level with their host microbiome when compared to *Nepenthes.*

Temporal patterns across Sarracenia

Previous studies examining the microbiome of *Sarracenia alata* and *purpurea* have found shifts in community composition and abundance across time (Koopman *et al*., 2010; Gray, 2012). We also saw a shift in community members and abundance, but there appears to be an interaction by species. This suggests that some species tend to shift their communities over time, while others remain more stable. Reasons for these successional shifts may be the result of many factors. In particular, temperature shifts across the season, pH differences from prey decomposition, disturbance, and age of pitcher may all influence community dynamics within pitchers (Buckley *et al*., 2010; Gray 2012). Additionally, community dynamics between not only conspecifics, but also trophic level interactions may be responsible for shifting these communities (Matz et al., 2005; Kneitel, 2007; Peterson *et al*., 2008; Glenn and Bodri, 2012; Gray, 2012).

We did not sample the same individuals over time because of the invasiveness of the sampling technique. Furthermore, many *Sarracenia* species put up a second flush of pitchers late in the summer and we were interested in whether the microbiome communities of these young

pitchers would mirror the young pitchers sampled in the spring. Even for pitchers of relatively similar ages across seasons there are community differences. One possible reason for these seasonal shifts may be the input of prey between spring and fall. Fall pitchers tend to have higher prey biomass and some species have shifts in prey composition with the addition of more Lepidoptera species in the fall (Stephens *et al*., 2015b; Stephens *et al*., in prep). These seasonal differences could shift the nutrient input and possible regional pool of microbes found on prey items, thus shifting community dynamics.

Conclusions

Recent research points to a complex and interdependent relationship among eukaryote hosts and their microbiomes, influencing many aspects of host biology and evolution (Ochman *et al*., 2010; McFall-Ngai *et al.*, 2013). Our understanding of plant microbiomes is leading to some interesting findings and applications for plant health (Berendsen et al., 2012; Turner *et al*., 2013; Berg *et al*., 2014), yet the diversity and complexity of these communities across hosts are still largely unknown. Here, we took a comparative approach to examine microbiome diversity and structure in a genus of carnivorous plants; the use of a common garden gives some experimental capabilities allowing us to hold some environmental factors constant. Our findings suggest that host species has a significant effect on community structure, and hence genetic differences between species may be regulating the microbiomes. These macroevolutionary patterns point to underlying mechanisms and processes that structure these communities and provide a promising avenue of future research. For example, how do host enzymes influence microbes or whether or not host species can alter environmental characteristics inside the pitcher (e.g., pH, water content). Furthermore, investigation into multitrophic interactions and their influence on community assembly in this system (i.e., host-prey-microbes) is needed, as well as the function

of microbes in decomposition and nutrient acquisition for the host species. Future research on host-specific mechanisms and interactions among species could better inform our understanding of carnivorous plant microbiomes and potentially elucidate steps in the evolution of carnivory in plants.

References

Alcaraz LD, Martínez-Sánchez S, Torres I, Ibarra-Laclette E, Herrera-Estrella L. (2016). The metagenome of *Utricularia gibba*'s traps: into the microbial input to a carnivorous plant. *PLoS ONE* **11**: E0148979.

Amaral-Zettler LA, McCliment EA, Ducklow HW, Huse SM. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS One* DOI: 10.1371/journal.pone.0006372

Berendsen RL, Pieterse CMJ, Bakker PAHM. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

Bittleston LS, Baker CCM, Strominger LB, Pringle A, Pierce NE. (2016). Metabarcoding as a tool for investigating arthropod diversity in *Nepenthes* pitcher plants. *Austral Ecology* **41**: 120–132.

Bledzki LA, Ellison AM. (2003). Diversity of rotifers from northeastern USA bogs with new species records for North America and New England. *Hydrobiologia* **497**: 53–62.

Brucker RM, Bordenstein SR. (2013). The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science* **341**: 661–669.

Buch F, Rott M, Rottloff S, Paetz C, Hilke I, Raessler M, *et al.* (2012). Secreted pitfall-trap fluid of carnivorous *Nepenthes* plants is unsuitable for microbial growth. *Annals of Botany* doi:10.1093/aob/mcs287

Buckley HL, Miller TE, Ellison AM, Gotelli NJ. (2010). Local- to continental-scale variation in the richness and composition of an aquatic food web. *Global Ecology and Biogeography* **19**: 711–723.

Butler JL, Gotelli NJ, Ellison AM. (2008). Linking the brown and green: Nutrient transformation and fate in the *Sarracenia* microecosystem. *Ecology* 89: 898–904.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, *et al*. (2010a). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335–336.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. (2010b). PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**: 266–267.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, *et al.* (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal.* **6**: 1621–1624.

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, *et al.* (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature* **488**: 178–185.

Collins SM, Surette M, Bercik P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews Microbiology* **10**: 735–742.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. (2015). Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Faith DP. (1992). Conservative evaluation and phylogenetic diversity. *Biological Conservation* **61**: 1–10.

Gallie DR, Chang S-C. (1997). Signal transduction in the carnivorous plant *Sarracenia purpurea. Plant Physiology* **115**: 1461–1471.

Givnish TJ. (2015). New evidence on the origin of carnivorous plants. *Proceedings of the National Academy of Sciences* **112**: 10–11.

Glenn A, Bodri MS. (2012). Fungal endophyte diversity in *Sarracenia*. *PLoS ONE* **7**: e32980.

Gotelli NJ, Ellison AM. (2006). Food-web models predict species abundances in response to habitat change. *PLoS Biology* 4: 1869–1873.

Gray SM. (2012). Succession in the aquatic *Sarracenia purpurea* community: deterministic or driven by contingency? *Aquatic Ecology* **46**: 487–499.

Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, *et al.* (2015). Microbiota and host nutrition across plant and animal kingdoms. *Cell Host & Microbe* **17**: 603–616.

Hatano N, Hamada T. (2012). Proteomic analysis of secreted protein induced by a component of prey in pitcher fluid of the carnivorous plant *Nepenthes alata. Journal of Proteomics* **75**: 4844–4852.

Harvey E, Miller TE. (1996). Variance in composition of inquiline communities in leaves of *Sarracenia purpurea* L. on multiple scales. *Oecologia* **108**: 562–566.

Kanokratana P, Mhuanthong W, Laothanachareon T, Tangphatsornruang S, Eurwilaichitr L, Kruetreepradit T, Mayes S, Champreda V. (2016). Comparative study of bacterial communities in *Nepenthes* pitchers and their correlation to species and fluid acidity. *Environmental Microbiology* **72**: 381–393.

Karagatzides JD, Butler JL, Ellison AM. (2009). The pitcher plant *Sarracenia purpurea* can directly acquire organic nitrogen and short-circuit the inorganic nitrogen cycle. *PLoS ONE* **4**: 1–9.

Kneitel JM. (2007). Intermediate-consumer identity and resources alter a food web with omnivory. *Journal of Animal Ecology* **76**: 651–659.

Koopman MM, Fuselier DM, Hird S, Carstens BC. (2010). The carnivorous pale pitcher plant harbors diverse, distinct, and time-dependent bacterial communities. *Applied and Environmental Microbiology* **76**:1851–1860.

Koopman MM, Carstens BC. (2011). The microbial phyllogeography of the carnivorous plant *Sarracenia alata*. *Microbial Ecology* **61**:750–758.

Krieger JR, Kourtev. (2012). Bacterial diversity in three distinct sub-habitats within the pitchers of the northern pitcher plant, *Sarracenia purpurea*. *FEMS Microbiology Ecology* **79**: 555–567.

Lane DJ. (1991). 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds.) *Nucleic Acid Techniques in Bacterial Systematics*. Wiley Press: New York, pp. 115–175.

Lodhi MA, Guang-Ning Y, Weeden NF, Reisch BI. (1994). A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. *Plant Molecular Biology Reporter* **12**: 6–13.

Lozupone C, Knight R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* **71**: 8228–8235.

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. (2011). UniFrac: An effective distance metric for microbial community comparison. *The ISME Journal* **5**: 169–172.

Matz C, McDougald D, Moreno AM, Yung PY, Yildiz FH, Kjelleberg S. (2005). Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae. Proceedings of the National Academy of Science USA* **102**: 16819–16824.

McDonald D, Price MN, Goodrich JA, Nawrocki EP, DeSantis TZ, Probst A, *et al.* (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal* **6**: 610–618.

McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, *et al.* (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Science, USA*. **110**: 3229–3236.

Medlin L, Elwood HJ, Stickel S, Sogin ML. (1988). The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**: 491–499.

Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM. (2014). Taxonomical and functional microbial community selection in soybean rhizosphere. *The ISME Journal* **8**: 1577–1587.

Miller TE, Cassill D, Johnson C, Kindell C, Leips J, McInnes D, Bevis T, Mehlman D, Richard B. (1994). Intraspecific and interspecific competition of *Wyeomyia smithii* (Coq.) (Culicidae) in pitcher plant communities. *American Midland Naturalist* **131**: 136–145.

Mouquet N, Daufresna T, Gray SM, Miller TE. (2008). Modelling the relationship between a pitcher plant (*Sarracenia purpurea*) and its phytotelma community: mutualism or parasitism? *Functional Ecology* **22**: 728–737.

Ochman H, Worobey M, Kuo CH, Hdjango JBN, Peeters M, Hahn BH, *et al.* (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biology* **8**, e1000546.

Oksanen J, Blanchet F, Kindt R, Legendre P, O'Hare RB, et al. (2017). Vegan: Community ecology package, version 2.4-2, https://cran.r-project.org/web/packages/vegan/index.html Accessed 1 March 2017.

Peterson CN, Day S, Wolfe BE, Ellison AM, Kolter R, Pringle A. (2008). A keystone predator controls bacteria diversity in the pitcher-plant (*Sarracenia purpurea*) microecosystem. *Environmental Microbiology* **10**: 2257–2266.

Price MN, Dehal PS, Arkin AP. (2010). FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS One* **5**, e9490.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al.* (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**: D590–D596.

Quinn G, Keough M. (2002). *Experimental Design and Data Analysis for Biologists.* Cambridge University Press, Cambridge.

R Development Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org.

Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, *et al*. (2011). *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* **6056**: 670–674.

Sickel W, Grafe TU, Meuche I, Steffan-Dewenter I, Keller A. (2016). Bacterial diversity and community structure in two Bornean *Nepenthes* species with differences in nitrogen acquisition strategies. *Microbial Ecology* **71:** 938–953.

Siragusa AJ, Swenson JE, Casamatta DA. (2007). Culturable bacteria present in the fluid of the hooded-pitcher plant *Sarracenia minor* based on 16S rDNA gene sequence data. *Microbial Ecology* **54**: 324–331.

Stephens JD, Rogers WL, Heyduk K, Cruse-Sanders JM, Determann RO, Glenn TC, *et al.* (2015a). Resolving phylogenetic relationships of the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Molecular Phylogenetics and Evolution* **85**: 76–87.

Stephens JD, Godwin RL, Folkerts DR. (2015b). Distinctions in pitcher morphology and prey capture of the Okefenokee variety within the carnivorous plant species *Sarracenia minor*. *Southeastern Naturalist* **14**: 254–266.

Stoeck T, Bass D, Nebel M, Christen R, Jones MD, Breiner H-W, *et al.* (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology* **19**: 21–31.

Takeuchi Y, Salcher MM, Ushio M, Shimizu-Inatsugi R, Kobayashi MJ, Diway B, von Mering C, Pernthaler J, Shimizu KK. (2011). *In situ* enzyme activity in the dissolved and particulate fraction of the fluid from four pitcher plant species in the genus *Nepenthes* *PLoS ONE* **6**: e25144.

Tremaroli V, Bäckhed F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature* **489**: 242–249.

Turner TR, James EK, Poole PS. (2013). The plant microbiome. *Genome Biology* **14**: 209.

Whitman RL, Byers SE, Shively DA, Ferguson DM, Byappanahalli M. (2005). Occurrence and growth characteristics of *Escherichia coli* and *enterococci* within the accumulated fluid of the northern pitcher plant (*Sarracenia purpurea* L.). *Canadian Journal of Microbiology* **51**: 1027–1037.

Yang J, Kloepper JW, Ryu CM. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* **14**: 1–4. doi: 10.1016/j.tplants.2008.10.004

Zhang J, Kassian K, Flouri T, Stamatakis A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–620.

Table 5.1. Species by season interaction Adonis results for bacteria communities.

	Unweighted	Weighted
2014	$F_{12, 170} = 1.95$, $R^2 = 0.09$, p = 0.001	$F_{12, 170} = 1.99$, $R^2 = 0.10$, p = 0.001
2015	$F_{14, 190} = 2.09$, $R^2 = 0.10$, p = 0.001	$F_{14, 190} = 1.97$, $R^2 = 0.10$, p = 0.001

Table 5.2. Species by season interaction Adonis results for eukaryote communities.

	Unweighted
2014	$F_{13, 149} = 2.49$, $R^2 = 0.10$, p = 0.001
2015	$F_{14, 177} = 3.12$, $R^2 = 0.13$, p = 0.001

Table 5.3. Species by year interaction Adonis result for bacteria communities.

	Unweighted	Weighted
Spring	$F_{12, 171} = 1.76$, $R^2 = 0.08$, p = 0.001	$F_{12, 171} = 2.49$, $R^2 = 0.11$, p = 0.001
Fall	$F_{13, 175} = 1.87$, $R^2 = 0.08$, p = 0.001	$F_{13, 175} = 1.82$, $R^2 = 0.09$, p = 0.001

Table 5.4. Species by year interaction Adonis result for eukaryote communities.

	Unweighted
Spring	$F_{13, 174} = 2.30$, $R^2 = 0.09$, p = 0.001
Fall	$F_{13, 139} = 2.36$, $R^2 = 0.10$, p = 0.001

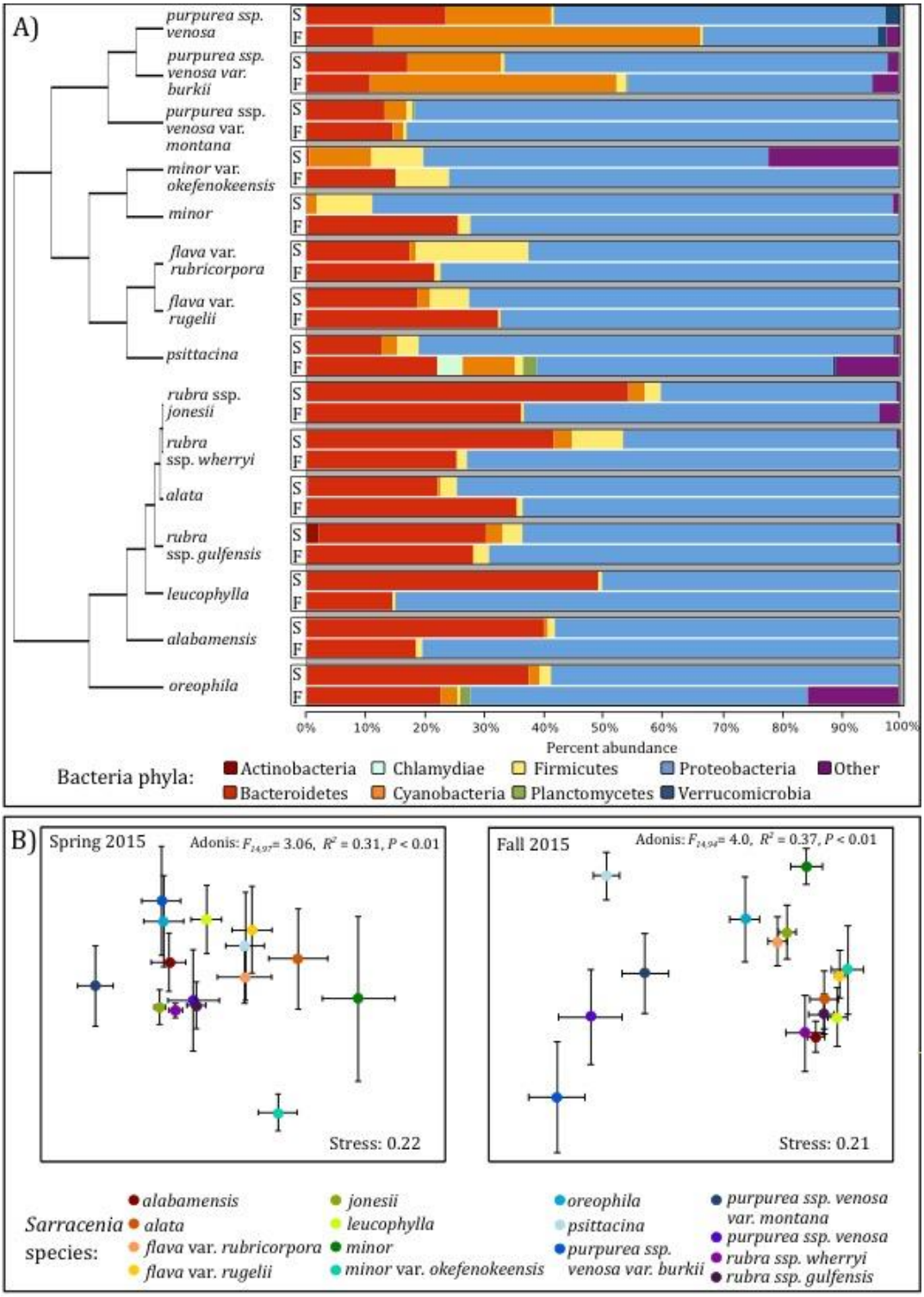

Figure 5.1. Bacterial composition of *Sarracenia* species for 2015 sampling period. A) Bacterial composition at the phylum level across the *Sarracenia* phylogeny (Stephens *et al*., 2015a). For each species spring (S) and fall (F) are shown. B) Non-metric multidimensional scaling (NMDS) plots at the 97% OTU level for bacterial communities with standard error measures.

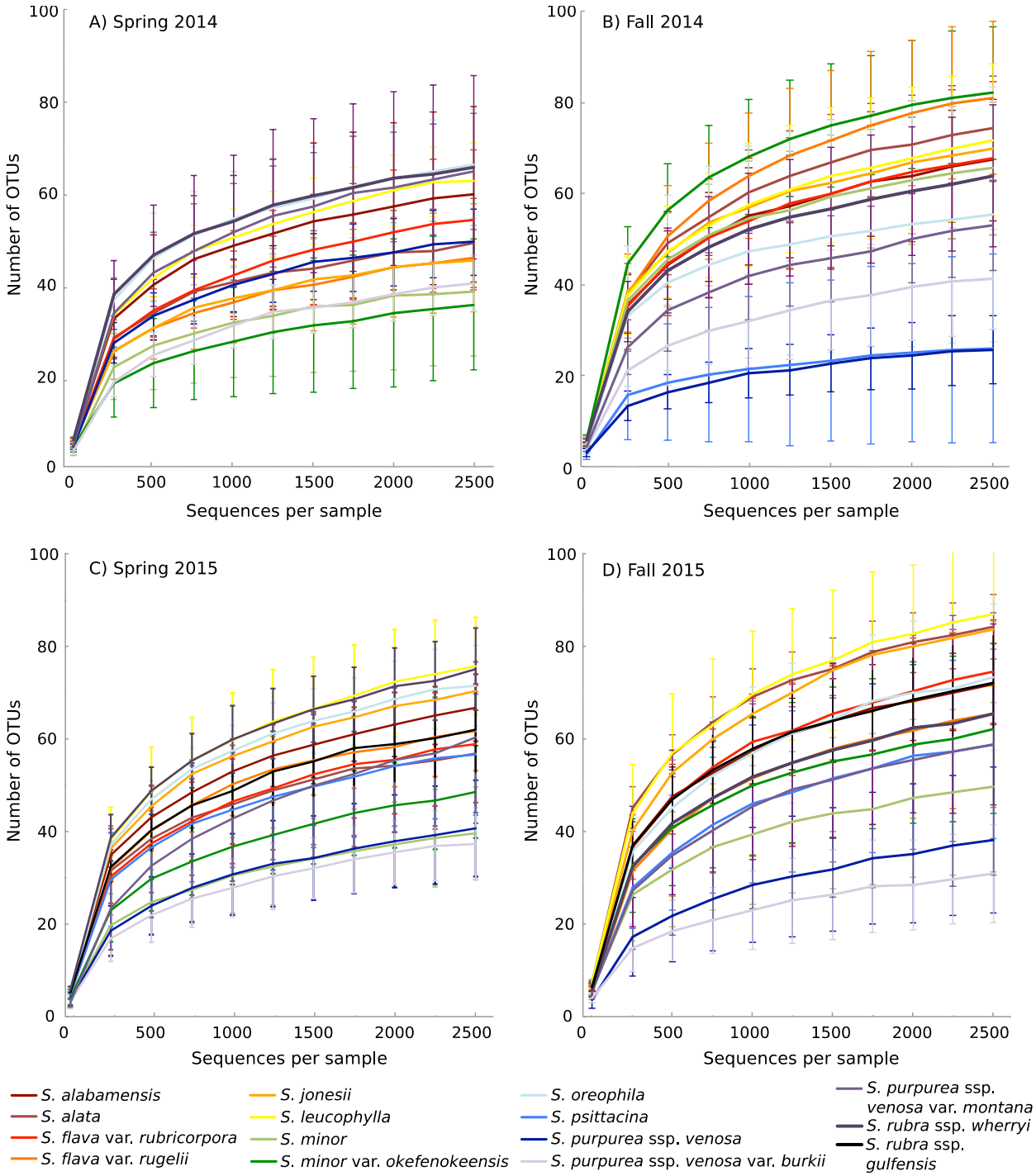

Figure 5.2. Rarefaction curves for the number of bacterial operational taxonomic units (OTUs) captured across fourteen *Sarracenia* species at a sampling depth of 2500 reads. Each panel designates a different sampling period (A) spring 2014 (B) fall 2014 (C) spring 2015 (D) fall 2015. An additional *Sarracenia* species, *S. rubra* ssp. *gulfensis*, was collected during the spring and fall 2015 sampling. *Sarracenia* species are represented with different colors as seen in the legend with within species sample variation represented by the error bars at each sampling point.

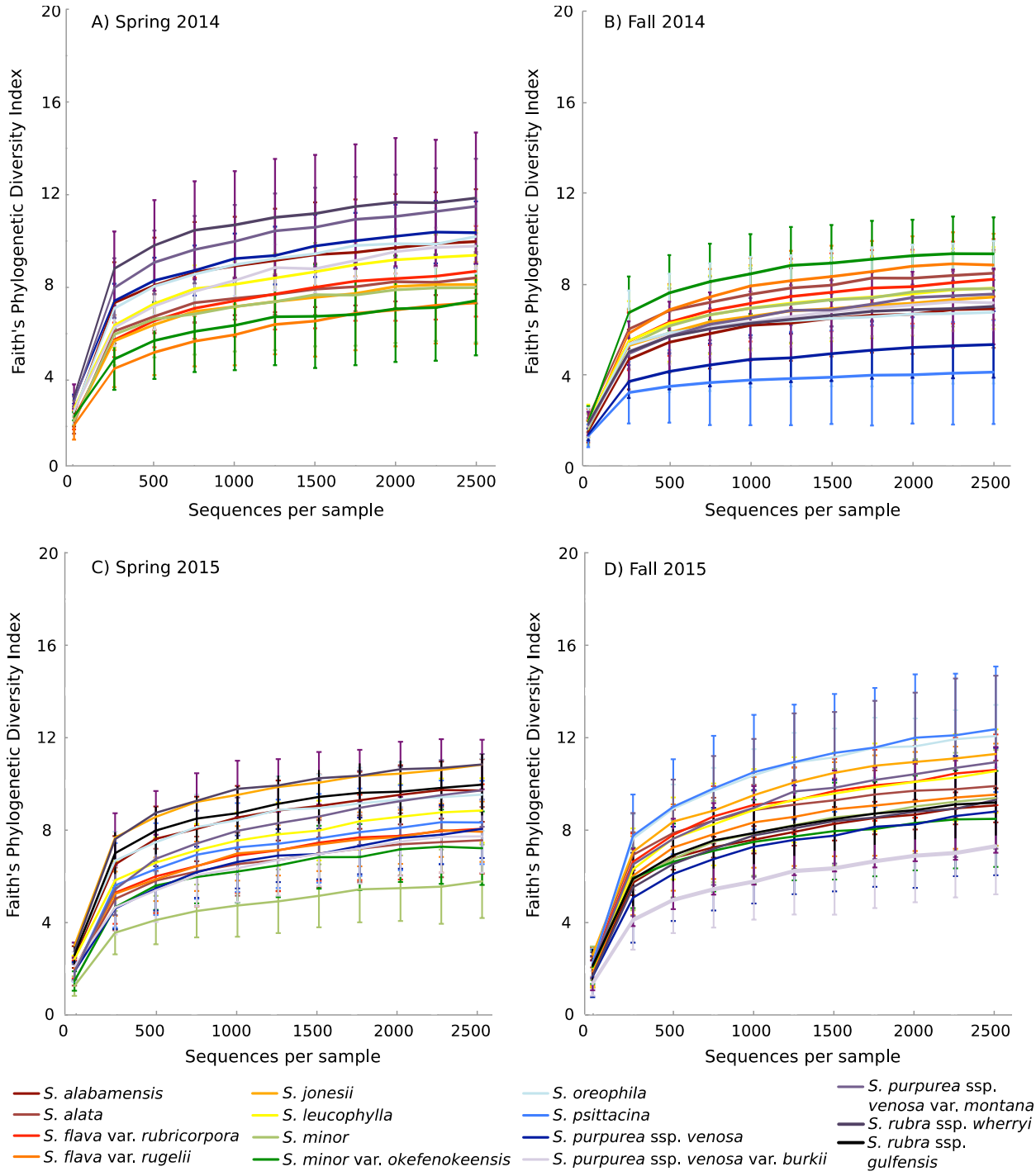

Figure 5.3. Rarefaction curves for Faith's metric of bacterial alpha diversity across fourteen *Sarracenia* species at a sampling depth of 2500 reads. Each panel designates a different sampling period (A) spring 2014 (B) fall 2014 (C) spring 2015 (D) fall 2015. An additional *Sarracenia* species, *S. rubra* ssp. *gulfensis*, was collected during the spring and fall 2015 sampling. *Sarracenia* species are represented with different colors as seen in the legend with within species sample variation represented by the error bars at each sampling point.

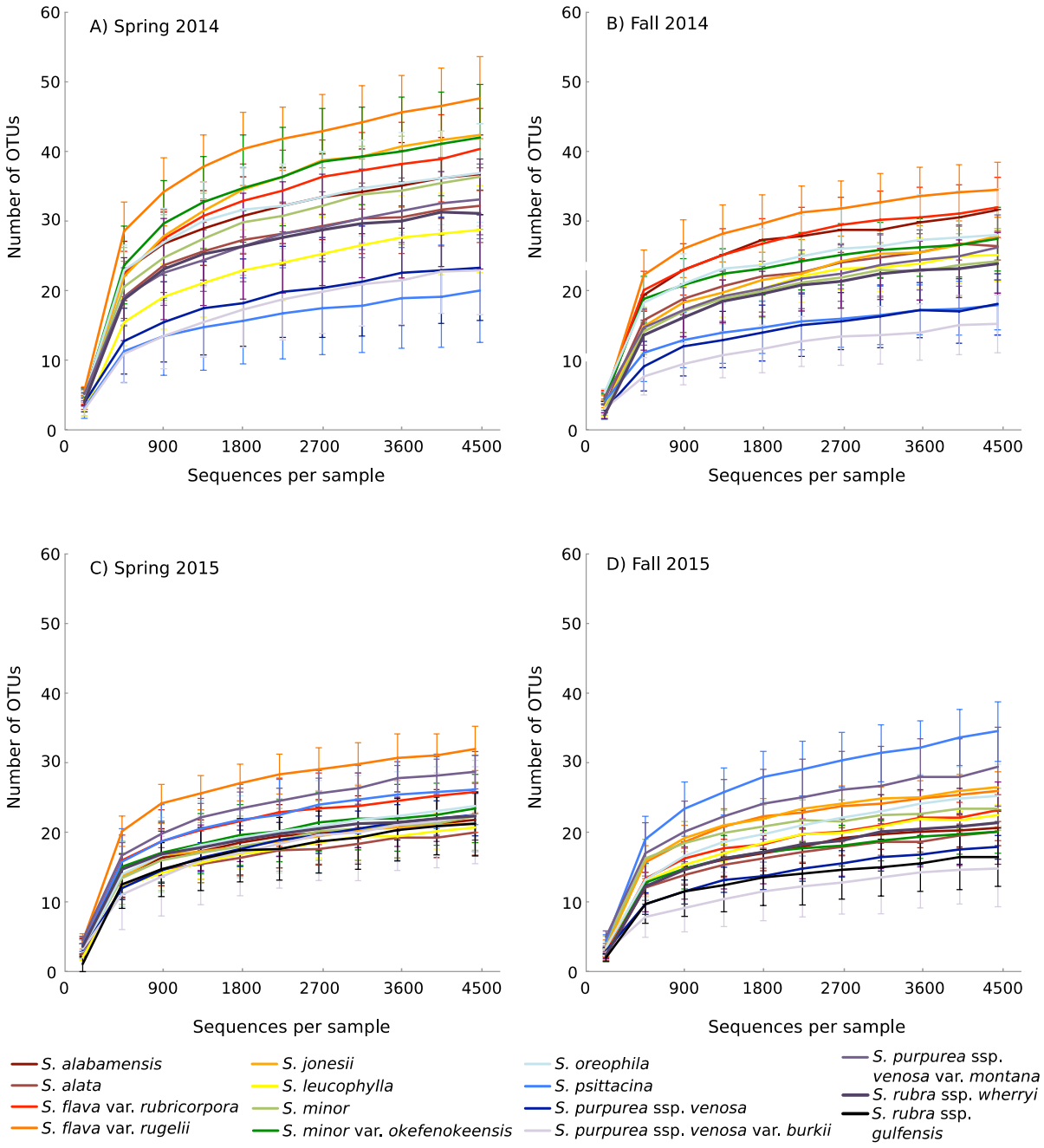

Figure 5.4. Rarefaction curves for the number of eukaryotes captured across fourteen *Sarracenia* species at a sampling depth of 4500 reads. Each panel designates a different sampling period (A) spring 2014 (B) fall 2014 (C) spring 2015 (D) fall 2015. An additional *Sarracenia* species, *S. rubra* ssp. *gulfensis*, was collected during the spring and fall 2015 sampling. *Sarracenia* species are represented with different colors as seen in the legend with within species sample variation represented by the error bars at each sampling point.

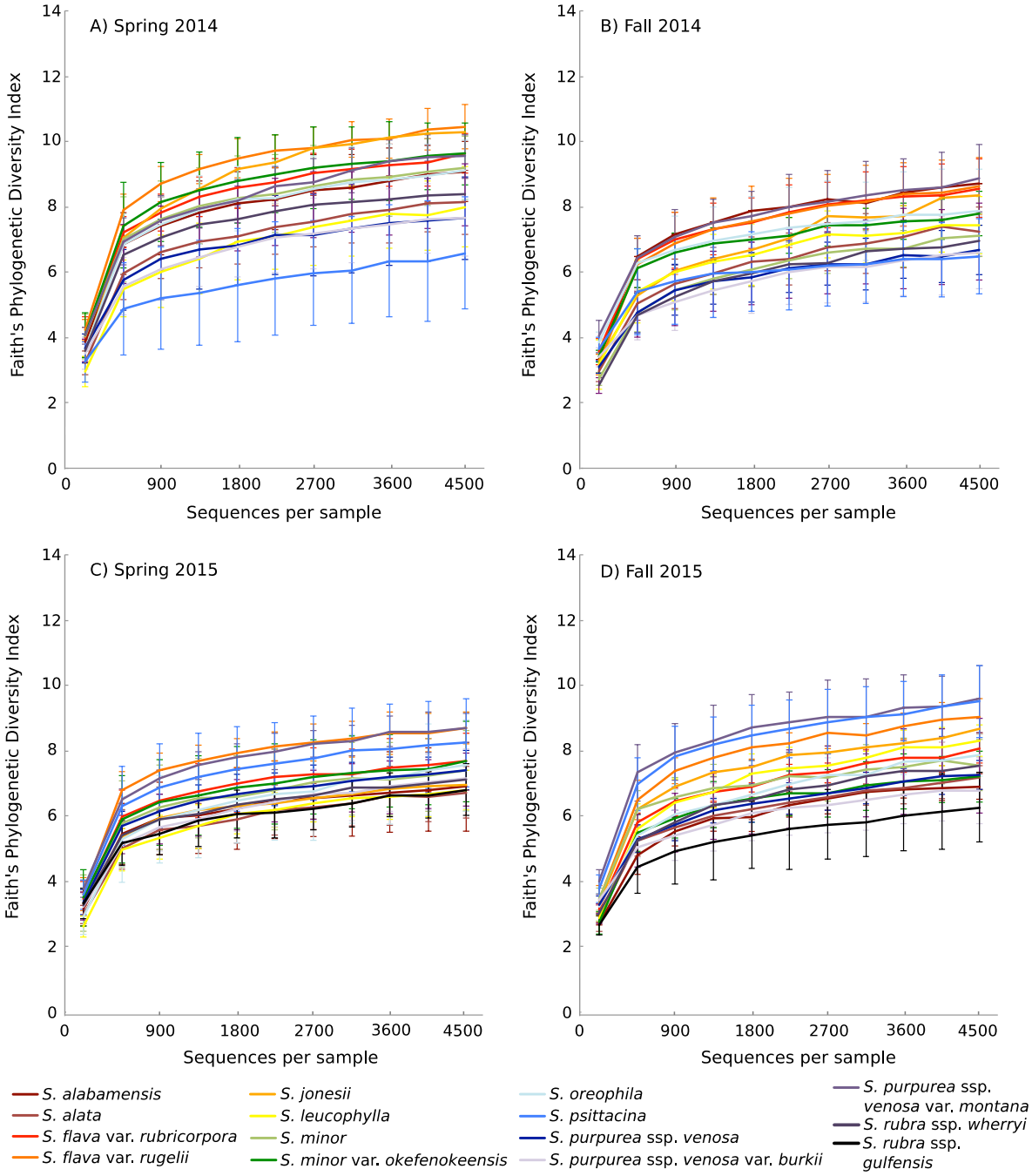

Figure 5.5. Rarefaction curves for Faith's metric of eukaryote alpha diversity across fourteen *Sarracenia* species at a sampling depth of 4500 reads. Each panel designates a different sampling period (A) spring 2014 (B) fall 2014 (C) spring 2015 (D) fall 2015. An additional *Sarracenia* species, *S. rubra* ssp. *gulfensis*, was collected during the spring and fall 2015 sampling. *Sarracenia* species are represented with different colors as seen in the legend with within species sample variation represented by the error bars at each sampling point.

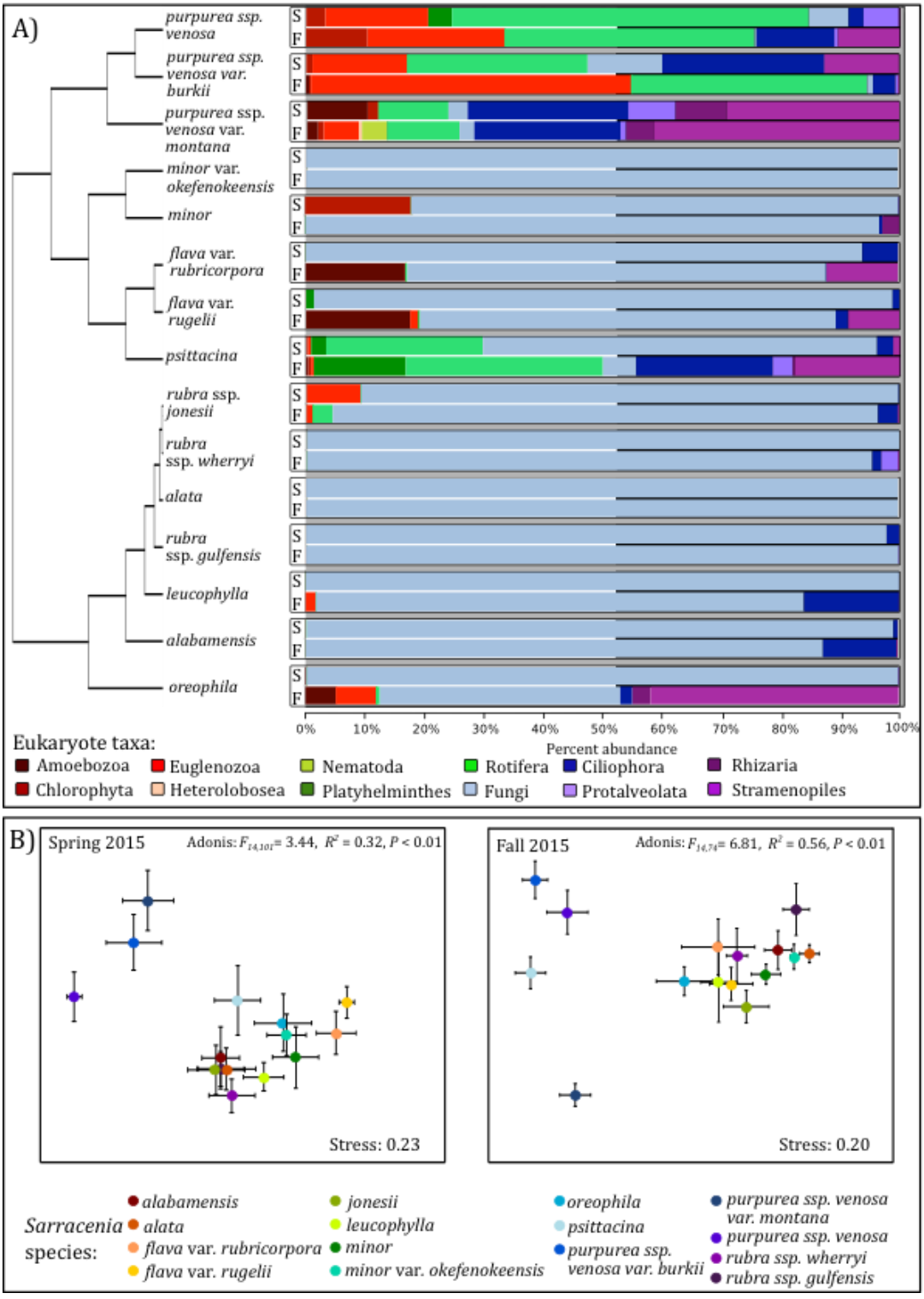

Figure 5.6. Eukaryote sequence composition of *Sarracenia* species for 2015 sampling period. A) Eukaryote composition at varying taxonomic levels across the *Sarracenia* phylogeny. For each species spring (S) and fall (F) are shown. B) Non-metric multidimensional scaling (NMDS) plots for eukaryote communities with standard error measures.

CHAPTER VI:

SHOW ME THE WAY: FUTURE FACULTY PREFER DIRECTIVE FEEDBACK WHEN TRYING ACTIVE LEARNING APPROACHES

Stephens JD, Battle D, Gormally C, Brickman P. Accepted to The Journal for College Science Teaching.

Abstract

Early training opportunities for future faculty, namely graduate students and postdoctoral researchers can better prepare them to use active learning approaches. We know that instructional feedback supports sustained change and motivates instructors to improve teaching practices. Here, we incorporated feedback as a key component of a pedagogical course for future faculty who had never taught or were currently novice teaching assistants. We studied (1) how future faculty's teaching beliefs changed over the course of the semester, (2) whether feedback varied between future faculty (peers) and facilitators (faculty and upper level graduate students), (3) future faculty's feedback preferences (i.e., written versus oral, peer versus facilitator), and (4) how to use those preferences to tailor feedback that encourages future faculty at all levels to adopt more active learning approaches. We found that future faculty made greater shifts in their teaching beliefs than more experienced facilitators, responding more favorably to direct feedback that informed them how to improve rather than simple encouragement.

Introduction

Early training opportunities for future faculty, namely graduate students and postdoctoral researchers, can better prepare them to use active learning approaches in teaching (Wise 2011; Ebert-May et al. 2015; Singer, Nielsen, and Schweingruber 2012). Numerous pedagogical development programs have been devised to support this goal, such as the American Society of Microbiology Science Teaching Fellows, the Center for the Integration of Research, Teaching, and Learning, and the Preparing Future Faculty Program (Markowitz and DuPre 2007; Marbach-Ad et al. 2012; Wyse, Long, and Ebert-May 2014). Since the majority of doctoral students seek faculty positions at primarily teaching institutions (Golde and Dore 2001; Austin 2002), focusing on early career instructors offers possibilities for sustained pedagogical development, contributes to STEM education reform, and influences how teaching is valued in science culture (Brownell and Tanner 2012; Austin et al. 2009). Yet, few studies have evaluated the effectiveness of these early training opportunities, specifically, the role of instructional feedback to support the transition to active learning approaches (Gormally, Evans, and Brickman 2014).

Instructional feedback motivates faculty to improve teaching practices (Sunal et al. 2001; Henderson, Beach, and Finkelstein 2011). Teachers develop more student-centered ideologies through practice, peer observation, mentoring, and feedback (Luft 2001), which affects subsequent teaching (Nespor 1987; Fang 1996; Wallace and Kang 2004), and instructional mentoring and coaching can improve teacher attitudes and knowledge (Stes, Coertjens, and Van Petegem 2010), However, beginning faculty receive little formative instructional feedback (Gormally et al., 2014). Yet, this career stage may be most critical: K-12 pre-service and beginning teachers have tentative teaching beliefs that are malleable to professional development (Fang 1996), while more experienced teachers have established, less malleable beliefs (Luft

2001). Thus, graduate pedagogy courses offer an opportunity to support the growth of student-centered teaching beliefs. However, there has been no research on the impact of providing feedback that identifies strengths as well as areas for improvement to future faculty as they create and practice presenting teaching materials.

Here, we incorporated feedback as a key component of a pedagogical course for future faculty who had never taught or were currently novice teaching assistants. We characterized all feedback given over the course of the semester in an effort to inform effective feedback practices for future faculty and lead to a better understanding of how future faculty's teaching beliefs and practices develop.

Methods

Study context

Future faculty participated in five weeks of pedagogical instruction followed by five weeks of practice developing and refining activities, modeled after the Summer Institutes to Improve University Science Teaching (Figure 6.1) (Pfund et al. 2009). This pedagogical instruction model has been implemented at other universities for graduate student professional development (Wyse, Long, and Ebert-May 2014; Zehnder 2016; Markowitz and DuPre 2007; Lederer et al. 2016). However, we are the first to assess feedback given during such a course. The course included three five-week modules. Module 1 introduced inquiry, active learning, inclusive teaching, cooperative learning, motivation, and backwards design. During module 2, future faculty collaborated in pairs to develop their own active learning exercise (Figure 6.1). Module 3 focused on professional development (e.g., resources provided at the university, developing a teaching philosophy).

Eight future faculty participated in the course (six women, two men). One student was pursuing a Master's degree and the others were seeking a Ph.D. Two future faculty were current laboratory teaching assistants and another had been a teaching assistant prior to the course. Of the eight future faculty, two were forestry majors, five plant biology, and one was a microbiology major. Finally, three future faculty spoke English as a second language. Four facilitators participated in the feedback module (the course instructor, JDS, did not participate in feedback): a tenured faculty member with 25+ years of teaching; a post-doctoral researcher with 4 years of experience; and two upper level graduate students with 5-7 years of teaching experience and heavy involvement in teaching resources throughout the University.

Measurement of Teaching Beliefs

Future faculty and facilitators completed the Teaching Beliefs Interview (TBI) (Luft and Roehrig 2007) during weeks 1 and 15 of the semester. The TBI is a series of seven questions used to examine how teachers' beliefs may shift along a spectrum from traditional to reform-based. This spectrum spans beliefs from (1) Traditional, where teachers view their role as transmitters of information; (2) Instructive, where teachers' goal is to provide experiences for students; (3) Transitional, where teachers focus on their rapport with students and how to engage them; (4) Responsive, where teachers focus on helping students take charge of their own learning; to (5) Reform, where teachers provide students with experiences that help them mediate prior knowledge and make sense of their own understanding. Each TBI interview was scored independently by three researchers. During the scoring process, codes were compared, and the last researcher was responsible for looking at the level of agreement between the first two coders. If discrepancies arose, all researchers looked back at the original data, examined prior codes, and

collaboratively made a final decision. Once the final codes were determined, the responses were converted into numeric scores and tabulated.

Collection of Feedback

When future faculty presented progress on their active learning activities, both peers and facilitators were asked to provide written feedback about what they liked and what needed improvement. Verbal feedback was also videotaped and transcribed. There was no discussion prior to or during the feedback sessions about what constitutes good feedback and no guidance given on verbal feedback. Prior to final presentations, future faculty received a copy of all the feedback they had received (average of 191 comments per student) and were asked to circle and rank the five most useful pieces of feedback. We used the ranked feedback (n = 40) to determine preferences. Each future faculty received different amounts of written versus oral feedback from each participant. In order to adjust for the probability that a particular piece of feedback was selected, we used proportions rather than absolute numbers for the ranked feedback selected. This number was then weighted by the rank of the feedback. We used a t-test to determine whether one type (written versus oral; facilitator versus peer) of feedback was preferred by future faculty. It should be noted that sample sizes are low and interpretation should be taken with caution. Lastly, future faculty were asked to describe why they selected these pieces of feedback to modify and improve their active learning activity.

Coding of Feedback Comments

Since this study is the first to characterize the type of instructional feedback given during professional development for future faculty, we began by considering factors from the literature on best practices for providing feedback from both organizational psychology and education (Gormally, Evans, and Brickman 2014). Three research-based themes formed our initial starting

point for analysis: (1) effective feedback identifies what might be deficient or lacking (critical) (Hattie and Timperley 2007; Scheeler, Ruhl, and McAfee 2004); (2) effective feedback provides direction for how to correct or improve deficiencies (directive) (Cossairt, Hall, and Hopkins 1973; O'Reilly and Renzaglia 1994); and (3) effective feedback encourages and praises positive aspects (supportive) (Jussim, Yen, and Aiello 1995; Cossairt, Hall, and Hopkins 1973; Podsakoff and Farh 1989).

We conducted a thematic analysis, searching all feedback statements to identify concepts, categories, and ultimately, themes or patterns within the data (Braun and Clarke 2006) using the ATLAS.ti software to import and code comments. Three researchers gauged the presence of reported best practices (and other practices) in initial coding, then met to exchange, read, and sort through the initial analyses to identify the presence of these themes collectively, and agreed with the other's analyses and generated additional codes as needed to describe all the instances within the data set (Saldana 2016). The same three researchers also discussed how to identify feedback that encouraged future faculty to revise their activities to encourage greater inclusion of active learning approaches. After several rounds of coding to insure that we could explain all feedback statements in some manner, we agreed upon two major categories of codes: (1) Nature of the Feedback (supportive, critical, directive, and non-directive; Figure 6.3) and (2) Cognitive Behaviors that the feedback recommends (ICAP; Figure 6.4). We selected many of the categories of the Interactive, Constructive, Active and Passive (ICAP) framework (Chi and Wylie 2014) to classify both written and oral feedback given by peers and facilitators for modifying and improving activities because it explained most of our codes (Table 6.1). The final categories included: Passive cognitive behaviors that promote storage of new information (e.g. watching a video, taking notes); Active cognitive behaviors that promote retrieving and

strengthening knowledge (e.g. highlighting sentences while reading, searching for information of copying a solution of a problem that the teacher explains on the board); Constructive cognition behaviors that promote reorganization and making inferences about new knowledge (e.g. drawing a concept map, solving a new problem, comparing and contrasting); and Interactive cognition behaviors that involve co-construction of knowledge with a partner (e.g. arguing or defending a position or reciprocal teaching) An additional category, teacher-centered, was added to account for feedback that specifically targeted how the future faculty taught (e.g. using the whiteboard or voice volume). This framework classifies feedback based on the cognitive behaviors that the future faculty's potential students would engage in during active learning. Once the final set of codes was established, two researchers not involved in course instruction independently coded the feedback comments (n = 764), then discussed any comments for which there were discrepancies, coding until consensus was reached on all codes. Finally, the three researchers coded the rationale that future faculty provided for why they selected the most useful feedback given to them. These reasons were divided into three groups based on shifts in Teaching Beliefs over the course of the semester. This research was approved by the IRB of University of Georgia (UGA) study# 00002589.

Results & Discussion

Future faculty beliefs changed substantially over the semester

Beginning teachers are at a critical juncture in the development of their teaching beliefs. At the beginning of the course, future faculty expressed primarily instructive and transitional beliefs (Figure 6.2), similar to beginning K-12 teachers (Luft, Fletcher, and Fortney 2005). Future faculty's teaching beliefs shifted over the semester (Figure 6.2) to a similar degree as to those of beginning K-12 teachers (Roehrig and Luft 2006). Some future faculty exhibited larger

shifts in teaching beliefs than others, but most (7 of 8) shifted toward more student-centered reform-based beliefs, with only one shifting toward more traditional, teacher-centered beliefs. We echo Roehrig and Luft (2006), cautioning those leading professional development efforts that teachers are more sensitive to feedback during the beginning of their career, and future teachers with more student-centered beliefs were more likely to enact active learning approaches, including inquiry activities (Luft et al. 2007; Roehrig and Luft 2006). While the course was designed for future faculty, the facilitators did participate in all aspects of the course. Given this, we did not see a shift into a new category of teaching beliefs even though three of the four facilitators were considered at or near the same teaching belief categories as the future faculty participants. This mirrors Roehrig and Luft's (2001) finding that experienced teachers do not exhibit the same degree of shifts in their teaching beliefs as novice teachers. However, we should note that the course was not designed to target experienced teachers.

Facilitators and peers provide similar types of feedback

A total of 765 feedback comments (including written and oral) were given through the semester. Facilitators provided approximately twice the number of comments as peers (406 from 4 facilitators; 359 from 8 peers) and their comments were longer on average (38 versus 27 words/comment). However, both facilitators and peers provided nearly the same proportion of Nature of Feedback comments (supportive, critical, directive and nondirective) and comments about Cognitive Behaviors (all non-significant using chi-square tests; Figure 6.3, A and B; Figure 6.4).

Future faculty preferred directive, critical feedback given by facilitators rather than peers

At the conclusion of Module 2, future faculty were asked to select the five most useful pieces of feedback to improve their learning activity that they received (Figure 6.1). These preferred

comments had a higher number of words per comment (57 words/comment). After accounting for total feedback given, future faculty tended to prefer feedback provided by facilitators over peer feedback (Table 6.2), however, only 5% of the preferred feedback was attributed to the more responsive facilitator. The type of feedback given by the responsive facilitator was not significantly different from the transitional facilitators, with the exception that the responsive facilitator gave less directive feedback ($X^2 = 6.125$, df = 1, p = 0.013). Additionally, there seemed to be no preference for written versus oral feedback. Of the preferred feedback, future faculty favored feedback that was more directive and critical and less supportive (Figure 6.3C; Table 6.2). This is consistent with K-12 teachers who also express a preference for concrete, specific feedback. Teachers are also more likely to change their behaviors (for example, questioning students or other pacing and prompting behaviors) when they are given specific direction rather than general information (Englert and Sugai 1983; Giebelhaus 1994; Hindman and Polsgrove 1988; O'Reilly and Renzaglia 1994). Furthermore, in the ICAP framework, future faculty did not prefer suggestions that were passive in nature (Figure 6.4).

Explanations from future faculty on why they responded to feedback positively

While future faculty overall preferred critical, directive feedback, we were also interested in whether this result was consistent for all future faculty across TBI levels. We focused on comparing individuals who made shifts in teaching beliefs (Figure 6.2; Table 6.3). The one future faculty who remained in TBI Traditional/Instructive) preferred directive feedback and was sensitive to critical feedback. The future faculty who made shifts in their teaching beliefs, ending in Transitional, preferred feedback that was feasible, directive, supportive, student-centered (n=3). While they appreciated criticism, at times these future faculty were defensive or lacked the pedagogical knowledge to implement the feedback they received. Finally, the future faculty

whose post-semester teaching beliefs were categorized as Responsive preferred critical, feasible, student-centered feedback (n=4). They also appreciated supported feedback, but critical feedback was preferred to supportive feedback. These future faculty used feedback that reinforced their teaching beliefs. At times, these future faculty wanted more directive feedback, and were defensive or lacked the pedagogical knowledge to use feedback they received.

Implications & Recommendations

Here, we suggest specific strategies to help mentors improve the teaching beliefs and likelihood that future faculty will embrace active learning approaches during professional development. First, we acknowledge the importance of the status of those providing feedback. Future faculty gave greater weight to mentors' feedback than to peers' feedback, although both provided similar types of feedback. Feedback that was directive, such as ideas and suggestions for improvement, was most desirable to future faculty across a range of teaching beliefs. Both oral and written feedback was equally useful to future faculty.

We recommend that mentors use the TBI to start a discussion with their future faculty about teaching beliefs. This can help mentors to identify future faculty who may be defensive and unwilling to accept feedback. Mentors should be aware that both pedagogical content knowledge and teaching beliefs can be critical barriers to accepting and responding to feedback. As a result, future faculty who hold more traditional beliefs may need more supportive and directive student-centered feedback to encourage their transition to using active learning approaches. Future faculty with less teaching experience and more traditional beliefs were more sensitive to criticism. This is typical of novices, who have lower self-esteem and prefer positive, supportive feedback that emphasizes what they are doing well rather than critical or correcting mistakes (Finkelstein and Fishbach 2012).

Mentors should recognize that the type of feedback to best support future faculty will change as their teaching beliefs become more student-centered. As future faculty's teaching beliefs shift, they will begin to seek out and appreciate more critical feedback (Finkelstein and Fishbach 2012). We found that future faculty were much more receptive to critical feedback when they shifted toward more student-centered teaching beliefs (Transitional and Responsive) through the semester. Mentors should be prepared to offer more critical feedback as future faculty develop pedagogical content knowledge and shift toward student-centered teaching beliefs.

Our study focused on future faculty's development of student-centered teaching beliefs and the impact of these beliefs on instructional feedback preferences. However, we need additional applied work to understand how these teaching beliefs translate into classroom teaching practices. Researchers should address whether future faculty will implement active learning practices in a real classroom setting once they assume faculty positions. Future work should focus on what type of mentoring instructors may need in order to retain more student-centered teaching beliefs or how much departmental culture and acceptance of active learning approaches might erode their beliefs.

References

Austin, A. E. 2002. "Preparing the professoriate of the future: Graduate student socialization for faculty role." In *Higher education: Handbook of theory and research* edited by J. C. Smart, 397–456. Secaucus, N.J.: Springer Publishers.

Austin, AE, Campa III H, Pfund C, Gillian-Daniel DL, Mathieu R, and Stoddart J. 2009. "Preparing STEM doctoral students for future faculty careers." In *New Directions for Teaching and Learning Monograph Series (Improving the Climate for Undergraduate Teaching and Learning in STEM Fields)*, 83-95.

Braun, Virginia, and Victoria Clarke. 2006. "Using thematic analysis in psychology." Review of. *Qualitative Research in Psychology* 3:77-101.

Brownell, Sara E., and Kimberly D. Tanner. 2012. "Barriers to Faculty Pedagogical Change: Lack of Training, Time, Incentives, and…Tensions with Professional Identity?" Review of. *CBE - Life Sciences Education* 11 (4):339-46.

Chi, Michelene TH, and Ruth Wylie. 2014. "The ICAP framework: Linking cognitive engagement to active learning outcomes." Review of. *Educational Psychologist* 49 (4):219-43.

Cossairt, A., R. V. Hall, and B. L. Hopkins. 1973. "Effects of Experimenters Instructions, Feedback, and Praise on Teacher Praise and Student Attending Behavior." Review of. *Journal of Applied Behavior Analysis* 6 (1):89-100. doi: Doi 10.1901/Jaba.1973.6-89.

Ebert-May, D., T. L. Derting, T. P. Henkel, J. M. Maher, J. L. Momsen, B. Arnold, and H. A. Passmore. 2015. "Breaking the Cycle: Future Faculty Begin Teaching with Learner-Centered Strategies after Professional Development." Review of. *CBE - Life Sciences Education* 14 (2):1-12.

Fang, Zhihui. 1996. "A Review of Research on Teacher Beliefs and Practices." Review of. *Educational Research* 38 (1):47-65.

Finkelstein, Stacey R., and Ayelet Fishbach. 2012. "Tell Me What I Did Wrong: Experts Seek and Respond to Negative Feedback." Review of. *Journal of Consumer Research* 39 (1):22-38. doi: 10.1086/661934.

Golde, Chris M., and Timothy M. Dore. 2001. "At Cross Purposes: What the Experiences of Today's Doctoral Students Reveal about Doctoral Education." In, 63. Madison, WI: Pew Charitable Trusts, Philadelphia, PA.

Gormally, Cara, Mara Evans, and Peggy Brickman. 2014. "Feedback about Teaching in Higher Ed: Neglected Opportunities to Promote Change." Review of. *CBE - Life Sciences Education* 13:187-99.

Hattie, John, and Helen Timperley. 2007. "The Power of Feedback." Review of. *Review of Educational Research* 77 (1):81-112.

Henderson, C., A. Beach, and N. Finkelstein. 2011. "Facilitating Change in Undergraduate STEM Instructional Practices: An Analytic Review of the Literature." Review of. *Journal of Research in Science Teaching* 48 (8):952-84.

Jussim, L., H. J. Yen, and J. R. Aiello. 1995. "Self-Consistency, Self-Enhancement, and Accuracy in Reactions to Feedback." Review of. *Journal of Experimental Social Psychology* 31 (4):322-56. doi: Doi 10.1006/Jesp.1995.1015.

Lederer, Alyssa M, Catherine M Sherwood-Laughlin, Katherine D Kearns, and Valerie D O'Loughlin. 2016. "Development and Evaluation of a Doctoral-Level Public Health Pedagogy Course for Graduate Student Instructors." Review of. *College Teaching* 64 (1):19-27.

Luft, J. A., and G. H. Roehrig. 2007. "Capturing Science Teachers' Epistemological Beliefs: The Development of the Teacher Beliefs Interview." Review of. *Electronic Journal of Science Education* 11 (2):38-63.

Luft, Julie A. 2001. "Changing Inquiry Practices and Beliefs: The Impact of an Inquiry-based Professional Development Programme on Beginning and Experienced Secondary Science Teachers." Review of. *International Journal of Science Education* 23 (5):517-34.

Luft, Julie A., Eunmi Lee, Steve Fletcher, and Gill Roehrig. 2007. "Growing or Wilting? Beginning Biology Teachers in an Induction Program for Science Teachers." Review of. *American Biology Teacher* 69 (6):341-6.

Luft, Julie, Steve Fletcher, and Brian Fortney. 2005. "Early Recruitment of Science Teachers: Promising or Problematic Strategy." Review of. *Science Educator* 14 (1):41-8.

Marbach-Ad, Gili, Kathryn L. Schaefer, Bryna C. Kumi, Lee A. Friedman, Katerina V. Thompson, and Michael P. Doyle. 2012. "Development and Evaluation of a Prep Course for Chemistry Graduate Teaching Assistants at a Research University." Review of. *Journal of Chemical Education* 89 (7):865-72. doi: 10.1021/ed200563b.

Markowitz, Dina G., and Michael J. DuPre. 2007. "Graduate Experience in Science Education: The Development of a Science Education Course for Biomedical Science Graduate Students." Review of. *CBE - Life Sciences Education* 6 (3):233-42.

Nespor, Jan. 1987. "The Role of Beliefs in the Practice of Teaching." Review of. *Journal of Curriculum Studies* 19 (4):317-28.

O'Reilly, Mark, and Adelle Renzaglia. 1994. "A Systematic Approach to Curriculum Selection and Supervision Strategies: A Preservice Practicum Supervision Model." Review of. *Teacher Education and Special Education* 17 (3):170-80.

Pfund, C., S. Miller, K. Brenner, P. Bruns, A. Chang, D. Ebert-May, A. P. Fagen, et al. 2009. "Summer Institute to Improve University Science Teaching." Review of. *Science* 324 (5926):470-1. doi: 10.1126/science.1170015.

Podsakoff, P. M., and J. L. Farh. 1989. "Effects of Feedback Sign and Credibility on Goal Setting and Task-Performance." Review of. *Organizational Behavior and Human Decision Processes* 44 (1):45-67. doi: Doi 10.1016/0749-5978(89)90034-4.

Roehrig, G. H., and J. A. Luft. 2006. "Does one size fit all? The induction experience of beginning science teachers from different teacher-preparation programs." Review of. *Journal of Research in Science Teaching* 43 (9):963-85. doi: Doi 10.1002/Tea.20103.

Saldana, Johnny. 2016. *The Coding Manual for Qualitative Researchers*. Third Edition ed: Sage Publishing.

Scheeler, Mary Catherine, Kathy L. Ruhl, and James K. McAfee. 2004. "Providing Performance Feedback to Teachers: A Review." Review of. *Teacher Education and Special Education* 27 (4):396-407.

Singer, Susan, R., Natalie Nielsen, R., and Heidi Schweingruber, A. 2012. "Discipline-Based Education Research: Understanding and Improving Learning in Undergraduate Science and Engineering." In *Board on Science Education*

Division of Behavioral, Social Sciences, and Education, edited by Contributions Committee on the Status, and Future Directions of Discipline-Based Education Research, National Research Council. Washington, D.C.: The National Academies Press.

Stes, Ann, Liesje Coertjens, and Peter Van Petegem. 2010. "Instructional Development for Teachers in Higher Education: Impact on Teaching Approach." Review of. *Higher Education: The International Journal of Higher Education and Educational Planning* 60 (2):187-204.

Sunal, Dennis W., Jeanelle Hodges, Cynthia S. Sunal, Kevin W. Whitaker, L. Michael Freeman, Leo Edwards, Ronald A. Johnston, and Michael Odell. 2001. "Teaching Science in Higher Education: Faculty Professional Development and Barriers to Change." Review of. *School Science and Mathematics* 101 (5):246-57.

Wallace, C. S., and N. H. Kang. 2004. "An investigation of experienced secondary science teachers' beliefs about inquiry: An examination of competing belief sets." Review of. *Journal of Research in Science Teaching* 41 (9):936-60. doi: 10.1002/tea.20032.

Wise, Alyssa. 2011. "Supporting Future Faculty in Developing Their Teaching Practices: An Exploration of Communication Networks among Graduate Teaching Assistants." Review of. *International Journal of Teaching and Learning in Higher Education* 23 (2):135-49.

Wyse, S. A., T. M. Long, and D. Ebert-May. 2014. "Teaching Assistant Professional Development in Biology: Designed for and Driven by Multidimensional Data." Review of. *Cbe-Life Sciences Education* 13 (2):212-23. doi: 10.1187/cbe-13-06-0106.

Zehnder, Caralyn. 2016. "Assessment of Graduate Teaching Assistants Enrolled in a Teaching Techniques Course." Review of. *Journal of College Science Teaching* 46 (1):76-83.

Table 6.1. Coding definitions with feedback examples and keywords/phrases. Rows highlighted in yellow represent Nature of the Feedback codes and rows highlighted in green are the Cognitive Quality of the Feedback codes. Directive and non-directive are mutually exclusive codes.

	Code	Definition	Examples
Nature of Feedback	Directive	Specifically tells or suggests to student something new	"Add a little more complexity to the exercise." "Maybe have a way to show more of the connections?"
	Non-directive	Questions that are considered more 'probing' than helpful. Overall commentary.	"How do you plan to arrange the lecture and discussion time?" "Perhaps this activity is too discussion driven."
	Critical	Explicitly identifies flaws in student actions or expresses doubt.	"Maybe focus a little less on the content and more on the activity itself." "The assignment is not terribly relevant."
	Supportive	Encourages or affirms actions of students	"I think your point is a valid one." "Really nice job!"
Cognitive Quality of Feedback	Towards passive	Encourages information delivery associated with passive learning	"A video on the mechanisms would be more vivid and interesting."
	Towards active	Encourages presenter to make learners more engaged with prior knowledge	"Think about questions for the students." "Make sure that students get the key points."
	Towards constructive	Encourages presenter to offer learners opportunities to create or reorganize new knowledge	"You may want to think about a way to lead the students to the conclusions." "Maybe have a way to show more of the connections?"
	Towards interactive	Encourages presenter to offer learners opportunities to interact with one another to build new knowledge or resolve discrepancies	"This would be good to start with a group activity."

Table 6.2. Future faculty preferred directive, critical feedback given by facilitators rather than peers

Feedback preference	Qualitative	Quantitative
Facilitator over peer feedback	- 67.5% of feedback came from facilitators - Facilitator feedback was preferred 87.5% of the time for the top 2 rankings	$t = 5.21$, $df = 7$, $p < 0.001$
No preference for written vs. oral	- Written feedback was preferred 45% of the time and 50% of the time in the top 2 rankings	$t = 0.99$, $df = 7$, $p = 0.353$
Preference for directive/critical and less supportive	- Directive/critical only made up 9% of given feedback, yet was 28% preferred by future faculty - Supportive feedback made up 37% and 46% of given feedback, yet was 13% of preferred feedback	$X^2 = 9.76$, $df = 1$, $p = 0.002$; $X^2 = 14.52$, $df = 1$, $p < 0.0001$

Table 6.3. Future Faculty Impressions of Instructional Feedback. Themes are listed in bold with direct quotes from future faculty explain their reasoning for selecting the most useful feedback. Quotes highlighted in red represent themes that were only identified in that category. All other themes were identified in other categories, but at lower rates.

Traditional (n = 1)	Instructive (n = 3)	Transitional (n = 4) Responsive
Directive: "Our topic was too broad and unclear/confusing, so we reduced the scope of our topic and gave some specific examples and hypotheses based on the feedback we receive." **Sensitive to criticism:** "I felt the feedback was too critical and it upset me."	**Appreciate criticism:** "...legitimate comments (although heartbreaking sometimes)... need to know how people think differently." **Supportive:** "I preferred feedback that rewarded the point that I worked hard on." **Feasibility:** "We felt that if it didn't coincide with what we were trying to accomplish (and it was too difficult/ distracting to add) then we generally just disregarded it." **Defensive:** "Some comments I didn't agree with."	**Lack of pedagogical knowledge:** "I want to do something to improve it, but I don't know how to do it." **Critical:** "I embraced the deep-cutting feedback." **Student-centered:** "Asking 'what will the student get out of this?'" **Reinforcing belief:** "I discussed with my partner what changes we both agreed on and we incorporated it if a lot of people told us the same feedback."

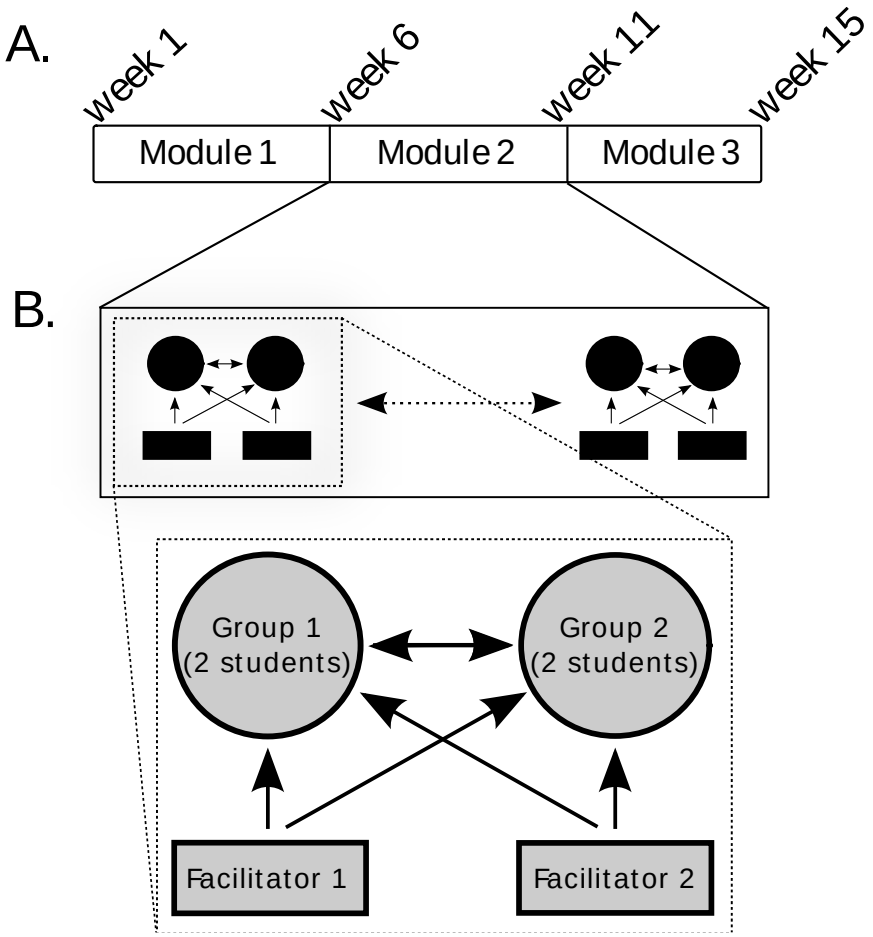

Figure 6.1. Course Design. During the second module of the course, future faculty collaborated in pairs to develop their own active learning exercises. Feedback sessions were designed to provide two separate groups with time to present ideas and receive feedback (arrows represent feedback direction) from another group (peers in circles) and two facilitators (squares) for 30 minutes (dotted box).

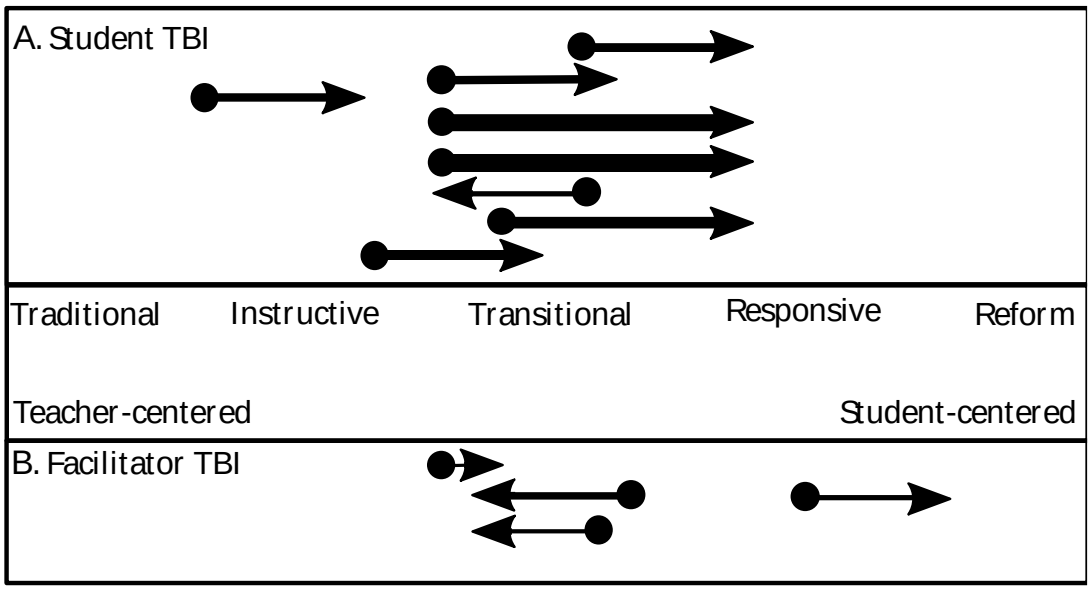

Figure 6.2. Teaching beliefs interview (TBI) scores. TBI scores for (A) future faculty and (B) facilitators at the beginning and end of the semester. Pre-class survey scores are represented by circles and post-class scores are represented by the arrows. Line thickness is relative to the amount of change over the course.

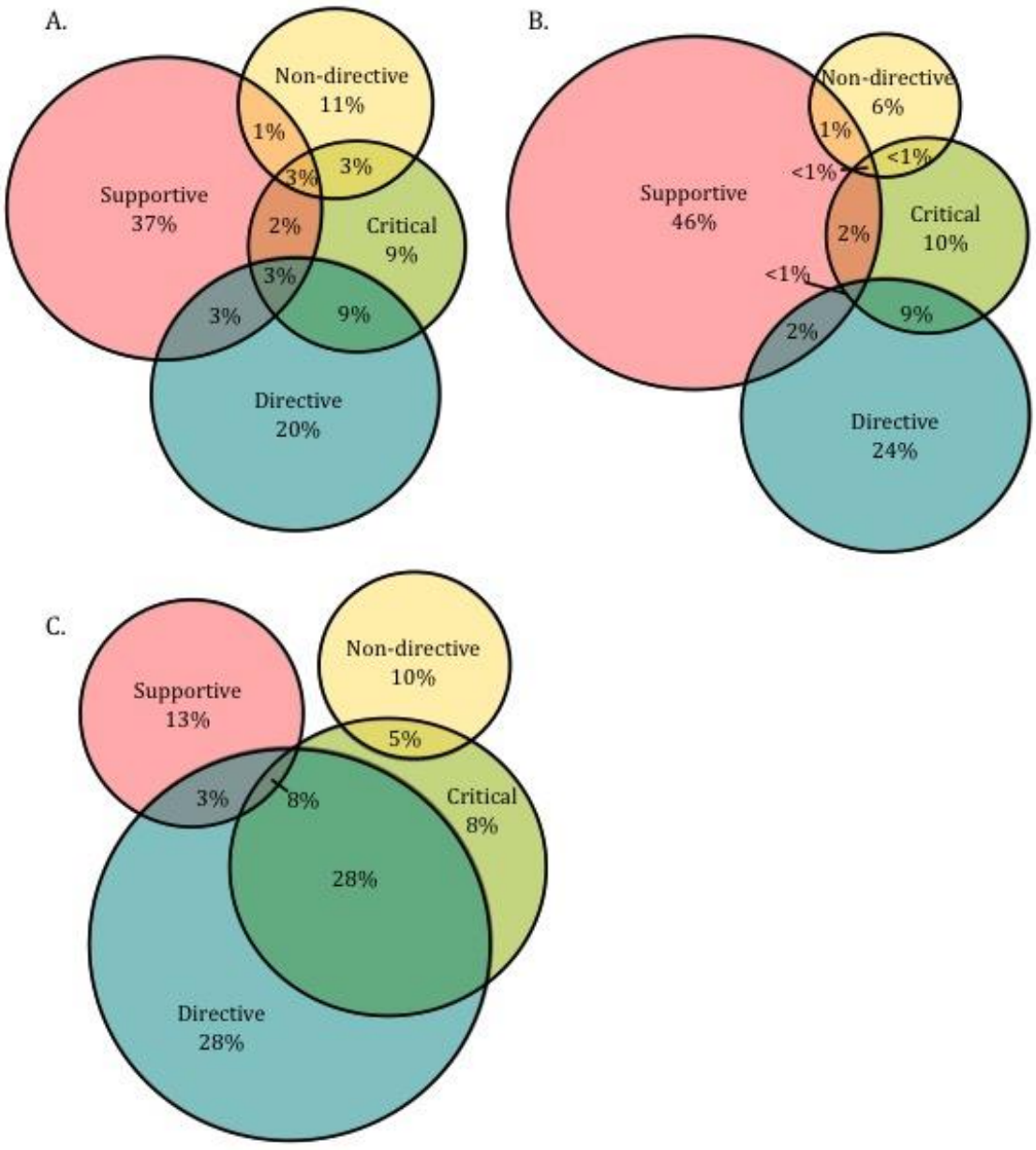

Figure 6.3. Types of feedback given to future faculty. Feedback given by (A) facilitators and (B) peers. (C) shows is the feedback types preferred by students. The four main categories are Supportive (encouraging, affirming of actions), Critical (identifying flaws or doubts), Directive (suggesting specific improvements), and Non-directive (posing questions or commentary without solutions). Percentages in overlapping areas represent feedback coded for multiple categories.

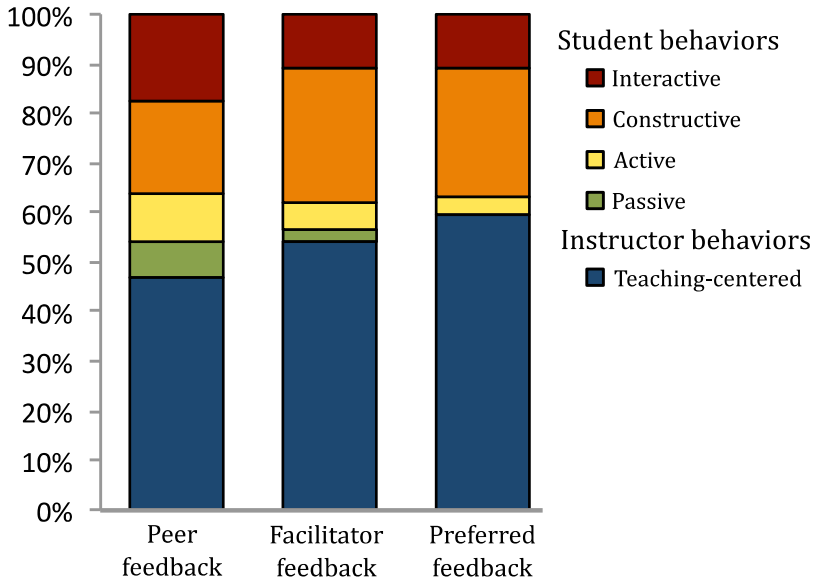

Figure 6.4. Analysis of the feedback along a continuum of practices from passive to interactive (ICAP). Comments were coded based on whether the feedback encouraged a change in the teaching activity.

CHAPTER VII:

CONCLUSION

My dissertation research interest has largely focused on understanding the ecological and evolutionary outcomes of plant interactions. Specifically, I have been interested in the interactions among plants, insects, and microbes using phylogenetic techniques.

Species tree estimation

Resolving species relationships among *Helianthus* and *Sarracenia* has proven to be difficult using few genes in the past. These previous attempts resulted in often, conflicting species relationships with numerous polytomies (e.g., *Helianthus:* Rieseberg et al. 1991, Schilling et al. 1998, Timme et al. 2007a; *Sarracenia*: Bayer et al. 1996, Ellison et al. 2012, Neyland and Merchant 2006), highlighting the difficulties inherent to species level relationships, especially in recently radiated groups with often, widespread hybridization (Timme et al. 2007b, Furches et al. 2013). To mitigate these issues, I undertook a multilocus approach (i.e., target enrichment), which is predicted to increase nodal support values in phylogenetic analyses by estimating more accurate model parameters (Maddison 1997, Song et al. 2012). This resulted in 170 genes for *Helianthus* and 199 genes for *Sarracenia* used for downstream analyses. Furthermore, I used the multispecies coalescence, which more accurately models sources of gene tree discordance within the species tree (Degnan and Rosenberg 2009, Knowles 2009, Liu et al. 2009). Together these approaches resulted in the most robust species level relationships for these two groups to date, emphasizing the utility of these methods for discerning species level relationships.

For both species level analyses there were few conflicts between the MP-EST accession tree and the concatenation tree. This was a surprising result simulations have shown that concatenation methods are predicted to produce inaccurate species trees when compared to the multispecies coalescent (Degnan and Rosenberg 2009, Edwards 2009, Liu et al. 2008, Liu et al. 2009, Liu et al. 2010). The reason for the mostly congruent approaches is not clear, but may have been the result of the robust taxon sampling in both genra (and possibly the use of multiple accessions), as concatenation is susceptible to missing taxa (Song et al. 2012). Additionally, I used a conservative approach to filtering genes that may have contributed to this congruence. Anomalous gene trees (i.e., a highly probable gene topology that conflicts with the species tree) that are the most commonly observed gene tree are additionally known to influence concatenation analyses (Degnan and Rosenberg 2006, Liu and Edwards 2009). Upon examination of gene trees in both studies we found no one dominant topology, possibly decreasing the likelihood of anomalous gene trees in downstream analyses.

While the multilocus strategy used in these studies is meant to target specific genomic regions, it is not uncommon for these approaches often yield off-target bonus sequences (i.e., high copy DNA from chloroplasts, mitochondria, and ribosomes; Weitmeier et al. 2014, Meiklejohn et al. 2014). Here, I was able to assemble a portion of the chloroplast genome for phylogenetic analyses. In both cases, the plastid tree was poorly supported with little resolution across trees. This most likely results from lack of informative sites as the chloroplast genome is more slowly evolving than the nuclear genome (Wolfe et al. 1987). There was support for some monophyletic clades in both *Helianthus* and *Sarracenia* that were in conflict with the nuclear phylogeny. This conflict is most likely a result of introgression of the maternally-inherited plastome in sympatric or commonly hybridizing species.

In these two studies, I used a multispecies coalescent approach to properly model gene tree discordance, however, it should be noted that the species tree analysis that was used only dealt with gene tree discordance as a result of incomplete lineage sorting. Given the rampant hybridization within both *Helianthus* (Timme et al. 2007b) and *Sarracenia* (Mellichamp 2009, Furches et al. 2013), further methods accounting for this hybridization may better inform the phylogenetic history of these two groups. These methods are currently being developed and are emerging as potential applications for future phylogenetic analyses (e.g., STEM-hy – Kubatko 2009; PhyloNet – Yu et al. 2011, Yu and Nakhleh 2015). In conclusion, target enrichment is highly applicable for non-model species level phylogenies as it is cost-efficient, and allows for a dramatic increase in genes available for phylogenetic analyses (Faircloth et al. 2012, Lemmon and Lemmon 2013). Furthermore, this approach combined with improved models of gene tree discordance has the potential to revolutionize current approaches to species tree resolution, which in turn lays the foundation for examining questions pertaining to evolution and speciation at this finer taxonomic scale.

Species interactions

Species do not live in a vacuum; they are subjected to multiple interactions that can range from daily interactions to complete symbiosis. These interactions can influence host development, community assembly, and evolutionary processes. Interactions can be especially important for driving host evolution for more sessile organisms, like plants. Plants have evolved mechanisms to attract pollinators and seed dispersers (Fenster et al. 2004, Sargent 2004), deter herbivores (Agrawal and Fishbein 2006), and facilitate nutrient acquisition (Turner et al. 2013). Carnivorous plants, while interacting with pollinators and herbivores, have a diverse array of interactions including as prey, parasites, capture interrupters, obligate insect associates, and

microbes involved in digestion of prey (Folkerts 1999). Our current understanding of how species interactions have influenced carnivorous plant diversification is not well known, but given the dependence on prey for nutrients (Ne'eman et al. 2006, Karagatzides et al. 2009) there should be strong selection on traits to attract, capture, retain, and digest prey items (Ellison and Gotelli 2001, Ellison and Gotelli 2009).

In this dissertation, I first examined the role that prey capture may have had in trap morphology across the genus *Sarracenia* in an outdoor common garden. To this end, I found the trapping traits are highly correlated into suites of traits and that these carnivorous syndromes were associated with the type of prey captured. Height was positively correlated with peristome width and negatively correlated with trichomes at the base of the pitchers. *Sarracenia* species that were taller with less trichomes tended to capture more flying prey types, while shorter species captured crawling prey. These patterns suggest that plant-prey interactions and competition for prey may have resulted in the large phenotypic diversity of trap morphology. Future experimental work can help elucidate the functionality of the traits in this study, while future work examining ecological character displacement can test the role that interspecific competition has on trapping traits and prey capture. Furthermore, more comparative studies of traits and prey can inform of convergent evolutionary patterns across carnivorous plants.

The traits measured in the dissertation are just a small portion of possible characteristics that could be influencing prey attraction and capture. Nectar, volatiles, and colors have all been suggested to influence prey attraction and capture (Joel 1986, Juniper et al. 1989). Nectar and pitcher color have been explored within species with conflicting results (Zamora 1995, Newell and Nastase 1998, Green and Horner 2007, Stephens et al. 2015). Plant volatile emissions have not been well characterized, but recent work suggests diversity in *Sarracenia* species volatile

profiles (Jürgens et al. 2009). Future comparative work examining volatile emissions across species can further our understanding of carnivorous syndromes and prey specialization. These plants are also predicted to use a combination of chemical, spatial, and temporal cues to avoid capturing their pollinators (Juniper et al. 1989, Jürgens et al. 2012). This pollinator-prey conflict is an additional interaction scenario predicted to drive selection in these plants.

The process of decomposition after prey capture should additionally be under strong selection, as these plants can gain up to 80% of their nitrogen from this process (Karagatzides et al. 2009). This dependence on nutrient absorption should not only influence plant traits that may facilitate digestion, but also microbe communities that aid in the digestion of these items. The results from this dissertation suggest that these communities are not random and that the plant host species accounts for the majority of the variation found in these microbiome communities. The exact mechanisms for this host specific community are unknown, but may be related to enzymes secreted by the plant to aid in digestion of prey (Takeuchi et al. 2011, Hatano and Hamada 2012, Buch et al. 2013). It may also be a product of host control over the environmental characteristics within pitchers (e.g., pH, water content). Future work examining the enzymes released by *Sarracenia* species can elucidate their role in this community assembly. Additionally, the function of microbes in releasing essential nutrients for the host is lacking. Experimental manipulations of the microbiome can additionally elucidate host-microbe interactions.

In conclusion, this dissertation sought to further our understanding of species interactions and their potential roles in carnivorous plant diversification. Results from this work point to multitrophic interactions that can influence functional host traits and community assembly in microbiome communities.

References

Agrawal, A.A., Fishbein, M., 2006. Plant defense syndromes. *Ecology* 87: S132–S149.

Bayer, R.J., Hufford, L., Soltis, D.E., 1996. Phylogenetic relationships in Sarraceniaceae based on rbcL and ITS sequences. *Systematic Botany* 21: 121–134.

Buch, F., Rott, M., Rottloff, S., Paetz, C., Hilke, I., Raessler, M., *et al.*, 2012. Secreted pitfall-trap fluid of carnivorous *Nepenthes* plants is unsuitable for microbial growth. *Annals of Botany* doi:10.1093/aob/mcs287

Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. *PLoS Genetics* doi:10.1371/journal.pgen.0020068.

Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and multispecies coalescent. *Trends in Ecology and Evolution* 24: 332–340.

Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? *Evolution* doi:10.1111/j.1558-5646.2008.00549.x

Ellison, A.M., Gotelli, N.J., 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology & Evolution* 16: 623–629.

Ellison, A.M., Gotelli, N.J., 2009. Energetics and the evolution of carnivorous plants–Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* 60: 19–42.

Ellison, A.M., Butler, E.D., Hicks, E.J., Naczi, R.F.C., Calie, P.J., Bell, C.D., Davis, C.C., 2012. Phylogeny and biogeography of the carnivorous plant family Sarraceniaceae. *PLoS ONE* doi:10.1371/journal.pone.0039291.

Faircloth, B.C., Glenn, T.C., McCormack, J.E., Harvey, M.G., Sorsenson, L., Alfaro, M., White, N., 2012. Target enrichment of Illumina libraries. Available at http://ultraconserved.org. Accessed 3 October 2014.

Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., Thomson, J.D., 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375–403.

Folkerts, D.R., 1999. Pitcher plant wetlands of the southeastern United States: Arthropod associates. Pp. 247–275, *In* Batzer DP, Rader RB, Wissinger SA (eds.). *Invertebrates in Freshwater Wetlands of North America*: *Ecology and Management.* John Wiley and Sons, New York, NY.

Furches, M.S., Small, R.L., Furches, A., 2013. Hybridization leads to interspecific gene flow in *Sarracenia* (Sarraceniaceae). *American Journal of Botany* 100: 2085–2091.

Green, M.L., Horner, J.D., 2007. The relationship between prey capture and characteristics of the carnivorous pitcher plant, *Sarracenia alata* Wood. *American Midland Naturalist* 158: 424–431.

Hatano, N., Hamada, T., 2012. Proteomic analysis of secreted protein induced by a component of prey in pitcher fluid of the carnivorous plant *Nepenthes alata. Journal of Proteomics* 75: 4844–4852.

Joel, D.M., 1986. Glandular structure in carnivorous plants: Their role in mutual exploitation of insects. pp. 219–234. *In:* Juniper BE, Southwood TRE (eds.). *Insects and Plant Surfaces*. Edward Arnold, London, UK.

Juniper, B.E., Robins, R.J., Joel, D.M., 1989. *The Carnivorous Plants*. Academic Press, New York, NY. 353 pp.

Jürgens, A., El-Sayad, A.M., Suckling, D.M., 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology* 23: 875–887.

Jürgens, A., Sciligo, A., Witt, T., El-Sayad, A.M., Suckling, D.M., 2012. Pollinator-prey conflict in carnivorous plants. *Biological Reviews* 87: 602–615.

Kanokratana, P., Mhuanthong, W., Laothanachareon, T., Tangphatsornruang, S., Eurwilaichitr, L., Kruetreepradit, T., Mayes, S., Champreda, V., 2016. Comparative study of bacterial communities in *Nepenthes* pitchers and their correlation to species and fluid acidity. *Environmental Microbiology* 72: 381–393.

Karagatzides, J.D., Butler, J.L., Ellison, A.M., 2009. The pitcher plant *Sarracenia purpurea* can directly acquire organic nitrogen and short-circuit the inorganic nitrogen cycle. *PLoS ONE* 4: 1–9.

Knowles, L.L., 2009. Estimating species trees: Methods of phylogenetic analysis when there is incongruence across genes. *Systematic Biology* 58: 463–467.

Kubatko, L.S., 2009. Identifying hybridization events in the presence of coalescence via model selection. *Systematic Biology* 58: 478–488.

Lemmon, E.M., Lemmon, A.R., 2013. High-throughput genomic data in systematics and phylogenetics. *Annual Review of Evolutionary Systematics* 44: 99–121.

Liu, L., Pearl, D.K., Brumfield, R.T., Edwards, S.V., 2008. Estimating species trees using multiple-allele DNA sequence data. *Evolution* 62: 2080–2091.

Liu, L., Edwards, S.V., 2009. Phylogenetic analysis in the anomaly zone. *Systematic Biology* doi:10.1093/sysbio/syp034.

Liu, L., Yu, L., Pearl, D.K., Edwards, S.V., 2009. Estimating species phylogenies using coalescence times among sequences. *Systematic Biology* 58: 468–477.

Liu, L., Yu, L., Edwards, S.V., 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. *BMC Evolutionary Biology* 10: 302.

Maddison, W.P., 1997. Gene trees in species trees. *Systematic Biology* 46: 523–536.

Meiklejohn, K.A., Danielson, M.J., Faircloth, B.C., Glenn, T.C., Braun, E.L., Kimball, R.T., 2014. Incongruence among different mitochondrial regions: A case study using complete mitogenomes. *Molecular Phylogenetics and Evolution* 78: 314-323.

Mellichamp, T.L., Case, F.W., 2009. Sarraceniaceae. *Flora of North America* 8: 348–363.

Ne'eman, G., Ne'eman, R., Ellison, A.M., 2006. Limits to reproductive success of *Sarracenia purpurea* (Sarraceniaceae). *American Journal of Botany* 93: 1660–1666.

Newell, S.J., Nastase, A.J., 1998. Efficiency of insect capture by *Sarracenia purpurea* (Sarraceniaceae), the northern pitcher plant. *American Journal of Botany* 85: 88–91.

Neyland, R., Merchant, M., 2006. Systematic relationships of Sarraceniaceae inferred from nuclear ribosomal DNA sequences. *Madroño* 53: 223–232.

Rieseberg, L. H., 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

Sargent, R.D., 2004. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society of London B* 271:603–608.

Schilling, E. E., Linder, C. R., Noyes R. D., Rieseberg, L. H., 1998. Phylogenetic relationships in *Helianthus* (Asteraceae) based on nuclear ribosomal DNA internal transcribed spacer region sequence data. *Systematic Botany* 23: 177–187.

Song, S., Liu, L., Edwards, S.V., Wu, S., 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. *Proceedings of the National Academy of Science* doi:10.1073/pnas.1211733109.

Stephens, J.D., Godwin, R.L., Folkerts, D.R., 2015. Distinctions in pitcher morphology and prey capture of the Okefenokee variety within the carnivorous plant species *Sarracenia minor*. *Southeastern Naturalist* 14: 254–266.

Takeuchi, Y., Salcher, M.M., Ushio, M., Shimizu-Inatsugi, R., Kobayashi, M.J., Diway, B., von Mering, C., Pernthaler, J., Shimizu, K.K., 2011. *In situ* enzyme activity in the dissolved and particulate fraction of the fluid from four pitcher plant species in the genus *Nepenthes* *PLoS ONE* 6: e25144.

Timme, R. E., Simpson, B. B., Linder, C. R., 2007a. High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18S-26S ribosomal DNA external transcribed spacer. *American Journal of Botany* 94: 1837–1852.

Timme, R. E., Kuehl, J. V., Boore, J. L., Jansen, R. K., 2007b. A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: identification of divergent regions and categorization of shared repeats. *American Journal of Botany* 94: 302–312.

Turner, T.R., James, E.K., Poole, P.S., 2013. The plant microbiome. *Genome Biology* 14: 209.

Weitmeier K., Straub S.C.K., Cronn R.C., Fishbein M., Schmickl R., McDonnell A., Liston A., 2014. Hyb-Seq: Combining target enrichment and genome skimming for plant phylogenomics. *Applications in Plant Sciences* doi:10.3732/apps.1400042.

Wolfe, K.H., Li, W.H., Sharp, P.M., 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Science* 84: 9054–9058.

Yu Y., Cuong T., Degnan J.H., Nakhleh L., 2011. Coalescent histories on phylogenetic networks and detection of hybridization despite incomplete lineage sorting. *Systematic Biology* 60: 138–149.

Yu Y., Nakhleh L., 2015. A distance-based method for inferring phylogenetic networks in the presence of incomplete lineage sorting. *Bioinformatics Research and Applications* 9096: 378–389.

Zamora, R., 1995. The trapping success of a carnivorous plant, *Pinguicula vallisneriifolia*: The cumulative effects of availability, attraction, retention, and robbery of prey. *Oikos* 73: 309–322.

APPENDIX A

SUPPLEMENTARY FIGURES AND TABLES FROM CHAPTER II

Table S2.1. Data summary and GRIN accession numbers for all accessions. Bold species names indicate accessions used for phylogenetic analyses. Short reads for all sequenced individuals were deposited in the NCBI Sequence Read Archive (BioProject PRJNA277479). Accessions of *H. petiolaris* ssp. *fallax* and *H. pumilus* were not included in the averages as they had few contigs that matched the targets. Vouchers for each GRIN accession were deposited in the UGA herbarium.

						Assemblies			Contigs aligned to targets					
Species	**GRIN Accession**	**Location**	**Latitude**	**Longitude**	**Number of trimmed reads[a]**	**Trinity contigs**	**Velvet contigs**	**Merged contigs**	**Total count**	**N50**	**Ave. coverage**	**Reads in contigs**	**Contigs "on-target"[b]**	**Reads "on-target"[c]**
H. agrestis	PI 673202	(GLA-1) FL	26.9510	-81.1349	1 762 860	7138	59 731	4565	580	367	6.5	14 310	13%	0.81%
H. agrestis	PI 673202	(GLA-5) FL	26.9510	-81.1349	1 776 320	7710	64 835	5075	596	537	6.2	18 555	12%	1.04%
H. agrestis	PI 673202	(GLA-8) FL	26.9510	-81.1349	1 366 095	6170	51 743	4011	473	365	6.3	11 315	12%	0.83%
H. agrestis	PI 673201	(HEN) FL	26.4231	-81.2484	999 372	5097	37 896	3340	511	397	6.5	8918	15%	0.89%
H. agrestis	PI 673205	(SCW) FL	28.7870	-81.1852	1 665 771	6381	49 637	4104	511	556	5.1	11 524	12%	0.69%
H. angustifolius	PI 673210	(CRP-1) FL	28.9543	-82.6489	1 363 778	6928	51 372	4605	444	440	8.0	14 686	10%	1.08%
H. angustifolius	PI 673210	(CRP-2) FL	28.9543	-82.6489	1 062 433	5759	43 227	3862	399	430	8.3	13 153	10%	1.24%
H. angustifolius	PI 673210	(CRP-3) FL	28.9543	-82.6489	2 790 267	10 356	96 045	6657	505	619	9.1	26 732	8%	0.96%
H. angustifolius	PI 649937	(MAN-4) GA	33.7595	-84.8555	1 152 938	6487	50 777	4416	403	439	6.6	10 657	9%	0.92%
H. angustifolius	PI 649937	(MAN-8) GA	33.7595	-84.8555	3 375 424	13 036	113 473	8532	499	671	9.7	29 538	6%	0.88%
H. annuus	PI 649859	(FIR-5) CA	36.8903	-120.5028	1 941 119	10 721	102 342	7879	408	1038	12.4	37 172	5%	1.91%
H. annuus	PI 649859	(FIR-6) CA	36.8903	-120.5028	2 089 702	11 512	111 123	8461	519	605	13.5	38 148	6%	1.83%
H. annuus	PI 649859	(FIR-7) CA	36.8903	-120.5028	1 586 273	9393	86 413	6961	444	995	11.0	36 705	6%	2.31%
H. annuus	PI 673304	(KON-1) KS	39.1021	-96.6103	1 165 460	7042	53 978	5161	516	857	7.5	24 781	10%	2.13%
H. annuus	PI 673304	(KON-2) KS	39.1021	-96.6103	2 060 229	9883	82 151	6900	500	719	11.8	36 410	7%	1.77%
H. annuus	PI 673304	(KON-4) KS	39.1021	-96.6103	1 967 944	9725	71 899	6786	423	1066	10.4	33 043	6%	1.68%
H. annuus	PI 673304	(KON-5) KS	39.1021	-96.6103	921 967	6063	43 612	4529	474	595	6.8	16 519	10%	1.79%
H. annuus	PI 673305	(UTA-1) UT	39.7160	-112.2070	2 105 891	9981	94 265	7301	508	621	10.5	28 304	7%	1.34%
H. annuus	PI 673305	(UTA-2) UT	39.7160	-112.2070	1 366 020	7613	67 817	5587	535	702	7.5	23 694	10%	1.73%

H. annuus	PI 673305	(UTA-5) UT	39.7160	-112.2070	2 298 111	10 329	86 868	7313	488	670	10.7	26 863	7%	1.17%
H. argophyllus	PI 673306	(FLB) TX	27.6594	-97.3132	927 355	5618	42 672	4224	556	625	8.0	24 545	13%	2.65%
H. argophyllus	PI 673306	(MUS-4) TX	27.8351	-97.0525	1 463 281	7334	54 173	5235	536	732	8.9	29 558	10%	2.02%
H. argophyllus	PI 673306	(MUS-6) TX	27.8351	-97.0525	993 263	6110	42 270	4429	510	757	7.0	19 799	12%	1.99%
H. arizonensis	PI 653549	(1) AZ	34.6097	-109.3206	1 804 440	8710	86 415	6205	492	714	9.4	31 012	8%	1.72%
H. arizonensis	PI 653549	(3) AZ	34.6097	-109.3206	2 251 034	10 056	102 689	7135	435	1820	10.4	33 704	6%	1.50%
H. arizonensis	PI 653549	(4) AZ	34.6097	-109.3206	2 357 284	10 468	103 765	7295	446	1130	10.5	37 096	6%	1.57%
H. atrorubens	PI 664731	(FMF-1) SC	33.1944	-79.5256	2 513 076	11 372	91 450	7487	542	568	8.1	22 062	7%	0.88%
H. atrorubens	PI 664731	(FMF-8) SC	33.1944	-79.5256	2 246 500	10 644	82 114	7002	469	957	7.8	24 637	7%	1.10%
H. atrorubens	PI 664694	(TOC-2) GA	34.6603	-83.3481	965 589	6205	40 274	4222	466	502	5.1	10 701	11%	1.11%
H. atrorubens	PI 664694	(TOC-4) GA	34.6603	-83.3481	1 485 827	8188	59 887	5421	583	550	6.0	18 099	11%	1.22%
H. atrorubens	PI 664694	(TOC-6) GA	34.6603	-83.3481	1 763 877	9416	72 480	6310	503	715	6.1	17 560	8%	1.00%
H. atrorubens	PI 649940	(WAR-1) AL	33.8906	-86.8258	2 286 194	10 811	82 382	7165	572	526	7.8	20 817	8%	0.91%
H. atrorubens	PI 649940	(WAR-2) AL	33.8906	-86.8258	2 059 462	9864	69 165	6422	498	878	7.0	22 333	8%	1.08%
H. atrorubens	PI 649940	(WAR-7) AL	33.8906	-86.8258	1 216 473	7323	50 880	5087	516	656	5.3	15 397	10%	1.27%
H. carnosus	PI 673310	(POT-1) FL	29.6097	-81.4716	7 308 797	20 710	256 006	16 877	355	829	24.1	59 809	2%	0.82%
H. carnosus	PI 673310	(POT-4) FL	29.6097	-81.4716	7 747 889	21 789	270 298	17 752	301	952	26.5	56 503	2%	0.73%
H. carnosus	PI 673310	(POT-6) FL	29.6097	-81.4716	3 980 154	11 776	150 072	9363	358	910	17.5	44 652	4%	1.12%
H. carnosus	PI 673310	(POT-7) FL	29.6097	-81.4716	4 542 200	14 180	158 068	9931	378	1103	21.0	56 382	4%	1.24%
H. carnosus	Ames 32168	(SOF-1) FL	29.3200	-81.3102	1 427 397	7890	62 966	5325	481	449	7.2	14 695	9%	1.03%
H. carnosus	Ames 32168	(SOF-2) FL	29.3200	-81.3102	1 616 868	8673	66 785	5903	569	515	6.6	17 781	10%	1.10%
H. carnosus	Ames 32168	(SOF-4) FL	29.3200	-81.3102	907 046	5716	41 478	3958	457	540	5.0	10 285	12%	1.13%
H. cusickii	PI 649966	(LIT-2) CA	40.4184	-120.2833	1 038 673	6722	50 201	4919	535	800	5.4	18 081	11%	1.74%
H. cusickii	PI 649966	(LIT-3) CA	40.4184	-120.2833	1 412 795	8583	78 697	6362	565	633	8.0	25 923	9%	1.83%
H. cusickii	PI 649966	(LIT-5) CA	40.4184	-120.2833	1 163 758	7020	56 866	5063	548	807	6.1	20 737	11%	1.78%
H. cusickii	PI 649959	(NCO-4) OR	44.6206	-120.2064	1 460 051	7958	61 482	5648	534	648	7.1	21 419	9%	1.47%
H. cusickii	PI 649959	(NCO-5) OR	44.6206	-120.2064	920 092	6205	44 140	4621	530	1108	6.2	22 127	11%	2.40%
H. cusickii	PI 649959	(NCO-7) OR	44.6206	-120.2064	1 934 257	8683	78 940	6178	547	645	8.0	25 039	9%	1.29%
H. cusickii	PI 649967	(RAV-3) CA	40.6760	-120.2853	1 527 649	8375	72 554	6135	517	927	7.2	26 196	8%	1.71%

H. cusickii	PI 649967	(RAV-4) CA	40.6760	-120.2853	1 526 994	8499	75 350	6145	546	729	6.0	19 756	9%	1.29%
H. cusickii	PI 649967	(RAV-5) CA	40.6760	-120.2853	1 680 706	9153	79 353	6637	581	661	7.7	27 077	9%	1.61%
H. cusickii	PI 649967	(RAV-7) CA	40.6760	-120.2853	1 521 751	8471	73 578	6109	525	871	6.6	22 885	9%	1.50%
H. debilis ssp. debilis	PI 435669	(1) FL	Hobe Sound Public Beach, Hobe Sound		1 271 223	6688	47 633	4761	496	894	6.7	22 669	10%	1.78%
H. debilis ssp. debilis	PI 435669	(2) FL	Hobe Sound Public Beach, Hobe Sound		1 548 458	8001	63 513	5649	564	571	8.3	23 008	10%	1.49%
H. debilis ssp. debilis	PI 435669	(4) FL	Hobe Sound Public Beach, Hobe Sound		2 984 574	3204	42 490	2809	399	308	134.8	163 661	14%	5.48%
H. debilis ssp. silvestris	PI 435651	(1) TX	8-10 km northwest of Henderson		2 824 571	12 657	111 766	8823	434	1185	11.3	37 085	5%	1.31%
H. debilis ssp. silvestris	PI 435651	(2) TX	8-10 km northwest of Henderson		1 250 412	7301	59 778	5291	532	846	6.6	21 606	10%	1.73%
H. debilis ssp. silvestris	PI 435651	(3) TX	8-10 km northwest of Henderson		2 986 190	14 196	132 956	10 353	443	687	13.4	37 112	4%	1.24%
H. debilis ssp. tardiflorus	PI 673213	(CDK-1) FL	29.1832	-83.0171	2 230 966	11 315	107 115	8375	412	1494	12.5	44 168	5%	1.98%
H. debilis ssp. tardiflorus	PI 673213	(CDK-3) FL	29.1832	-83.0171	1 799 959	9548	88 270	7076	447	960	10.4	36 605	6%	2.03%
H. debilis ssp. tardiflorus	PI 673213	(CDK-4) FL	29.1832	-83.0171	2 489 227	11 757	116 532	8724	442	859	13.5	44 746	5%	1.80%
H. debilis ssp. tardiflorus	Ames 32170	(PAN-4) FL	30.0161	-84.3682	2 094 275	9272	73 773	6444	510	665	9.2	29 760	8%	1.42%
H. debilis ssp. tardiflorus	Ames 32170	(PAN-7) FL	30.0161	-84.3682	1 999 946	10 319	94 668	7818	513	1145	7.3	24 727	7%	1.24%
H. debilis ssp. tardiflorus	PI 673310	(PSJ-1) FL	29.8060	-85.3021	1 380 123	8326	71 223	6117	520	679	6.6	18 877	9%	1.37%
H. debilis ssp. tardiflorus	PI 673310	(PSJ-2) FL	29.8060	-85.3021	1 793 296	9845	83 324	7091	494	1135	7.9	26 692	7%	1.49%
H. debilis ssp. tardiflorus	PI 673310	(PSJ-4) FL	29.8060	-85.3021	1 891 766	10 219	92 681	7374	552	670	8.3	26 899	7%	1.42%
H. debilis ssp. tardiflorus	PI 673310	(PSJ-8) FL	29.8060	-85.3021	1 018 164	6671	50 563	4881	534	704	5.7	18 367	11%	1.80%
H. divaricatus	Ames 28236	(LCN) OH	41.5911	-83.7651	3 709 848	14 451	131 459	9925	476	804	11.0	36 030	5%	0.97%
H. divaricatus	Ames 32172	(PEM-6) IL	41.0899	-87.5640	1 332 410	6948	55 205	4697	509	391	6.6	13 195	11%	0.99%
H. divaricatus	Ames 32172	(PEM-8) IL	41.0899	-87.5640	1 259 637	7192	54 112	4915	518	700	5.2	14 384	11%	1.14%
H. exilis	PI 649895	(1) CA	41.6431	-122.7472	5 639 715	15 061	185 581	12 735	334	928	26.8	72 485	3%	1.29%
H. exilis	PI 649895	(2) CA	41.6431	-122.7472	3 988 965	12 477	143 166	9276	391	814	20.0	54 388	4%	1.36%
H. exilis	PI 649895	(4) CA	41.6431	-122.7472	4 241 618	12 889	152 168	10 938	393	805	19.5	53 835	4%	1.27%
H. floridanus	PI 673197	(APL-1) FL	29.7147	-85.0252	2 570 123	11 572	112 495	7632	482	855	8.5	29 277	6%	1.14%
H. floridanus	PI 673197	(APL-2) FL	29.7147	-85.0252	2 759 133	12 189	122 351	8263	499	656	9.1	27 206	6%	0.99%
H. floridanus	PI 673197	(APL-3) FL	29.7147	-85.0252	2 329 779	10 865	105 096	7229	489	752	8.5	26 132	7%	1.12%

H. floridanus	Ames 32740	(OCK-3) FL	29.0652	-81.9508	1 272 997	7506	55 139	5055	424	481	6.4	11 487	8%	0.90%
H. floridanus	Ames 32740	(OCK-4) FL	29.0652	-81.9508	1 418 155	7514	54 671	4963	551	518	7.3	20 315	11%	1.43%
H. floridanus	Ames 32740	(OCK-6) FL	29.0652	-81.9508	3 189 801	11 153	107 984	7417	400	1262	10.7	34 362	5%	1.08%
H. floridanus	PI 673204	(VOL-1) FL	28.6750	-80.9761	1 537 118	8728	72 088	5952	514	802	6.4	20 499	9%	1.33%
H. floridanus	PI 673204	(VOL-5) FL	28.6750	-80.9761	1 682 214	9538	77 845	6491	533	711	6.1	19 398	8%	1.15%
H. floridanus	PI 673204	(VOL-6) FL	28.6750	-80.9761	1 507 871	8694	70 499	5960	515	793	6.0	17 157	9%	1.14%
H. floridanus	PI 673204	(VOL-7) FL	28.6750	-80.9761	2 390 048	11 282	100 179	7582	528	665	8.5	26 035	7%	1.09%
H. giganteus	PI 664710	(BUR-1) NC	35.8117	-82.1972	2 007 906	10 301	87 471	7192	486	995	8.1	27 649	7%	1.38%
H. giganteus	PI 664711	(BUR-4) NC	35.8117	-82.1972	2 334 150	11 586	102 333	8152	473	1131	9.0	28 668	6%	1.23%
H. giganteus	PI 664712	(BUR-6) NC	35.8117	-82.1972	2 365 020	11 881	114 945	8537	522	644	9.2	27 906	6%	1.18%
H. giganteus	PI 664647	(LCN-5) OH	41.5911	-83.7651	2 704 719	10 460	91 668	7118	411	836	11.6	32 717	6%	1.21%
H. giganteus	PI 664648	(LCN-8) OH	41.5911	-83.7651	3 044 889	10 774	105 921	7482	348	870	15.1	39 579	5%	1.30%
H. giganteus	Ames 32741	(SPA-1) NC	36.5491	-81.2166	1 304 424	8436	67 181	6058	517	1369	6.4	19 414	9%	1.49%
H. giganteus	Ames 32741	(SPA-2) NC	36.5491	-81.2166	1 519 750	9200	77 950	6537	509	805	6.7	21 700	8%	1.43%
H. giganteus	Ames 32741	(SPA-5) NC	36.5491	-81.2166	2 308 776	11 576	100 037	8099	531	447	9.7	22 639	7%	0.98%
H. giganteus	Ames 32741	(SPA-7) NC	36.5491	-81.2166	1 476 755	8746	71 613	6196	504	896	6.9	22 898	8%	1.55%
H. gracilentus	PI 649987	(1) CA	34.2375	-117.4750	3 202 392	9114	111 266	6583	385	1136	16.3	54 667	6%	1.71%
H. gracilentus	PI 649987	(2) CA	34.2375	-117.4750	4 558 185	12 294	144 482	9052	385	950	19.4	61 258	4%	1.34%
H. gracilentus	PI 649987	(3) CA	34.2375	-117.4750	4 696 907	12 504	148 012	9141	363	931	20.8	58 686	4%	1.25%
H. gracilentus	PI 649987	(4) CA	34.2375	-117.4750	2 121 344	6906	89 838	5934	431	918	12.1	37 449	7%	1.77%
H. grosseserratus	PI 673315	(SAN-2) IL	41.0691	-87.6755	1 440 596	7802	65 502	5548	502	694	6.8	20 258	9%	1.41%
H. grosseserratus	PI 673315	(SAN-5) IL	41.0691	-87.6755	993 019	6089	44 995	4375	502	774	5.7	16 773	11%	1.69%
H. grosseserratus	PI 673315	(SAN-6) IL	41.0691	-87.6755	1 608 272	8375	67 829	5832	486	815	7.7	24 648	8%	1.53%
H. grosseserratus	PI 673315	(SAN-7) IL	41.0691	-87.6755	1 494 272	7906	67 080	5555	545	602	7.5	22 850	10%	1.53%
H. heterophyllus	N/A[d]	(ANS-2) FL	30.0588	-85.0155	1 871 283	9600	79 011	6393	520	438	6.9	14 869	8%	0.79%
H. heterophyllus	N/A[d]	(ANS-4) FL	30.0588	-85.0155	1 174 462	6781	54 689	4614	421	458	6.3	10 952	9%	0.93%
H. heterophyllus	N/A[d]	(ANS-6) FL	30.0588	-85.0155	1 694 411	8860	73 643	5872	454	499	6.7	13 218	8%	0.78%
H. heterophyllus	PI 673162	(RAM-2) LA	30.5311	-90.1491	2 064 074	10 518	73 800	6698	539	672	6.9	22 282	8%	1.08%
H. heterophyllus	PI 673162	(RAM-3) LA	30.5311	-90.1491	1 645 429	8900	63 957	5955	494	803	6.4	20 737	8%	1.26%

H. heterophyllus	PI 673162	(RAM-8) LA	30.5311	-90.1491	1 327 205	7681	53 532	5176	488	697	5.9	15 658	9%	1.18%
H. heterophyllus	PI 664732	(SUP) NC	34.0686	-78.2936	1 829 010	5964	76 483	4522	419	315	21.4	29 589	9%	1.62%
H. laciniatus	PI 653562	(1) NM	32.2428	-107.4697	3 944 757	13 251	164 289	9503	383	827	18.0	46 930	4%	1.19%
H. laciniatus	PI 653562	(2) NM	32.2428	-107.4697	6 839 936	22 921	250 019	16 957	345	981	27.2	79 999	2%	1.17%
H. laciniatus	PI 653562	(3) NM	32.2428	-107.4697	3 174 335	10 332	128 861	7393	402	1451	16.9	50 552	5%	1.59%
H. laciniatus	PI 653562	(4) NM	32.2428	-107.4697	1 754 387	7329	89 157	5472	503	531	14.1	32 764	9%	1.87%
H. longifolius	PI 664680	(FLR-3) AL	34.7578	-85.6964	2 936 601	11 171	116 450	7769	404	832	14.4	40 186	5%	1.37%
H. longifolius	PI 664680	(FLR-4) AL	34.7578	-85.6964	4 101 329	15 151	155 323	10 687	396	914	17.8	55 127	4%	1.34%
H. longifolius	PI 664680	(FLR-5) AL	34.7578	-85.6964	1 720 250	8492	78 908	6017	483	897	12.5	35 495	8%	2.06%
H. longifolius	PI 664680	(FLR-6) AL	34.7578	-85.6964	2 683 882	11 232	118 984	7981	470	794	15.7	41 195	6%	1.53%
H. longifolius	PI 650001	(FTP-1) AL	34.4319	-85.6753	1 581 574	8538	64 634	5702	506	725	6.5	18 949	9%	1.20%
H. longifolius	PI 650001	(FTP-2) AL	34.4319	-85.6753	1 250 793	7703	54 868	5240	487	604	7.0	16 564	9%	1.32%
H. longifolius	PI 650001	(FTP-3) AL	34.4319	-85.6753	1 473 016	7907	56 377	5185	550	590	6.8	19 997	11%	1.36%
H. longifolius	PI 650001	(FTP-5) AL	34.4319	-85.6753	1 367 997	7547	56 100	4920	513	733	5.9	18 458	10%	1.35%
H. maximiliani	Ames 32178	(KON-1) KS	39.1100	-96.5625	2 486 733	9614	84 132	6413	436	970	8.7	27 916	7%	1.12%
H. maximiliani	Ames 32178	(KON-5) KS	39.1100	-96.5625	2 754 017	10 728	88 634	7186	474	717	9.4	27 507	7%	1.00%
H. maximiliani	Ames 32742	(KYL-6) TX	29.9667	-97.8761	1 371 805	7584	60 294	5356	579	472	7.0	18 389	11%	1.34%
H. maximiliani	Ames 32742	(KYL-7) TX	29.9667	-97.8761	965 975	5903	44 012	4135	429	482	5.6	10 205	10%	1.06%
H. maximiliani	PI 613794	(LAW-1) IA	42.4597	-96.1942	1 544 513	7966	61 548	5552	518	746	6.1	18 127	9%	1.17%
H. maximiliani	PI 613794	(LAW-2) IA	42.4597	-96.1942	1 658 813	8619	66 521	6022	495	1390	5.8	17 420	8%	1.05%
H. maximiliani	PI 613794	(LAW-7) IA	42.4597	-96.1942	1 668 041	8869	66 724	6141	549	581	6.7	18 959	9%	1.14%
H. microcephalus	N/A[d]	(DYS-2) NC	35.5869	-81.8247	2 459 597	9967	86 952	6599	524	668	7.8	24 907	8%	1.01%
H. microcephalus	PI 664743	(IVA-1) SC	34.2624	-82.6627	2 238 612	10 918	96 410	7576	454	950	8.6	25 693	6%	1.15%
H. microcephalus	PI 664743	(IVA-2) SC	34.2624	-82.6627	2 949 841	13 540	131 340	9783	474	632	12.8	35 164	5%	1.19%
H. microcephalus	PI 664743	(IVA-3) SC	34.2624	-82.6627	1 806 144	10 048	85 981	7179	479	783	7.3	21 685	7%	1.20%
H. microcephalus	PI 673317	(MTR-2) SC	34.9475	-83.0892	1 102 199	6510	47 561	4708	515	619	5.5	14 934	11%	1.35%
H. microcephalus	PI 673317	(MTR-6) SC	34.9475	-83.0892	1 217 012	6882	50 446	4867	563	515	5.9	16 214	12%	1.33%
H. microcephalus	PI 664703	(SUN-3) SC	34.9611	-82.8450	1 352 048	7904	56 974	5512	580	523	5.8	15 807	11%	1.17%
H. microcephalus	PI 664703	(SUN-4) SC	34.9611	-82.8450	1 673 091	9295	72 472	6330	480	769	5.9	13 191	8%	0.79%
H. mollis	PI 673147	(DAR-2) OH	39.8934	-83.2006	8 973 712	17 422	227 775	12 877	419	335	63.8	104 306	3%	1.16%

H. mollis	PI 673147	(DAR-6) OH	39.8934	-83.2006	2 204 859	10 161	87 517	6865	464	806	9.4	28 193	7%	1.28%
H. mollis	PI 673147	(DAR-7) OH	39.8934	-83.2006	2 032 580	9820	85 701	6589	500	717	7.3	19 954	8%	0.98%
H. mollis	PI 673318	(PEM-5) IL	41.0899	-87.5659	1 809 073	8865	88 373	5966	540	406	6.8	14 499	9%	0.80%
H. mollis	PI 673318	(PEM-7) IL	41.0899	-87.5659	1 046 068	6290	47 377	4397	478	560	5.1	11 598	11%	1.11%
H. mollis	PI 673318	(PEM-8) IL	41.0899	-87.5659	1 009 305	6315	44 384	4424	479	550	5.2	11 096	11%	1.10%
H. neglectus	PI 673320	(KER-3) TX	31.8258	-103.0781	2 590 394	10 508	110 343	7515	407	766	15.1	40 259	5%	1.55%
H. neglectus	PI 673320	(KER-4) TX	31.8258	-103.0781	5 164 616	18 565	197 578	13 804	293	893	27.1	54 700	2%	1.06%
H. neglectus	PI 673320	(KER-5) TX	31.8258	-103.0781	1 727 782	7681	77 376	5568	449	687	10.8	29 567	8%	1.71%
H. neglectus	PI 673320	(KER-6) TX	31.8258	-103.0781	517 163	3501	32 309	2756	458	451	6.7	11 977	17%	2.32%
H. neglectus	PI 673321	(MON-1) TX	31.6315	-102.8100	1 557 664	7924	70 312	5630	488	804	8.0	24 792	9%	1.59%
H. neglectus	PI 673321	(MON-4) TX	31.6315	-102.8100	897 893	5650	46 177	4119	521	1029	6.4	20 101	13%	2.24%
H. neglectus	PI 673321	(MON-6) TX	31.6315	-102.8100	2 363 528	10 734	102 623	7770	455	732	13.0	40 409	6%	1.71%
H. neglectus	PI 673321	(MON-8) TX	31.6315	-102.8100	1 159 780	4499	60 274	3636	443	470	20.4	37 933	12%	3.27%
H. niveus ssp. canescens	PI 649905	(1) AZ	32.1014	-113.4511	3 185 594	11 151	104 942	7763	487	782	12.8	41 901	6%	1.32%
H. niveus ssp. canescens	PI 649905	(3) AZ	32.1014	-113.4511	3 042 077	10 466	104 037	7288	415	889	11.1	32 125	6%	1.06%
H. niveus ssp. canescens	PI 649905	(4) AZ	32.1014	-113.4511	2 110 465	8659	82 801	6135	491	950	8.2	27 112	8%	1.28%
H. niveus ssp. tephrodes	PI 650020	(IVS-1) CA	32.7375	-114.9133	3 427 431	13 444	129 872	9801	383	950	21.1	62 780	4%	1.83%
H. niveus ssp. tephrodes	PI 650021	(IVS-2) CA	32.7375	-114.9133	2 271 674	10 075	85 026	7091	442	1185	12.1	47 048	6%	2.07%
H. niveus ssp. tephrodes	PI 650022	(IVS-5) CA	32.7375	-114.9133	2 782 971	8357	74 371	6137	434	1154	10.9	38 648	7%	2.32%
H. niveus ssp. tephrodes	PI 650023	(IVS-6) CA	32.7375	-114.9133	1 251 422	11 782	112 992	8604	376	1234	15.8	49 784	4%	1.79%
H. nuttalli ssp. nuttalli	PI 531053	MT	46.5333	-110.9167	11 805 196	45 221	318 327	33 233	303	426	5.0	6807	1%	0.06%
H. occidentalis	PI 673323	(OQK-3) IL	41.0293	-90.9270	1 778 145	9081	66 761	6079	528	624	7.4	21 781	9%	1.22%
H. occidentalis	PI 673323	(OQK-4) IL	41.0293	-90.9270	1 423 301	7890	58 220	5480	507	711	6.2	18 373	9%	1.29%
H. petiolaris	PI 673325	(GSD) CO	37.7675	-105.5150	1 893 306	8018	66 083	5590	501	704	9.0	28 925	9%	1.53%
H. petiolaris	PI 673325	(GAR-2) IN	41.6180	-87.2686	2 127 616	9501	95 031	6767	490	859	9.5	29 115	7%	1.37%
H. petiolaris	PI 673325	(GAR-4) IN	41.6180	-87.2686	2 564 244	10 983	110 728	7784	480	671	12.1	35 060	6%	1.37%
H. petiolaris	PI 673325	(GAR-6) IN	41.6180	-87.2686	1 972 833	9174	92 345	6717	462	811	10.3	31 799	7%	1.61%
H. petiolaris ssp. fallax	PI 435838	(1) UT	37.1750	-113.2900	33 532	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

H. petiolaris ssp. fallax	PI 435838	(2) UT	37.1750	-113.2900	10 100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
H. petiolaris ssp. fallax	PI 435838	(3) UT	37.1750	-113.2900	25 654	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
H. porteri	PI 673331	(CMR-1) GA	33.2507	-85.1466	3 574 877	11 669	101 592	8187	460	813	11.8	38 827	6%	1.09%
H. porteri	PI 673331	(CMR-6) GA	33.2507	-85.1466	2 702 530	9771	92 496	7082	501	708	10.4	30 531	7%	1.13%
H. porteri	PI 673332	(HR-2) GA	33.5396	-82.2514	1 351 950	5835	43 536	4184	615	525	5.1	15 483	15%	1.15%
H. porteri	PI 673332	(HR-6) GA	33.5396	-82.2514	599 005	3230	23 731	2424	346	394	6.4	8925	14%	1.49%
H. pumilus	PI 650070	(1) WY	42.4511	-105.3500	3 723 114	3657	68 330	2930	3	242	10.0	177	0%	0.01%
H. pumilus	PI 650070	(2) WY	42.4511	-105.3500	874 823	1000	19 417	853	0	N/A	N/A	N/A	N/A	N/A
H. pumilus	PI 650070	(3) WY	42.4511	-105.3500	4 889 420	5340	127 079	4474	7	304	15.1	310	0.16%	0.01%
H. praecox ssp. hirtus	PI 435855	(1) TX	1.6 km west of Carrizo Springs		2 573 730	10 197	98 830	7185	408	1008	13.2	40 218	6%	1.56%
H. praecox ssp. hirtus	PI 435855	(3) TX	1.6 km west of Carrizo Springs		8 372 764	14 041	237 681	11 249	406	324	104.8	151 249	4%	1.81%
H. praecox ssp. hirtus	PI 435855	(4) TX	1.6 km west of Carrizo Springs		3 414 755	13 336	127 809	9315	425	943	13.2	36 568	5%	1.07%
H. praecox ssp. praecox	PI 435847	TX	South La Porte, north of Seabrook.		1 426 468	7431	63 857	5356	612	475	7.4	20 652	11%	1.45%
H. praecox ssp. runyonii	PI 673328	(FBA-1) TX	27.6593	-97.3112	4 269 542	15 806	164 088	11 064	445	712	15.7	42 723	4%	1.00%
H. praecox ssp. runyonii	PI 673328	(FBA-2) TX	27.6593	-97.3112	2 787 383	10 564	94 696	7248	384	840	12.6	34 725	5%	1.25%
H. praecox ssp. runyonii	PI 673329	(FBB-1) TX	27.6494	-97.3089	1 812 130	8141	70 329	5737	522	605	7.3	20 056	9%	1.11%
H. praecox ssp. runyonii	PI 673329	(FBB-3) TX	27.6494	-97.3089	1 961 564	8012	59 270	5318	525	880	7.3	23 464	10%	1.20%
H. praecox ssp. runyonii	PI 673329	(FBB-8) TX	27.6494	-97.3089	2 256 662	9006	68 337	6007	537	589	8.1	22 792	9%	1.01%
H. radula	PI 664738	(HAR-2) SC	32.2281	-81.0744	2 688 746	11 085	82 821	7253	465	863	8.1	24 464	6%	0.91%
H. radula	PI 664738	(HAR-4) SC	32.2281	-81.0744	4 256 688	15 233	134 881	10 135	423	801	12.9	35 643	4%	0.84%
H. radula	PI 664738	(HAR-7) SC	32.2281	-81.0744	5 232 710	12 608	162 645	9278	440	350	41.0	67 761	5%	1.29%
H. radula	PI 673163	(RAM-6) LA	30.5311	-90.1594	2 198 473	10 104	68 693	6393	511	733	5.0	14 979	8%	0.68%
H. radula	PI 673163	(RAM-7) LA	30.5311	-90.1594	3 051 819	13 400	104 077	8993	556	584	7.4	22 221	6%	0.73%
H. radula	PI 673218	(RLR-2) FL	29.6316	-81.7038	3 690 235	14 708	114 253	9831	434	1046	10.0	32 250	4%	0.87%
H. radula	PI 673218	(RLR-4) FL	29.6316	-81.7038	2 219 052	10 232	73 179	6699	471	875	7.8	21 142	7%	0.95%
H. radula	PI 673218	(RLR-7) FL	29.6316	-81.7038	2 102 153	9839	73 616	6594	518	594	7.9	20 958	8%	1.00%
H. salicifolius	PI 664780	(NOW-1) OK	36.6992	-95.4750	1 719 526	8938	75 668	6286	427	1077	10.1	29 222	7%	1.70%
H. salicifolius	PI 664781	(NOW-2)	36.6992	-95.4750	2 195 311	9994	90 959	6995	393	1230	11.9	38 033	6%	1.73%

		OK (NOW-4)												
H. salicifolius	PI 664782	OK	36.6992	-95.4750	1 800 528	9256	78 387	6483	409	940	10.6	31 021	6%	1.72%
		(NOW-5)												
H. salicifolius	PI 664783	OK	36.6992	-95.4750	2 450 258	11 238	100 372	7821	422	848	13.6	41 472	5%	1.69%
H. salicifolius	PI 664768	(PAO-1) KS	38.5633	-94.7906	1 052 503	5898	51 004	4308	431	476	8.8	15 605	10%	1.48%
H. salicifolius	PI 664769	(PAO-5) KS	38.5633	-94.7906	3 080 939	11 860	114 366	8202	550	564	12.8	36 879	7%	1.20%
H. salicifolius	PI 664770	(PAO-6) KS	38.5633	-94.7906	5 557 452	18 971	179 662	13 259	366	732	18.8	42 440	3%	0.76%
H. silphioides	PI 673156	(COL) LA	32.3255	-92.2083	3 139 963	12 431	108 225	8098	589	465	6.7	17 598	7%	0.56%
H. silphioides	PI 664795	(PAR) AR	36.1894	-90.5442	2 986 653	12 222	109 452	8218	585	451	9.0	22 073	7%	0.74%
H. silphioides	PI 664793	(WEP) MO	36.6633	-91.6956	7 397 398	23 800	226 553	17 282	516	523	21.4	55 410	3%	0.75%
H. verticillatus	PI 650110	(ALV-3) AL	34.1418	-85.4372	1 335 713	7205	49 966	4985	514	1066	6.3	19 614	10%	1.57%
H. verticillatus	PI 650110	(ALV-4) AL	34.1418	-85.4372	1 666 219	7568	54 185	5263	497	672	6.4	17 099	9%	1.28%
H. verticillatus	PI 650109	(TNV) TN	35.4848	-88.7113	1 872 074	7760	80 302	5304	396	321	15.3	19 404	8%	1.04%
Phoebanthus tenuifolius	Ames 32195	(BSP-1) FL	30.0441	-85.0119	2 856 698	12 665	107 609	8170	424	937	9.0	29 320	5%	1.03%
Phoebanthus tenuifolius	Ames 32195	(BSP-3) FL	30.0441	-85.0119	3 467 452	13 446	121 364	8523	431	786	10.1	31 267	5%	0.90%
Phoebanthus tenuifolius	Ames 32195	(BSP-4) FL	30.0441	-85.0119	2 592 023	11 151	92 322	7141	442	850	9.0	27 553	6%	1.06%
Phoebanthus tenuifolius	Ames 32195	(BSP-7) FL	30.0441	-85.0119	1 009 836	6336	43 433	4268	473	606	5.5	13 688	11%	1.36%
Phoebanthus tenuifolius	Ames 32196	(FRC-4) FL	30.1613	-85.0670	1 694 993	9207	67 721	5943	504	724	5.7	17 059	8%	1.01%
Phoebanthus tenuifolius	Ames 32196	(FRC-5) FL	30.1613	-85.0670	2 223 688	9768	75 507	6034	465	859	8.0	24 825	8%	1.12%
Phoebanthus tenuifolius	Ames 32196	(FRC-7) FL	30.1613	-85.0670	2 059 971	9610	69 213	6060	541	635	7.3	23 242	9%	1.13%
Average (median)					**2 334 252 (1 937 688)**	**9904 (9344)**	**89 686 (78 924)**	**6983 (6417.5)**	**477 (487.5)**	**751 (724.5)**	**11 (8.1)**	**29 497 (24 808.5)**	**8% (8%)**	**1% (1.3%)**

[a]This is the resulting reads after removal of adapter contamination, quality trimming, and reads with ambiguous bases

[b]Contigs "on-target" is the percentage of contigs that match targeted exons from total merged contigs

[c]Reads "on-target" is the percentage of reads from Total contigs divided by Number of trimmed reads

[d]no accession, wild-collected

Table S2.2. Data summary and GRIN accession numbers for all accessions used in chloroplast analysis. The aligned chloroplast sequences were deposited in Dryad (http://dx.doi.org/10.5061/dryad.4n28n) and individual annotations in Verdant (http://verdant.iplantcollaborative.org).

Species	GRIN Accession	Location	Latitude	Longitude	YASRA contigs	Ave. coverage	Total bp
H. agrestis	PI 673202	(GLA-1) FL	26.9510	-81.1349	134	8.0	95 803
H. agrestis	PI 673202	(GLA-5) FL	26.9510	-81.1349	73	6.4	94 314
H. agrestis	PI 673201	(HEN) FL	26.4231	-81.2484	144	3.1	49 216
H. agrestis	PI 673205	(SCW) FL	28.7870	-81.1852	67	5.9	93 699
H. angustifolius	PI 673210	(CRP-3) FL	28.9543	-82.6489	236	6.2	88 659
H. angustifolius	PI 649937	(MAN-8) GA	33.7595	-84.8555	148	4.9	86 392
H. annuus	PI 649859	(FIR-6) CA	36.8903	-120.5028	12	15.8	96 731
H. annuus	PI 673304	(KON-1) KS	39.1021	-96.6103	25	8.1	96 315
H. annuus	PI 673305	(UTA-2) UT	39.7160	-112.2070	18	11.9	96 697
H. argophyllus	PI 673306	(FLB) TX	27.6594	-97.3132	158	3.8	64 529
H. argophyllus	PI 673306	(MUS-4) TX	27.8351	-97.0525	50	6.8	95 476
H. arizonensis	PI 653549	(1) AZ	34.6097	-109.3206	11	74.9	96 734
H. arizonensis	PI 653549	(4) AZ	34.6097	-109.3206	13	65.0	96 742
H. atrorubens	PI 664731	(FMF-1) SC	33.1944	-79.5256	19	19.4	96 692
H. atrorubens	PI 664694	(TOC-4) GA	34.6603	-83.3481	56	13.3	96 580
H. atrorubens	PI 649940	(WAR-1) AL	33.8906	-86.8258	17	18.2	96 760
H. carnosus	PI 673310	(POT-7) FL	29.6097	-81.4716	13	32.2	96 758
H. carnosus	Ames 32168	(SOF-2) FL	29.3200	-81.3102	56	6.8	96 098
H. cusickii	PI 649966	(LIT-3) CA	40.4184	-120.2833	63	14.6	95 788
H. cusickii	PI 649966	(LIT-5) CA	40.4184	-120.2833	36	13.3	96 611
H. cusickii	PI 649959	(NCO-4) OR	44.6206	-120.2064	14	43.9	96 747
H. cusickii	PI 649959	(NCO-7) OR	44.6206	-120.2064	20	26.0	96 694
H. cusickii	PI 649967	(RAV-4) CA	40.6760	-120.2853	18	31.6	96 722
H. cusickii	PI 649967	(RAV-5) CA	40.6760	-120.2853	30	18.3	96 592

H. debilis ssp. debilis	PI 435669	(2) FL	Hobe Sound Public Beach, Hobe Sound		4	19.3	96 732
H. debilis ssp. silvestris	PI 435651	(2) TX	8-10 km northwest of Henderson		28	9.1	96 581
H. debilis ssp. tardiflorus	PI 673213	(CDK-3) FL	29.1832	-83.0171	7	21.2	96 723
H. debilis ssp. tardiflorus	Ames 32170	(PAN-7) FL	30.0161	-84.3682	4	22.9	96 720
H. debilis ssp. tardiflorus	PI 673310	(PSJ-4) FL	29.8060	-85.3021	9	19.2	96 708
H. divaricatus	Ames 28236	(LCN) OH	41.5911	-83.7651	85	11.5	95 855
H. divaricatus	Ames 32172	(PEM-8) IL	41.0899	-87.5640	149	4.1	84 715
H. exilis	PI 649895	(2) CA	41.6431	-122.7472	15	38.1	96 712
H. exilis	PI 649895	(4) CA	41.6431	-122.7472	13	44.2	96 713
H. floridanus	PI 673197	(APL-2) FL	29.7147	-85.0252	131	4.9	84 263
H. floridanus	Ames 32740	(OCK-4) FL	29.0652	-81.9508	129	4.5	87 825
H. floridanus	PI 673204	(VOL-5) FL	28.6750	-80.9761	162	3.6	66 608
H. giganteus	PI 664712	(BUR-6) NC	35.8117	-82.1972	50	11.8	96 533
H. giganteus	PI 664647	(LCN-5) OH	41.5911	-83.7651	137	4.7	86 906
H. giganteus	Ames 32741	(SPA-5) NC	36.5491	-81.2166	149	4.4	86 842
H. gracilentus	PI 649987	(1) CA	34.2375	-117.4750	11	48.7	96 742
H. gracilentus	PI 649987	(2) CA	34.2375	-117.4750	8	77.6	96 744
H. gracilentus	PI 649987	(4) CA	34.2375	-117.4750	12	33.6	96 742
H. grosseserratus	PI 673315	(SAN-5) IL	41.0691	-87.6755	124	3.1	56 242
H. grosseserratus	PI 673315	(SAN-7) IL	41.0691	-87.6755	158	3.4	59 287
H. heterophyllus	N/A[d]	(ANS-2) FL	30.0588	-85.0155	153	4.2	85 522
H. heterophyllus	N/A[d]	(ANS-6) FL	30.0588	-85.0155	103	4.7	92 100
H. heterophyllus	PI 673162	(RAM-2) LA	30.5311	-90.1491	155	3.3	67 751
H. heterophyllus	PI 673162	(RAM-3) LA	30.5311	-90.1491	161	3.2	64 672
H. heterophyllus	PI 664732	(SUP) NC	34.0686	-78.2936	126	6.0	51 196
H. laciniatus	PI 653562	(3) NM	32.2428	-107.4697	10	64.3	96 743
H. laciniatus	PI 653562	(4) NM	32.2428	-107.4697	8	45.1	96 739
H. longifolius	PI 664680	(FLR-5) AL	34.7578	-85.6964	26	13.1	96 724
H. longifolius	PI 650001	(FTP-3) AL	34.4319	-85.6753	21	23.3	96 764
H. maximiliani	Ames 32178	(KON-1) KS	39.1100	-96.5625	21	15.4	96 775

H. maximiliani	Ames 32178	(KON-5) KS	39.1100	-96.5625	7	46.6	96 779
H. maximiliani	Ames 32742	(KYL-6) TX	29.9667	-97.8761	60	6.3	95 763
H. maximiliani	Ames 32742	(KYL-7) TX	29.9667	-97.8761	83	9.0	96 277
H. maximiliani	PI 613794	(LAW-1) IA	42.4597	-96.1942	52	7.2	96 342
H. maximiliani	PI 613794	(LAW-7) IA	42.4597	-96.1942	185	5.7	71 797
H. microcephalus	N/A[d]	(DYS-2) NC	35.5869	-81.8247	41	8.1	96 565
H. microcephalus	PI 664743	(IVA-3) SC	34.2624	-82.6627	78	5.8	94 926
H. microcephalus	PI 673317	(MTR-6) SC	34.9475	-83.0892	129	5.0	88 264
H. microcephalus	PI 664703	(SUN-3) SC	34.9611	-82.8450	42	7.7	96 393
H. mollis	PI 673147	(DAR-6) OH	39.8934	-83.2006	163	7.9	93 312
H. mollis	PI 673147	(DAR-7) OH	39.8934	-83.2006	167	7.1	95 088
H. mollis	PI 673318	(PEM-5) IL	41.0899	-87.5659	32	9.0	96 575
H. mollis	PI 673318	(PEM-8) IL	41.0899	-87.5659	150	5.2	80 985
H. neglectus	PI 673320	(KER-6) TX	31.8258	-103.0781	28	16.2	96 661
H. neglectus	PI 673321	(MON-4) TX	31.6315	-102.8100	18	12.1	96 713
H. niveus ssp. canescens	PI 649905	(4) AZ	32.1014	-113.4511	14	32.6	96 723
H. niveus ssp. tephrodes	PI 650021	(IVS-2) CA	32.7375	-114.9133	11	35.2	96 731
H. niveus ssp. tephrodes	PI 650022	(IVS-5) CA	32.7375	-114.9133	8	58.5	96 708
H. nuttalli ssp. nuttalli	PI 531053	MT	46.5333	-110.9167	6	80.9	96 783
H. occidentalis	PI 673323	(OQK-3) IL	41.0293	-90.9270	216	6.8	91 990
H. occidentalis	PI 673323	(OQK-4) IL	41.0293	-90.9270	63	6.9	96 058
H. petiolaris	PI 673325	(GSD) CO	37.7675	-105.5150	3	61.0	96 746
H. petiolaris	PI 673325	(GAR-2) IN	41.6180	-87.2686	3	74.5	96 744
H. porteri	PI 673331	(CMR-6) GA	33.2507	-85.1466	26	17.7	96 666
H. porteri	PI 673332	(HR-2) GA	33.5396	-82.2514	96	4.8	92 065
H. praecox ssp. hirtus	PI 435855	(1) TX	1.6 km west of Carrizo Springs		10	28.1	96 706
H. praecox ssp. hirtus	PI 435855	(4) TX	1.6 km west of Carrizo Springs		9	23.9	96 710
H. praecox ssp. praecox	PI 435847	TX	South La Porte, north of Seabrook.		18	25.8	96 729
H. praecox ssp. runyonii	PI 673328	(FBA-1) TX	27.6593	-97.3112	3	120.3	96 721

H. praecox ssp. runyonii	PI 673329	(FBB-3) TX	27.6494	-97.3089	6	17.9	96 745
H. praecox ssp. runyonii	PI 673329	(FBB-8) TX	27.6494	-97.3089	4	23.1	96 747
H. radula	PI 664738	(HAR-2) SC	32.2281	-81.0744	52	11.9	96 718
H. radula	PI 673163	(RAM-7) LA	30.5311	-90.1594	40	7.3	96 518
H. radula	PI 673218	(RLR-7) FL	29.6316	-81.7038	27	9.8	96 663
H. salicifolius	PI 664780	(NOW-1) OK	36.6992	-95.4750	51	26.3	96 668
H. salicifolius	PI 664769	(PAO-5) KS	38.5633	-94.7906	11	30.3	96 778
H. silphioides	PI 673156	(COL) LA	32.3255	-92.2083	68	10.0	96 235
H. silphioides	PI 664795	(PAR) AR	36.1894	-90.5442	29	15.4	96 465
H. silphioides	PI 664793	(WEP) MO	36.6633	-91.6956	6	37.0	96 765
H. verticillatus	PI 650110	(ALV-3) AL	34.1418	-85.4372	136	4.1	84 776
H. verticillatus	PI 650110	(ALV-4) AL	34.1418	-85.4372	156	4.0	80 219
H. verticillatus	PI 650109	(TNV) TN	35.4848	-88.7113	124	5.2	91 343
Phoebanthus tenuifolius	Ames 32195	(BSP-1) FL	30.0441	-85.0119	18	18.9	96 758
Phoebanthus tenuifolius	Ames 32195	(BSP-3) FL	30.0441	-85.0119	15	29.5	96 774
Phoebanthus tenuifolius	Ames 32195	(BSP-4) FL	30.0441	-85.0119	11	19.0	96 763
Phoebanthus tenuifolius	Ames 32195	(BSP-7) FL	30.0441	-85.0119	34	8.6	96 435
Phoebanthus tenuifolius	Ames 32196	(FRC-4) FL	30.1613	-85.0670	57	6.3	95 697
Phoebanthus tenuifolius	Ames 32196	(FRC-5) FL	30.1613	-85.0670	32	9.1	96 647
Phoebanthus tenuifolius	Ames 32196	(FRC-7) FL	30.1613	-85.0670	152	6.7	93 633
Average (median)					**62 (32)**	**20.1 (11.9)**	**91 868(96 581)**

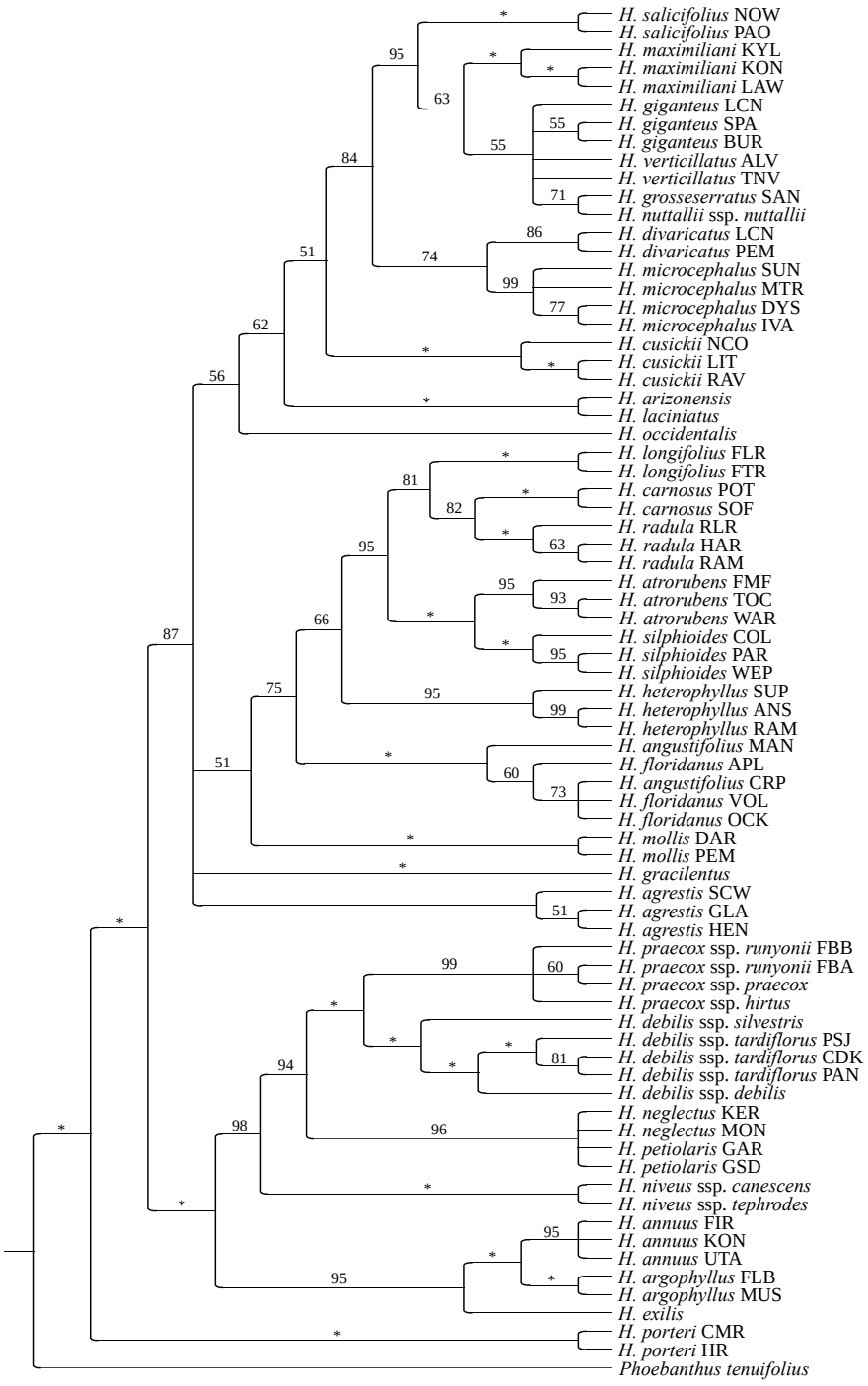

Figure S2.1. *Helianthus* MP-EST population tree based on 170 nuclear genes across 103 individuals. Nodes with <50 bootstrap support are collapsed; asterisks indicate bootstrap support of 100; all other support is listed at the nodes.

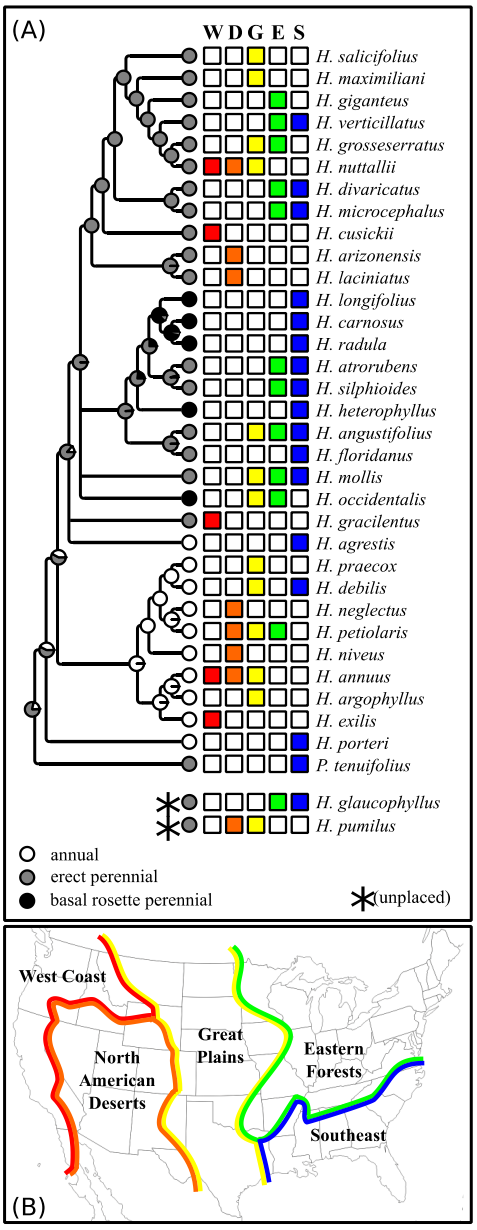

Figure S2.2. Geographic distribution of diploid *Helianthus* and the evolution of growth form and life history across the genus. (A) Presence-absence occupation of five major biogeographical regions of North America (W- West Coast, D – North America Deserts, G – Great Plains, E – Eastern Forests, S – Southeast) and stochastic character mapping (Huelsenbeck et al., 2003; Bollback, 2006) of growth form/life history on the *Helianthus* phylogeny. Mapping was performed using 100 simulations in the *make.simmap* function in the R package phytools (Revell, 2012). Note that *H. glaucophyllus* and *H. pumilus* were not able to be included in the phylogeny and remain unplaced. (B) The five major North American biogeographical regions used in Panel A (*sensus* Udvardy and Udvardy, 1975).

APPENDIX B

SUPPLEMENTARY FIGURES AND TABLES FROM CHAPTER III

Table S3.1. Summary of the assemblies for all individuals.

				Assemblies						Contigs aligned to targets			
Individual	**SRA Accession**	**Voucher Location[a]**	**Location**	**Trimmed Reads[b]**	**Trinity Contigs**	**Velvet Contigs**	**Merged Contigs**	**Total Count**	**N50**	**Ave. Cov.**	**Reads in contigs**	**Contigs "on-target"[c]**	**Reads "on-target"[d]**
Darlingtonia californica	SAMN03354578	UGA66	Oregon (OR)	2,012,780	6,778	46,331	3,620	393	558	8.1	34,999	10.9%	1.7%
Darlingtonia californica	SAMN03354579	N/A	Unknown (UN1)	1,824,513	6,019	48,941	3,828	370	545	10.8	61,000	9.7%	3.3%
Darlingtonia californica	SAMN03354580	UGA54	Unknown (UN2)	1,842,393	5,603	12,728	3,294	353	566	8.9	16,771	10.7%	0.9%
Heliamphora minor	SAMN03354581	UGA55	Venezuela (VE)	2,440,615	4,019	88,900	3,357	516	560	17.4	62,295	15.4%	2.6%
Sarracenia alabamensis	SAMN03354582	UGA19	Alabama (AL)	907,820	2,560	20,926	2,690	384	339	6.2	9,528	14.3%	1.1%
Sarracenia alata	SAMN03354583	UGA21	Mississippi (MS1)	2,378,412	5,534	67,029	5,338	483	391	7.1	13,119	9.0%	0.6%
Sarracenia alata	SAMN03354584	N/A	Mississippi (MS2)	2,404,916	5,298	21,526	1,457	528	442	9.7	57,157	36.2%	2.4%
Sarracenia alata	SAMN03354585	UGA67	Louisiana (LA1)	2,065,520	5,518	46,399	6,182	492	410	7.1	13,028	8.0%	0.6%
Sarracenia alata	SAMN03354586	TAES253951	Texas (TX)	3,270,898	6,101	43,888	3,047	602	474	8.0	24,617	19.8%	0.8%
Sarracenia alata	SAMN03354587	UGA60	Louisiana (LA2)	2,196,825	4,636	36,238	5,311	598	470	7.7	27,103	11.3%	1.2%
Sarracenia flava	SAMN03354588	UGA15	Georgia (GA)	2,803,219	5,779	47,388	3,245	583	474	7.8	41,239	18.0%	1.5%
Sarracenia flava	SAMN03354589	UGA65	Florida (FL)	5,215,450	10,666	121,668	13,701	636	498	10.4	29,473	4.6%	0.6%
Sarracenia flava	SAMN03354590	UGA48	North Carolina (NC1)	3,108,978	7,346	62,020	8,452	499	397	7.9	13,826	5.9%	0.4%
Sarracenia flava	SAMN03354591	UGA45	South Carolina (SC)	5,583,207	11,673	124,865	8,538	625	541	14.7	65,233	7.3%	1.2%
Sarracenia flava	SAMN03354592	UGA50	North Carolina (NC2)	4,950,868	10,945	108,601	6,633	636	547	12.0	47,393	9.6%	1.0%
Sarracenia flava	SAMN03354593	UGA64	Virginia (VA)	6,706,537	12,287	144,350	8,738	622	480	11.1	82,062	7.1%	1.2%
Sarracenia flava var. *rubricorpora*	SAMN03354594	UGA18	Florida (FL1)	3,118,859	6,670	50,177	3,447	543	422	8.3	38,122	15.8%	1.2%
Sarracenia flava var. *rubricorpora*	SAMN03354595	UGA18	Florida (FL2)	3,939,839	8,195	109,209	8,283	645	512	9.9	52,845	7.8%	1.3%
Sarracenia flava var. *rugelii*	SAMN03354596	UGA51	Alabama (AL)	5,795,290	10,247	17,869	1,930	499	617	11.4	69,665	25.9%	1.2%
Sarracenia flava var. *rugelii*	SAMN03354597	UGA26	Georgia (GA1)	3,741,044	7,129	80,047	10,213	599	468	9.5	23,154	5.9%	0.6%
Sarracenia flava var. *rugelii*	SAMN03354598	UGA44	Georgia (GA2)	2,254,871	5,092	52,041	5,566	564	453	12.2	29,601	10.1%	1.3%
Sarracenia jonesii	SAMN03354599	UGA32	South Carolina (SC1)	3,297,283	6,625	18,297	1,841	528	588	11.2	63,466	28.7%	1.9%
Sarracenia jonesii	SAMN03354600	UGA31	North Carolina (NC1)	6,303,334	11,787	96,568	12,735	607	660	11.0	35,388	4.8%	0.6%
Sarracenia jonesii	SAMN03354601	UGA33	North Carolina (NC2)	3,616,505	7,119	65,957	9,078	536	478	9.0	22,326	5.9%	0.6%
Sarracenia jonesii	SAMN03354602	UGA30	South Carolina (SC2)	3,930,604	6,744	59,789	7,660	550	534	8.9	23,547	7.2%	0.6%
Sarracenia leucophylla	SAMN03354603	UGA57	Florida (FL1)	3,489,950	7,139	65,388	8,791	597	482	10.3	31,105	6.8%	0.9%
Sarracenia leucophylla	SAMN03354604	UGA40	Alabama (AL1)	3,475,019	8,584	106,356	7,130	620	515	11.2	89,357	8.7%	2.6%
Sarracenia leucophylla	SAMN03354605	UGA17	Georgia (GA)	6,171,711	12,834	138,106	10,080	598	638	11.6	38,748	5.9%	0.6%
Sarracenia leucophylla	SAMN03354606	UGA56	Florida (FL2)	5,079,847	14,371	168,595	16,456	635	520	18.4	55,060	3.9%	1.1%
Sarracenia leucophylla	SAMN03354607	UGA52	Alabama (AL2)	6,537,778	12,423	162,697	12,204	651	613	20.4	216,278	5.4%	3.3%
Sarracenia leucophylla	SAMN03354608	UGA6	Florida (FL3)	3,791,319	7,799	62,644	9,065	567	490	10.8	28,235	6.3%	0.7%
Sarracenia minor	SAMN03354609	N/A	Georgia (GA1)	2,819,321	6,422	56,759	4,656	531	462	8.2	46,562	11.4%	1.7%
Sarracenia minor	SAMN03354610	UGA8	Georgia (GA2)	1,317,400	3,251	15,733	1,177	324	375	21.6	25,596	27.5%	1.9%
Sarracenia minor	SAMN03354611	UGA39	Georgia (GA3)	1,915,939	4,021	28,318	2,018	532	497	26.7	86,295	26.4%	4.5%
Sarracenia minor	SAMN03354612	UGA46	South Carolina (SC1)	2,980,563	6,889	45,125	3,345	544	489	9.0	68,047	16.3%	2.3%
Sarracenia minor	SAMN03354613	UGA13	South Carolina (SC2)	2,528,957	5,016	50,787	4,737	521	482	6.4	19,506	11.0%	0.8%

S. minor var. *okefenokeensis*	SAMN03354614	UGA23	Georgia (GA)	3,639,814	7,901	87,683	7,452	611	499	14.8	337,087	8.2%	9.3%
Sarracenia oreophila	SAMN03354615	UGA2	Alabama (AL1)	4,103,540	8,384	79,196	7,127	579	578	8.2	24,340	8.1%	0.6%
Sarracenia oreophila	SAMN03354616	UGA28	Alabama (AL2)	3,248,174	6,986	42,656	3,579	593	574	12.4	108,635	16.6%	3.3%
Sarracenia oreophila	SAMN03354617	UGA27	Alabama (AL3)	1,555,872	3,046	23,554	3,248	369	400	6.3	7,926	11.4%	0.5%
Sarracenia oreophila	SAMN03354618	UGA20	North Carolina (NC)	2,640,804	5,740	61,477	5,414	427	393	8.1	12,436	7.9%	0.5%
Sarracenia oreophila	SAMN03354619	UGA24	Alabama (AL4)	3,156,070	6,062	31,602	2,452	541	468	9.3	54,454	22.1%	1.7%
Sarracenia oreophila	SAMN03354620	UGA22	Georgia (GA)	3,333,665	7,054	75,195	6,551	483	452	8.6	32,054	7.4%	1.0%
Sarracenia psittacina	SAMN03354621	UGA43	Georgia (GA1)	3,117,132	5,766	54,115	3,739	630	574	9.9	35,755	16.8%	1.2%
Sarracenia psittacina	SAMN03354622	UGA9	Georgia (GA2)	2,333,477	5,706	73,775	5,141	550	457	7.4	32,718	10.7%	1.4%
Sarracenia psittacina	SAMN03354623	UGA11	Alabama (AL1)	3,048,249	6,603	72,562	3,920	577	422	7.6	25,872	14.7%	0.9%
Sarracenia psittacina	SAMN03354624	UGA10	Georgia (GA3)	4,004,895	8,822	64,394	3,609	611	591	12.2	84,652	16.9%	2.1%
Sarracenia psittacina	SAMN03354625	UGA1	Alabama (AL2)	2,645,312	6,557	49,137	3,185	636	469	8.6	46,534	20.0%	1.8%
Sarracenia psittacina	SAMN03354626	UGA35	Florida (FL)	1,079,396	2,372	23,914	1,908	283	346	6.7	15,310	14.8%	1.4%
Sarracenia psittacina	SAMN03354627	UGA53	Alabama (AL3)	4,265,795	9,463	62,559	4,411	589	491	8.2	57,227	13.4%	1.3%
Sarracenia psittacina	SAMN03354628	UGA59	Louisiana (LA)	1,249,566	3,623	39,336	2,088	356	340	5.3	10,456	17.0%	0.8%
S. purpurea ssp. *purpurea*	SAMN03354629	UGA61	Nova Scotia (NS)	1,384,141	4,059	38,178	2,604	477	349	5.3	9,272	18.3%	0.7%
S. purpurea ssp. *purpurea*	SAMN03354630	UGA47	Wisconsin (WI1)	4,641,910	5,061	95,910	4,127	552	445	19.2	93,248	13.4%	2.0%
S. purpurea ssp. *purpurea*	SAMN03354631	UGA47	Wisconsin (WI2)	6,128,348	9,978	203,332	10,043	624	503	16.3	94,214	6.2%	1.5%
S. purpurea ssp. *venosa*	SAMN03354632	UGA49	North Carolina (NC)	2,818,567	6,473	75,643	7,426	605	468	8.1	19,873	8.1%	0.7%
S. purpurea ssp. *venosa*	SAMN03354633	UGA62	Maryland (MD)	3,714,370	6,089	79,041	4,836	591	515	14.6	127,942	12.2%	3.4%
S. purpurea ssp. *venosa*	SAMN03354634	UGA63	Virginia (VA)	2,095,824	5,179	52,916	5,673	529	420	6.7	16,119	9.3%	0.8%
S. purpurea ssp. *venosa*	SAMN03354463	UGA12	Georgia (GA)	4,071,252	7,528	66,429	9,139	607	534	7.9	22,909	6.6%	0.6%
S. purpurea ssp. *venosa* var. *montana*	SAMN03354635	UGA34	North Carolina (NC)	2,270,005	5,657	46,834	6,543	531	429	9.4	14,876	8.1%	0.7%
S. purpurea ssp. *venosa* var. *montana*	SAMN03354636	UGA41	Georgia (GA)	2,733,046	7,178	56,440	8,235	583	481	8.5	20,514	7.1%	0.8%
S. rosea (*S. purpurea* ssp. *venosa* var. *burkii*)	SAMN03354637	UGA16	Florida (FL1)	959,292	2,750	21,142	1,290	321	349	5.8	12,035	24.9%	1.3%
S. rosea (*S. purpurea* ssp. *venosa* var. *burkii*)	SAMN03354638	UGA4	Alabama (AL)	3,218,785	6,641	58,834	4,487	611	509	9.9	78,936	13.6%	2.5%
S. rosea (*S. purpurea* ssp. *venosa* var. *burkii*)	SAMN03354639	UGA7	Mississippi (MS)	3,429,597	8,334	73,002	5,034	616	433	7.2	54,894	12.2%	1.6%
S. rosea (*S. purpurea* ssp. *venosa* var. *burkii*)	SAMN03354640	UGA5	Florida (FL2)	3,462,222	7,680	20,432	1,966	552	559	8.2	24,617	28.1%	0.7%
Sarracenia rubra	SAMN03354641	UGA42	Georgia (GA1)	4,306,451	9,641	68,012	4,981	630	560	12.4	102,209	12.6%	2.4%
Sarracenia rubra	SAMN03354642	UGA58	Georgia (GA2)	6,131,091	12,893	92,549	6,184	619	616	10.0	39,539	10.0%	0.6%
Sarracenia rubra	SAMN03354643	UGA37	Georgia (GA3)	7,058,643	11,006	114,837	5,910	603	716	24.4	224,356	10.2%	3.2%
Sarracenia rubra	SAMN03354644	UGA36	Georgia (GA4)	6,873,311	10,270	119,354	5,361	596	712	21.6	230,558	11.1%	3.4%
Sarracenia rubra	SAMN03354645	UGA36	Georgia (GA5)	4,507,115	7,159	91,534	5,922	595	658	13.4	97,204	10.0%	2.2%
Sarracenia rubra	SAMN03354646	UGA14	Georgia (GA6)	2,569,200	3,703	11,442	1,523	559	571	11.1	36,931	36.7%	1.4%
Sarracenia rubra	SAMN03354661	N/A	South Carolina (SC)	6,557,369	11,568	194,176	13,702	641	694	14.5	51,152	4.7%	0.8%
S. rubra ssp. *gulfensis*	SAMN03354647	UGA3	Florida (FL1)	3,532,833	5,514	40,406	2,866	590	590	15.6	155,947	20.6%	4.4%
S. rubra ssp. *gulfensis*	SAMN03354648	UGA29	Florida (FL2)	1,603,721	3,431	11,744	1,130	404	436	7.2	22,587	35.8%	1.4%
S. rubra ssp. *gulfensis*	SAMN03354649	UGA25	Florida (FL3)	3,391,802	6,597	50,304	4,517	579	486	9.8	78,747	12.8%	2.3%
S. rubra ssp. *wherryi*	SAMN03354650	UGA38	Alabama (AL)	3,294,081	6,715	76,146	5,459	607	616	12.1	103,787	11.1%	3.2%
Average				**3,466,575**	**7,124**	**67,894**	**5,608**	**546**	**503**	**10.9**	**57,062**	**13.2%**	**1.6%**

[a]UGA refers to the University of Georgia Herbarium and is followed by the collection number, N/A refers to individuals that died before vouchers were taken, TAES is the Texas A&M Herbarium

[b]This is the resulting reads after removal of adapter contamination, quality trimming, and reads with ambiguous bases

[c]Contigs "on-target" is the percentage of contigs that match targeted exons from total merged contigs

[d]Reads "on-target" is the percentage of reads from Total Contigs divided by Number of Trimmed Reads

APPENDIX C

SUPPLEMENTARY FIGURES AND TABLES FROM CHAPTER IV

Table S4.1. Mean prey proportions for each studied taxa by season and year. Orders were used for all analyses with the exception of Formicidae being distinguished from Hymenoptera, as this family has been known to be a primary food source for *Sarracenia* (see Ellison & Gotelli, 2009). Prey that comprised < 0.01 are not listed here, but were included in analyses. Examples of prey that were identified at lower taxomonic levels are listed under their respective orders. For analyses, prey was further divided into functional groups by either crawling or flying prey types. Coleoptera, Orthoptera, and Hemiptera were not included in these designations.

	Spring 2014		Fall 2014		Spring 2015		Fall 2015	
Species	Prey Types	Functional groups	Prey Types	Functional groups	Prey Types	Functional groups	Prey Types	Functional groups
S. alabamensis	Diptera – 0.42 Hymenoptera - 0.06 Vespidae, *Bombus sp.* Coleoptera – 0.30 Coccinellidae, Elateridae, Curculionidae, Hemiptera – 0.20 Arachnida – 0.02	Crawling – 0.02 Flying – 0.51	Lepidoptera – 0.71 Diptera – 0.19 Hymenoptera - 0.04 Braconidae, Vespidae, *Scolia dubia* Coleoptera – 0.03 Coccinellidae, Elateridae Hemiptera – 0.02 *Prosapia bicincta* Dermaptera – 0.01	Crawling – 0.01 Flying – 0.94	Hemiptera – 0.27 Coleoptera – 0.23 Coccinellidae Hymenoptera - 0.20 Diptera – 0.15 Formicidae – 0.12 Lepidoptera – 0.03	Crawling – 0.12 Flying – 0.38	Diptera – 0.77 Lepidoptera – 0.15 Coleoptera – 0.04 Curculionidae, Coccinellidae Hymenoptera - 0.02 Vespidae Diplopoda – 0.01	Crawling – 0.01 Flying – 0.94
S. alata	Diptera – 0.38 Coleoptera – 0.17 Coccinellidae, Cantharidae Hemiptera - 0.13 Formicidae – 0.09 Dermaptera – 0.07 Arachnida – 0.04 Hymenoptera – 0.04 Neuroptera – 0.04 Lepidoptera – 0.03 Isopoda – 0.02 Armadillidiiae	Crawling – 0.21 Flying – 0.45	Lepidoptera – 0.87 Diptera – 0.05 Formicidae – 0.03 Arachnida – 0.02 Hymenoptera – 0.01 Vespidae Coleoptera – 0.01 Curculionidae Dermaptera - 0.01	Crawling – 0.06 Flying – 0.93	Coleoptera – 0.39 Coccinellidae, Elateridae, *Cotinis nitida* Diptera – 0.20 Hymenoptera – 0.15 Hemiptera – 0.14 Formicidae – 0.07 Lepidoptera – 0.05	Crawling – 0.07 Flying – 0.40	Diptera – 0.65 Lepidoptera – 0.18 Formicidae – 0.11 Coleoptera – 0.05 Coccinellidae, Curculionidae Hymenoptera – 0.01 Hemiptera – 0.01	Crawling – 0.11 Flying – 0.83
S. flava var. rubricorpora	Diptera – 0.38 Coleoptera – 0.22 Coccinellidae,	Crawling – 0.07 Flying – 0.51	Lepidoptera – 0.51 Coleoptera – 0.18 Coccinellidae,	Crawling – 0.05 Flying – 0.67	Coleoptera – 0.42 Coccinellidae, Curculionidae,	Crawling – 0.09 Flying – 0.20	Lepidoptera – 0.35 Diptera – 0.29 Coleoptera – 0.11	Crawling – 0.08 Flying – 0.73

	Lampyridae, Ceranbycidae, Cantharidae, Elateridae, *Cotinis nitida* Hemiptera – 0.20 Lepidoptera – 0.08 Hymenoptera – 0.05 Halticidae, *Sphecius speciosus* Formicidae – 0.05 Arachnida – 0.02 Blattodea – 0.01		Curculionidae, *Cotinis nitida* Hemiptera – 0.10 *P. bicincta* Hymenoptera – 0.09 Halticidae Diptera – 0.06 Formicidae – 0.04		Elateridae, *Megacopta cribraria* Hemiptera – 0.29 Diptera – 0.13 Formicidae – 0.09 Hymenoptera – 0.04 Halticidae, *Dolichovespula masculata* Lepidoptera – 0.03		Coccinellidae, Curculionidae, Elateridae, *Cotinis nitida* Hemiptera – 0.08 Formicidae – 0.08 Hymenoptera – 0.08 Halticidae	
S. flava var. rugelii	Diptera – 0.49 Tipulidae Hemiptera – 0.20 Reduviidae Coleoptera – 0.18 Coccinellidae, Ceranbycidae, Cantharidae, Elateridae, Curculionidae Lepidoptera – 0.06 Hymenoptera – 0.03 Halticidae Formicidae – 0.02 Arachnida – 0.02	Crawling – 0.04 Flying – 0.58	Lepidoptera – 0.65 Hymenoptera – 0.10 Halticidae, Apidae, *S. dubia* Hemiptera – 0.09 Pentatomidae, *Megacopta cribraria*, *P. bicincta* Coleoptera – 0.07 Coccinellidae Diptera – 0.06 Formicidae – 0.02	Crawling – 0.02 Flying – 0.81	Hemiptera – 0.33 Coleoptera – 0.30 Coccinellidae, Elateridae Diptera – 0.15 Formicidae – 0.11 Hymenoptera – 0.08 Lepidoptera – 0.03	Crawling – 0.11 Flying – 0.26	Lepidoptera – 0.44 Diptera – 0.33 Coleoptera – 0.08 Coccinellidae, Elateridae Hemiptera – 0.06 Formicidae – 0.05 Hymenoptera – 0.05 *Bombus sp.*	Crawling – 0.05 Flying – 0.82
S. leucophylla	Lepidoptera – 0.06 *Papilio glaucus* Diptera – 0.13 Hymenoptera – 0.37 Halticidae, Vespidae, *Bombus sp.* Coleoptera – 0.40 Coccinellidae, Cantharidae, Elateridae, Curculionidae, Scarabaeidae Formicidae – 0.02 Hemiptera – 0.01	Crawling – 0.03 Flying – 0.57	Lepidoptera – 0.41 Diptera – 0.35 Hymenoptera – 0.19 Halticidae, Apidae, Vespidae, *S. dubia* Coleoptera – 0.02 Curculionidae Formicidae – 0.02	Crawling – 0.02 Flying – 0.95	Coleoptera – 0.42 Coccinellidae, Curculionidae, *Cotinis nitida* Hymenoptera – 0.40 Halticidae, *Bombus sp.* Formicidae – 0.07 Hemiptera – 0.05 Diptera – 0.04 Lepidoptera – 0.02	Crawling – 0.07 Flying – 0.46	Diptera – 0.45 Syrphidae Lepidoptera – 0.27 Hesperiidae, *Papilio glaucus*, *Agraulis vanillae* Hymenoptera – 0.17 Halticidae, Apidae, *Bombus sp.* Coleoptera – 0.06 Coccinellidae, Curculionidae, *Cotinis nitida* Hemiptera – 0.03	Crawling – 0.01 Flying – 0.90

							Formicidae – 0.01	
S. minor	Diptera – 0.66 Formicidae – 0.25 Hymenoptera – 0.05 Coleoptera – 0.02 Dermaptera – 0.02	Crawling – 0.27 Flying – 0.71	Diptera – 0.69 Lepidoptera – 0.18 Formicidae – 0.07 Coleoptera – 0.02 Carabidae Orthoptera – 0.02 Gryllidae Hymenoptera – 0.01 Halticidae Arachnida – 0.01 Hemiptera – 0.01	Crawling – 0.08 Flying – 0.87	Diptera – 0.37 Formicidae – 0.25 Hemiptera – 0.22 Coleoptera – 0.12 Coccinellidae Lepidoptera – 0.02 Arachnida – 0.02 Orthoptera – 0.01	Crawling – 0.27 Flying – 0.38	Diptera – 0.72 Formicidae – 0.15 Lepidoptera – 0.06 Hymenoptera – 0.04 Coleoptera – 0.03 Arachnida – 0.02 Hemiptera – 0.01 Orthoptera – 0.01	Crawling – 0.15 Flying – 0.81
S. minor var. okefenokeensis	Diptera – 0.73 Syrphidae Formicidae – 0.11 Hemiptera – 0.10 Coleoptera – 0.03 Dermaptera – 0.02 Lepidoptera – 0.01	Crawling – 0.14 Flying – 0.74	Diptera – 0.50 Tipulidae Lepidoptera – 0.42 Formicidae – 0.03 Coleoptera – 0.03 Coccinellidae, Curculionidae Hymenoptera – 0.02 Halticidae, Braconidae, Vespidae Hemiptera – 0.01 Reduviidae, *P. bicincta*	Crawling – 0.03 Flying – 0.94	Diptera – 0.44 Hemiptera – 0.22 Formicidae – 0.16 Hymenoptera – 0.09 Halticidae Coleoptera – 0.05 Lepidoptera – 0.04 Neuroptera – 0.01	Crawling – 0.16 Flying – 0.57	Diptera – 0.83 Lepidoptera – 0.11 Formicidae – 0.04 Hymenoptera – 0.01 Coleoptera – 0.01 Coccinellidae, Curculionidae	Crawling – 0.04 Flying – 0.95
S. oreophila	Diptera – 0.34 Syrphidae Coleoptera – 0.23 Coccinellidae, Curculionidae, Elateridae, Cantharidae, Scarabaeidae Hymenoptera – 0.22 *Bombus sp.* Lepidoptera – 0.09 *Agraulis vanillae* Formicidae – 0.09 Hemiptera – 0.03 Arachnida – 0.01 Salticidae	Crawling – 0.10 Flying – 0.65	Diptera – 0.55 Lepidoptera – 0.16 Formicidae – 0.10 Hymenoptera – 0.06 Halticidae, Braconidae Hemiptera – 0.06 *P. bicincta* Coleoptera – 0.02 Curculionidae Orthoptera – 0.02 Arachnida – 0.01 Gastropoda – 0.01	Crawling – 0.12 Flying – 0.77	Hymenoptera – 0.27 Halticidae, *Bombus sp.*, *Sphecius speciosus* Formicidae – 0.21 Lepidoptera – 0.13 Diptera – 0.08 Hemiptera – 0.07 Coleoptera – 0.02 Curculionidae, Coccinellidae, Elateridae, *Cotinis nitida*	Crawling – 0.21 Flying – 0.47	Diptera – 0.55 Formicidae – 0.12 Hymenoptera – 0.09 Gastropoda – 0.08 Coleoptera – 0.08 Lepidoptera – 0.07 Hemiptera – 0.02 Reduviidae	Crawling – 0.20 Flying – 0.71

S. psittacina	Collembola – 0.50 Isopoda – 0.50 Armadillidiiae	Crawling – 1.00 Flying – 0.00	Formicidae – 0.50 Diptera – 0.25 Diplopoda – 0.25	Crawling – 0.75 Flying – 0.25	Formicidae – 0.89 Arachnida– 0.11	Crawling – 1.00 Flying – 0.00	Formicidae – 1.00	Crawling – 1.00 Flying – 0.00
S. purpurea ssp. venosa	Gastropoda – 0.47 Formicidae – 0.22 Collembola – 0.18 Isopoda – 0.06 Armadillidiiae Diplopoda – 0.04 Coleoptera – 0.01 Elateridae Arachnida – 0.01	Crawling – 0.99 Flying – 0.00	Formicidae – 0.49 Gastropoda – 0.12 Coleoptera – 0.09 Carabidae Hymenoptera – 0.07 Diptera – 0.07 Hemiptera – 0.07 Reduviidae Diplopoda – 0.05 Arachnida – 0.02 Salticidae Orthoptera – 0.02	Crawling – 0.68 Flying – 0.14	Formicidae – 0.61 Arachnida – 0.14 Diplopoda – 0.11 Diptera – 0.05 Gastropoda – 0.04 Coleoptera – 0.03 Hymenoptera – 0.02	Crawling – 0.90 Flying – 0.07	Formicidae – 0.74 Gastropoda – 0.08 Orthoptera – 0.07 Coleoptera – 0.06 Diptera – 0.05 Hymenoptera – 0.02	Crawling – 0.82 Flying – 0.05
S. purpurea ssp. venosa var. burkii	Gastropoda – 0.47 Formicidae – 0.15 Diplopoda – 0.14 Diptera – 0.14 Hemiptera – 0.05 *P. bicincta* Coleoptera – 0.02 Arachnida – 0.02 Orthoptera – 0.01 Lepidoptera – 0.01	Crawling – 0.78 Flying – 0.15	Formicidae – 0.63 Gastropoda – 0.13 Diptera – 0.09 Hemiptera – 0.08 Reduviidae, *P. bicincta* Coleoptera – 0.03 Carabidae Arachnida – 0.02 Salticidae Diplopoda – 0.01 Orthoptera – 0.01	Crawling – 0.80 Flying – 0.09	Formicidae – 0.62 Diplopoda – 0.13 Gastropoda – 0.12 Hemiptera – 0.07 Coleoptera – 0.06 Scarabaeidae	Crawling – 0.87 Flying – 0.00	Formicidae – 0.50 Orthoptera – 0.15 Gastropoda – 0.13 Diplopoda – 0.12 Coleoptera – 0.08 Scarabaeidae Hemiptera – 0.03 Lepidoptera – 0.01	Crawling – 0.75 Flying – 0.01
S. purpurea ssp. venosa var. montana	Diplopoda – 0.34 Formicidae – 0.33 Arachnida – 0.14 Diptera – 0.10 Gastropoda – 0.06 Coleoptera – 0.03 Arachnida – 0.01 *Vaejovis carolinianus*	Crawling – 0.87 Flying – 0.10	Formicidae – 0.48 Gastropoda – 0.18 Diplopoda – 0.16 Coleoptera – 0.09 Carabidae Orthoptera – 0.08 Gryllidae	Crawling – 0.82 Flying – 0.00	Formicidae – 0.52 Gastropoda – 0.31 Diplopoda – 0.07 Coleoptera – 0.05 *Cotinis nitida* Orthoptera – 0.03 Arachnida – 0.01 Isopoda – 0.01 Armadillidiiae	Crawling – 0.92 Flying – 0.00	Formicidae – 0.41 Hymenoptera – 0.01 Diptera – 0.02 Lepidoptera – 0.01 Hemiptera – 0.01 Gastropoda – 0.28 Diplopoda – 0.15 Coleoptera – 0.07 Isopoda – 0.01 Armadillidiiae	Crawling – 0.89 Flying – 0.03
S. rubra ssp. gulfensis	n/a	n/a	n/a	n/a	Formicidae – 0.23 Diptera – 0.09 Hemiptera – 0.19 Hymenoptera – 0.08 Lepidoptera – 0.15 Coleoptera – 0.26 Coccinellidae,	Crawling – 0.23 Flying – 0.32	Diptera – 0.52 Formicidae – 0.26 Lepidoptera – 0.13 Hymenoptera – 0.06 Coleoptera – 0.03	Crawling – 0.26 Flying – 0.71

					Elateridae			
S. rubra ssp. jonesii	Coleoptera – 0.37 Diptera – 0.28 Lepidoptera – 0.17 Neuroptera – 0.13 Hymenoptera – 0.03 Hemiptera – 0.03	Crawling – 0.00 Flying – 0.60	Lepidoptera – 0.50 Diptera – 0.48 Hymenoptera – 0.01 Coleoptera – 0.01	Crawling – 0.00 Flying – 0.99	Diptera – 0.32 Hemiptera – 0.28 Hymenoptera – 0.23 Lepidoptera – 0.08 Coleoptera – 0.08 Coccinellidae	Crawling – 0.00 Flying – 0.64	Diptera – 0.61 Lepidoptera – 0.32 Hymenoptera – 0.05 Vespidae, Halticidae, *Bombus sp*. Formicidae – 0.02 Coleoptera – 0.01	Crawling – 0.02 Flying – 0.97
S. rubra ssp. wherryi	Diptera – 0.49 Hemiptera – 0.29 Dermaptera – 0.14 Coleoptera – 0.04 Elateridae Arachnida – 0.04	Crawling – 0.18 Flying – 0.51	Lepidoptera – 0.62 Diptera – 0.23 Bombyliidae Hymenoptera – 0.16 Vespidae, Halticidae, Apidae	Crawling – 0.00 Flying – 1.00	Formicidae – 0.49 Coleoptera – 0.18 Hymenoptera – 0.08 Diptera – 0.07 Arachnida – 0.06 Hemiptera – 0.06 Gastropoda – 0.04 Lepidoptera – 0.03	Crawling – 0.59 Flying – 0.17	Lepidoptera – 0.28 Coleoptera – 0.23 Diptera – 0.21 Orthoptera – 0.13 Formicidae – 0.13 Hymenoptera – 0.03	Crawling – 0.13 Flying – 0.53
Total	Diptera – 0.34 Coleoptera – 0.15 Formicidae – 0.10 Hemiptera – 0.09 Gastropoda – 0.08 Hymenoptera – 0.07 Lepidoptera – 0.04 Diplopoda – 0.04 Arachnida – 0.02 Dermaptera – 0.02 Isopoda – 0.02 Collembola – 0.02 Neuroptera – 0.01 Orthoptera – <0.01	Crawling – 0.30 Flying – 0.45	Lepidoptera – 0.37 Diptera – 0.27 Formicidae – 0.16 Hymenoptera – 0.06 Coleoptera – 0.04 Hemiptera – 0.03 Gastropoda – 0.03 Diplopoda – 0.03 Arachnida – 0.01 Orthoptera – 0.01 Dermaptera – <0.01 Neuroptera – <0.01 Blattodea – <0.01	Crawling – 0.22 Flying – 0.70	Formicidae – 0.29 Coleoptera – 0.19 Hemiptera – 0.15 Diptera – 0.14 Hymenoptera – 0.11 Lepidoptera – 0.04 Gastropoda – 0.03 Diplopoda – 0.02 Arachnida – 0.02 Isopoda – <0.01 Neuroptera – <0.01 Orthoptera – <0.01 Blattodea – <0.01	Crawling – 0.36 Flying – 0.30	Diptera – 0.42 Formicidae – 0.20 Lepidoptera – 0.17 Coleoptera – 0.07 Gastropoda – 0.04 Hymenoptera – 0.04 Hemiptera – 0.03 Diplopoda – 0.02 Orthoptera – 0.02 Arachnida – 0.01 Neuroptera – <0.01 Isopoda – <0.01	Crawling – 0.26 Flying – 0.63

Table S4.2. Mean phenotypic traits of the studied taxa.

Species	Total height (cm)	Height to peristome (cm)	Peristome width (cm)	Ala width (cm)	Bottom trichome density (per 1.5mm^2)	Middle trichome density (per 1.5mm^2)	Lip trichome density (per 1.5mm^2)	Top hood trichome density (per 1.5mm^2)	Bottom hood trichome density (per 1.5mm^2)
S. alabamensis	42.06	37.19	3.14	1.00	0.09	3.00	13.06	1.81	17.28
S. alata	47.58	43.77	2.37	0.96	0.41	2.66	13.63	4.72	20.78
S. flava var. rubricorpora	60.86	54.59	5.00	0.69	0.00	1.47	14.94	6.19	66.03
S. flava var. rugelii	60.41	54.34	5.18	1.00	0.00	2.72	17.50	7.03	68.47
S. leucophylla	67.48	61.66	4.01	0.67	0.00	0.00	11.00	10.44	5.84
S. minor	22.11	19.83	1.40	1.18	2.78	8.47	16.25	3.84	21.91
S. minor var. okefenokeensis	48.56	45.06	2.00	1.82	0.00	0.84	8.19	1.25	18.44
S. oreophila	31.06	26.70	3.21	0.45	2.91	8.81	15.84	3.53	28.81
S. psittacina	2.79	1.12	0.53	1.39	0.00	0.00	0.03	0.00	4.65
S. purpurea ssp. venosa	4.34	2.60	2.22	1.14	4.85	9.36	12.15	7.39	2.94
S. purpurea ssp. venosa var. burkii	5.90	3.79	2.88	2.05	7.50	12.13	14.03	9.94	3.44
S. purpurea ssp. venosa var. montana	6.01	3.65	2.40	2.84	0.45	6.94	10.19	5.77	3.23
S. rubra ssp. gulfensis	41.46	39.08	1.88	1.00	0.00	0.00	8.88	0.00	16.56
S. rubra ssp. jonesii	50.62	46.85	2.19	0.54	0.00	0.16	16.63	5.47	22.47
S. rubra ssp. wherryi	28.93	25.73	1.84	0.88	2.00	6.09	19.97	5.72	21.09

Table S4.3. Results from the perMANOVA analysis comparing trait variation within between seasons and years. Bolded underline values indicate significance at 1000 permutations.

	Year by year comparison		Season (Fall vs. Spring) within year comparison	
Species	Spring vs. Spring	Fall vs. Fall	2014	2015
alabamensis	**F=2.93, r^2=0.17, p=0.04**	F=1.94, r^2=0.12, p=0.16	**F=4.76, r^2=0.25, p=0.01**	**F=8.38, r^2=0.37, p<0.01**
alata	F=2.20, r^2=0.14, p=0.10	F=1.89, r^2=0.12, p=0.13	**F=4.47, r^2=0.24, p<0.01**	F=1.50, r^2=0.10, p=0.26
flava var. *rubricorpora*	F=2.59, r^2=0.16, p=0.06	F=2.57, r^2=0.16, p=0.07	F=0.65, r^2=0.04, p<0.58	F=1.04, r^2=0.07, p=0.32
flava var. *rugelii*	**F=6.63, r^2=0.32, p<0.01**	F=2.47 r^2=0.15, p=0.07	F=1.18, r^2=0.08, p=0.33	F=1.84, r^2=0.12, p=0.20
leucophylla	**F=14.14, r^2=0.50, p<0.01**	**F=6.07 r^2=0.30, p<0.01**	**F=14.93, r^2=0.52, p<0.01**	**F=6.90, r^2=0.33, p<0.01**
minor	F=1.03, r^2=0.07, p=0.35	F=1.77 r^2=0.11, p=0.15	**F=2.96 r^2=0.17, p=0.03**	F=0.96, r^2=0.06, p=0.37
minor var. *okefenokeensis*	**F=11.81, r^2=0.46, p<0.01**	**F=6.20, r^2=0.31, p=0.01**	F=1.71, r^2=0.11, p=0.18	F=2.73, r^2=0.16, p=0.06
oreophila	F=2.32, r^2=0.14, p=0.10	F=1.55, r^2=0.10, p=0.21	**F=22.59, r^2=0.62, p<0.01**	**F=7.58, r^2=0.35, p<0.01**
psittacina	F=1.60, r^2=0.10, p=0.21	F=2.49, r^2=0.14, p=0.07	F=1.31, r^2=0.09, p=0.28	F=0.88, r^2=0.05, p=0.40
purpurea ssp. *venosa*	F=2.18, r^2=0.13, p=0.09	F=0.70, r^2=0.04, p=0.63	F=0.16, r^2=0.01, p=0.88	F=0.56, r^2=0.04, p=0.70
purpurea ssp. *venosa* var. *burkii*	F=1.80, r^2=0.11, p=0.13	F=0.98, r^2=0.07, p=0.39	F=1.10, r^2=0.07, p=0.34	F=0.14, r^2=0.09, p=0.24
purpurea ssp. *venosa* var. *montana*	F=1.95, r^2=0.13, p=0.12	F=0.33, r^2=0.02, p=0.90	F=1.60, r^2=0.10, p=0.14	**F=7.23, r^2=0.36, p<0.01**
rubra ssp. *jonesii*	**F=14.00, r^2=0.50, p<0.01**	**F=4.23, r^2=0.23, p=0.03**	F=1.06, r^2=0.07, p=0.39	F=2.44, r^2=0.15, p=0.09
rubra ssp. *gulfensis*	*n/a*	*n/a*	*n/a*	F=1.83, r^2=0.12, p=0.16
rubra ssp. *wherryi*	**F=3.36, r^2=0.19, p=0.04**	**F=6.67, r^2=0.32, p<0.01**	**F=7.23, r^2=0.34, p<0.01**	F=5.17, r^2=0.27, p=0.09

Table S4.4. Results from the perMANOVA analysis comparing within species variation in prey composition between seasons and years. Bolded underline values indicate significance at 1000 permutations.

	Year by year comparison		Season (Fall vs. Spring) within year comparison	
Species	Spring vs. Spring	Fall vs. Fall	2014	2015
alabamensis	F=1.12, r^2=0.08, p=0.36	**F=51.93, r^2=0.79, p<0.01**	**F=14.71, r^2=0.53, p<0.01**	**F=10.34, r^2=0.43, p<0.01**
alata	**F=2.74, r^2=0.16, p=0.04**	**F=38.74, r^2=0.75, p<0.01**	**F=29.31, r^2=0.69, p<0.01**	**F=11.36, r^2=0.45, p<0.01**
flava var. *rubricorpora*	**F=7.28, r^2=0.34, p<0.01**	**F=3.01 r^2=0.19, p=0.04**	**F=19.15, r^2=0.60, p<0.01**	**F=9.81, r^2=0.41, p<0.02**
flava var. *rugelii*	**F=8.07, r^2=0.36, p<0.01**	**F=3.75 r^2=0.22, p=0.04**	**F=28.24, r^2=0.68, p<0.01**	**F=15.21, r^2=0.52, p<0.01**
leucophylla	F=0.85, r^2=0.06, p=0.46	**F=4.93 r^2=0.28, p=0.01**	**F=9.90, r^2=0.43, p<0.01**	**F=13.82, r^2=0.50, p<0.01**
minor	F=2.23, r^2=0.14, p=0.12	F=1.84 r^2=0.12, p=0.16	**F=2.84, r^2=0.17, p=0.05**	**F=3.95, r^2=0.22, p<0.01**
minor var. *okefenokeensis*	F=2.11, r^2=0.14, p=0.11	**F=22.64, r^2=0.64, p<0.01**	**F=13.60, r^2=0.53, p<0.01**	**F=5.40, r^2=0.28, p<0.01**
oreophila	F=2.03, r^2=0.13, p=0.12	F=0.62, r^2=0.04, p=0.64	**F=3.96, r^2=0.22, p<0.01**	**F=6.19, r^2=0.31, p<0.01**
psittacina	F=4.99, r^2=0.38, p=0.06	F=0.80, r^2=0.17, p=1.00	F=1.33, r^2=0.25, p=0.47	F=0.35, r^2=0.04, p=0.84
purpurea ssp. *venosa*	**F=5.23, r^2=0.29, p<0.01**	F=0.86, r^2=0.06, p=0.49	**F=2.86, r^2=0.18, p=0.02**	F=0.75, r^2=0.05, p=0.63
purpurea ssp. *venosa* var. *burkii*	**F=5.40, r^2=0.28, p=0.01**	F=1.28, r^2=0.08, p=0.26	**F=5.79, r^2=0.29, p<0.01**	F=0.52, r^2=0.04, p=0.82
purpurea ssp. *venosa* var. *montana*	**F=4.63, r^2=0.26, p<0.01**	F=2.45, r^2=0.17, p=0.07	**F=3.70, r^2=0.24, p<0.01**	F=1.07, r^2=0.08, p=0.42
rubra ssp. *jonesii*	**F=2.71, r^2=0.16 p=0.04**	F=3.38, r^2=0.19, p=0.09	**F=5.64, r^2=0.29, p<0.01**	**F=6.00, r^2=0.30, p<0.01**
rubra ssp. *gulfensis*	*n/a*	*n/a*	*n/a*	**F=3.45, r^2=0.20, p=0.02**
rubra ssp. *wherryi*	**F=4.84, r^2=0.27, p<0.01**	**F=2.70, r^2=0.16, p=0.03**	**F=5.64, r^2=0.29, p<0.01**	**F=6.01, r^2=0.30, p<0.01**

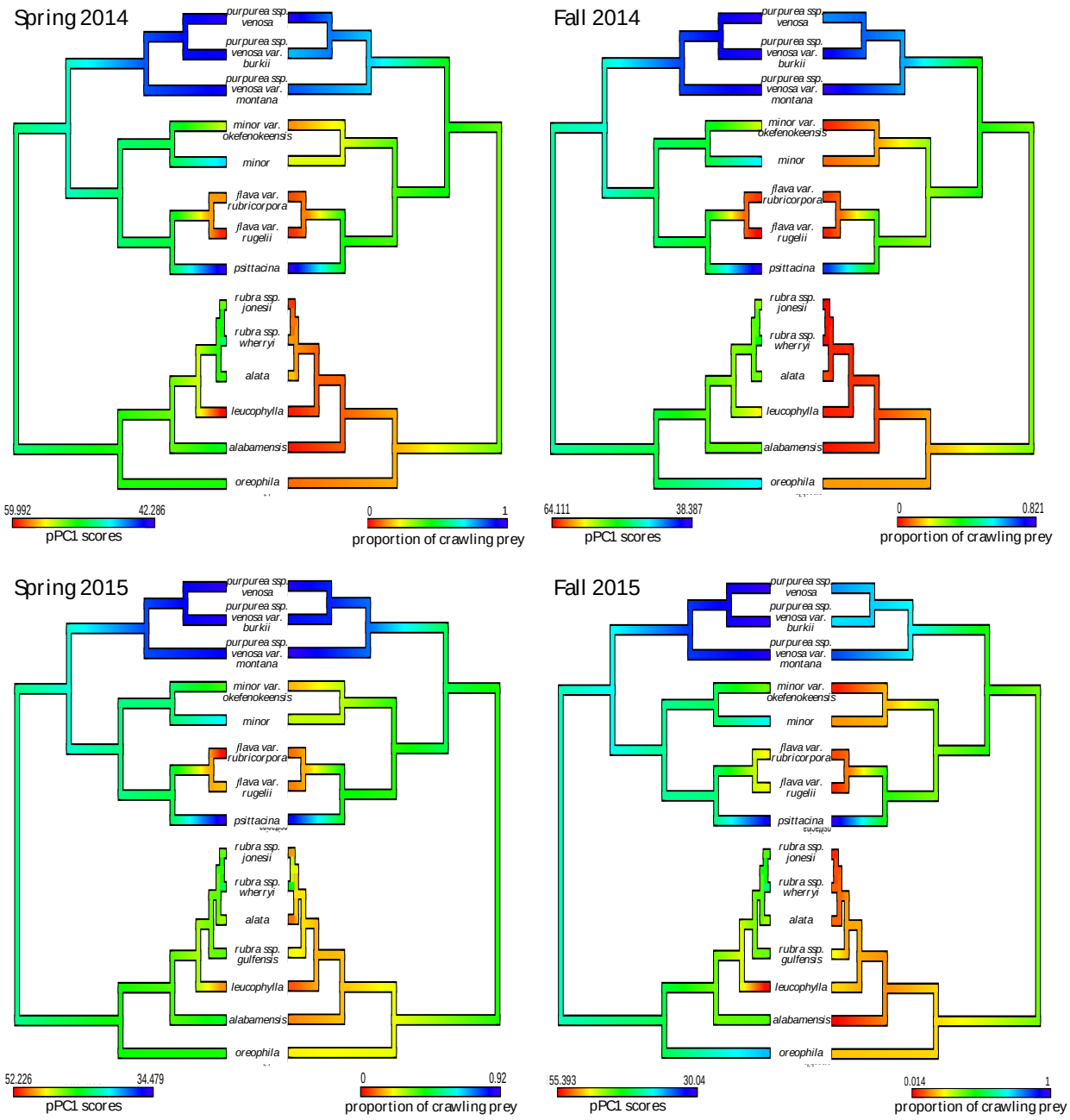

Figure S4.1. Visual representation of pPC1 scores for each species from Fig 4.4 (left side of figure) and average of the proportion of crawling prey captured for each species (right side of figure). Visuals for each sampling time point are depicted.

APPENDIX D

SUPPLEMENTARY FIGURES FROM CHAPTER V

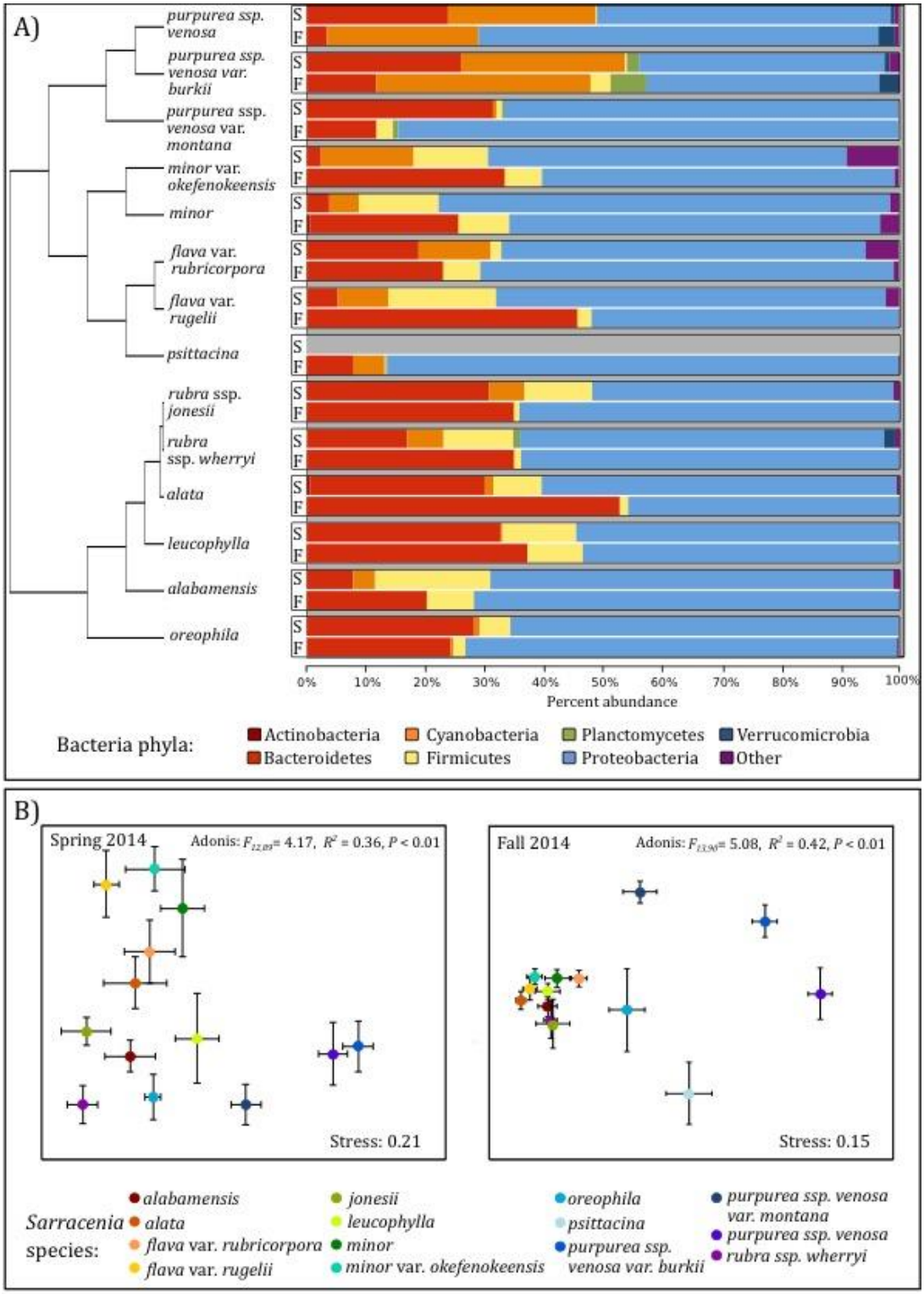

Figure S5.1. Bacterial composition of *Sarracenia* species for 2014 sampling period. A) Bacterial composition at the phylum level across the *Sarracenia* phylogeny. For each species spring (S) and fall (F) are shown. B) Non-metric multidimensional scaling (NMDS) plots at the 97% OTU level for unweighted UniFrac bacterial communities with standard error measures.

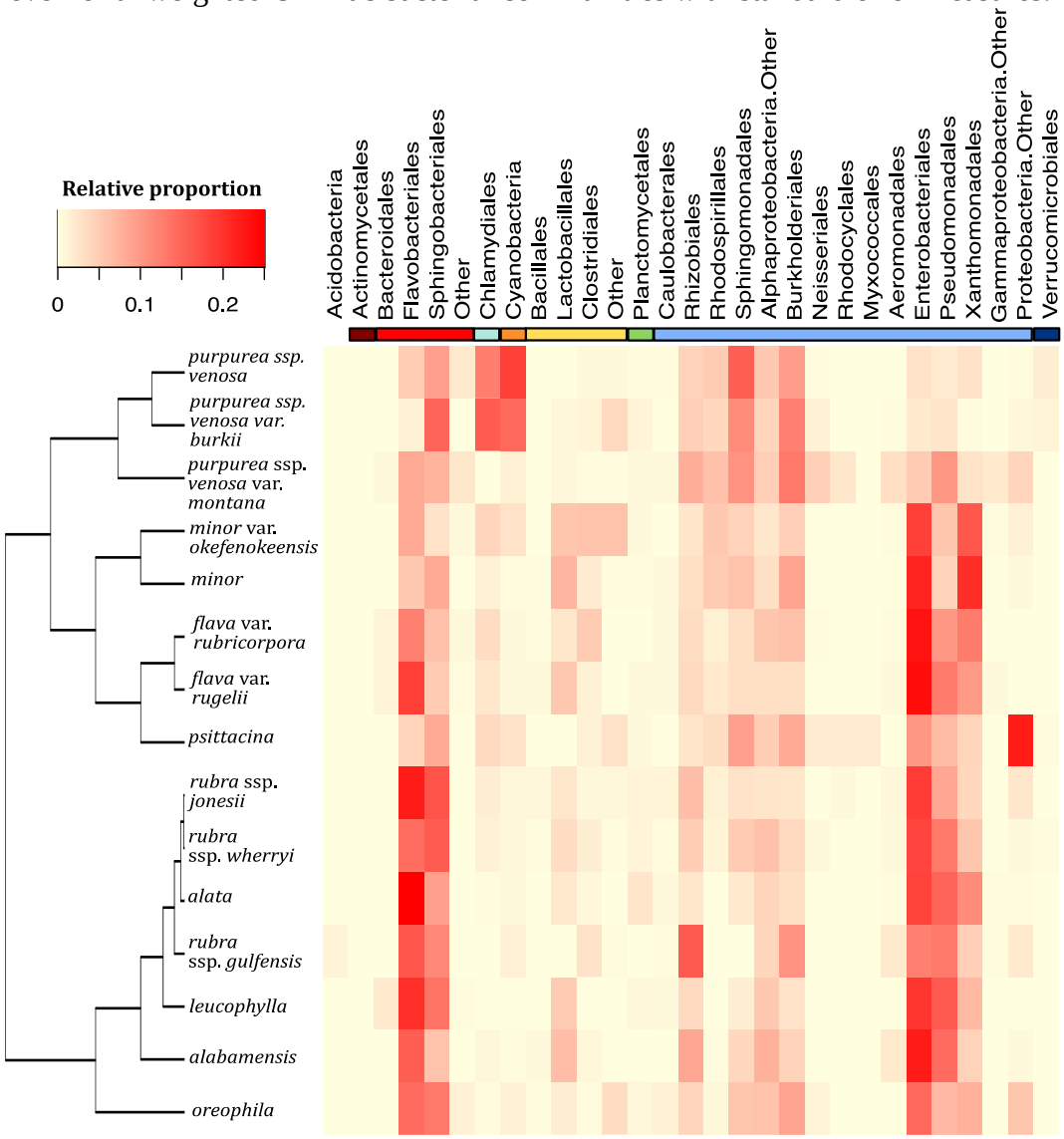

Figure S5.2. Heat map of relative abundance of bacteria orders over all samples for each *Sarracenia* species. Phylogenetic relationships of *Sarracenia* are depicted on the left. Bacteria phyla are represented under the order names. Colors correspond to Figure 5.1.

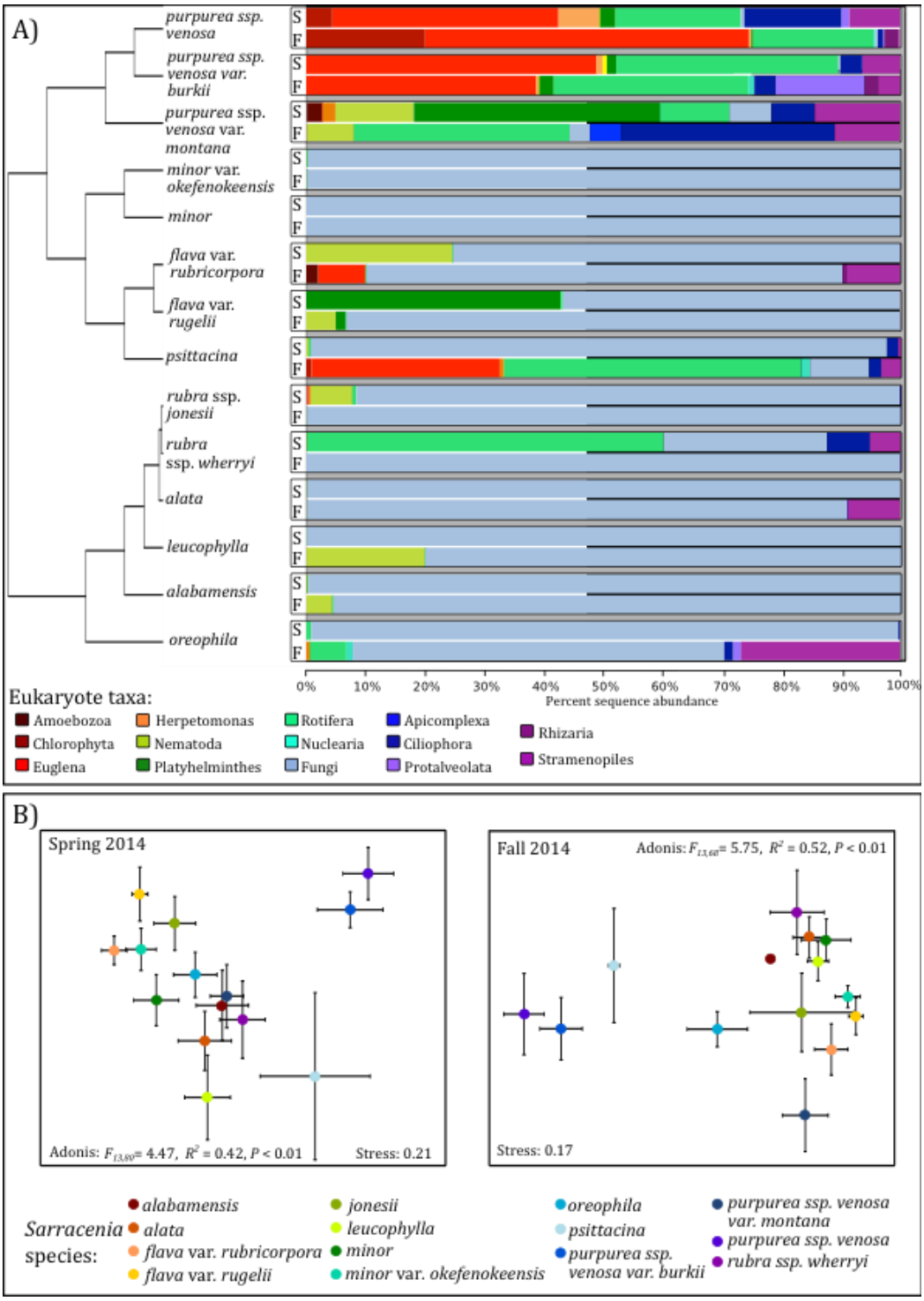

Figure S5.3. Eukaryote sequence composition of *Sarracenia* species for 2014 sampling period. A) Eukaryote composition at varying taxonomic levels across the *Sarracenia* phylogeny. For each species spring (S) and fall (F) are shown. B) Non-metric multidimensional scaling (NMDS) plots for eukaryote communities with standard error measures. There are no standard error measurements for *S. alabamensis* in the fall sampling period due to low sequence reads.

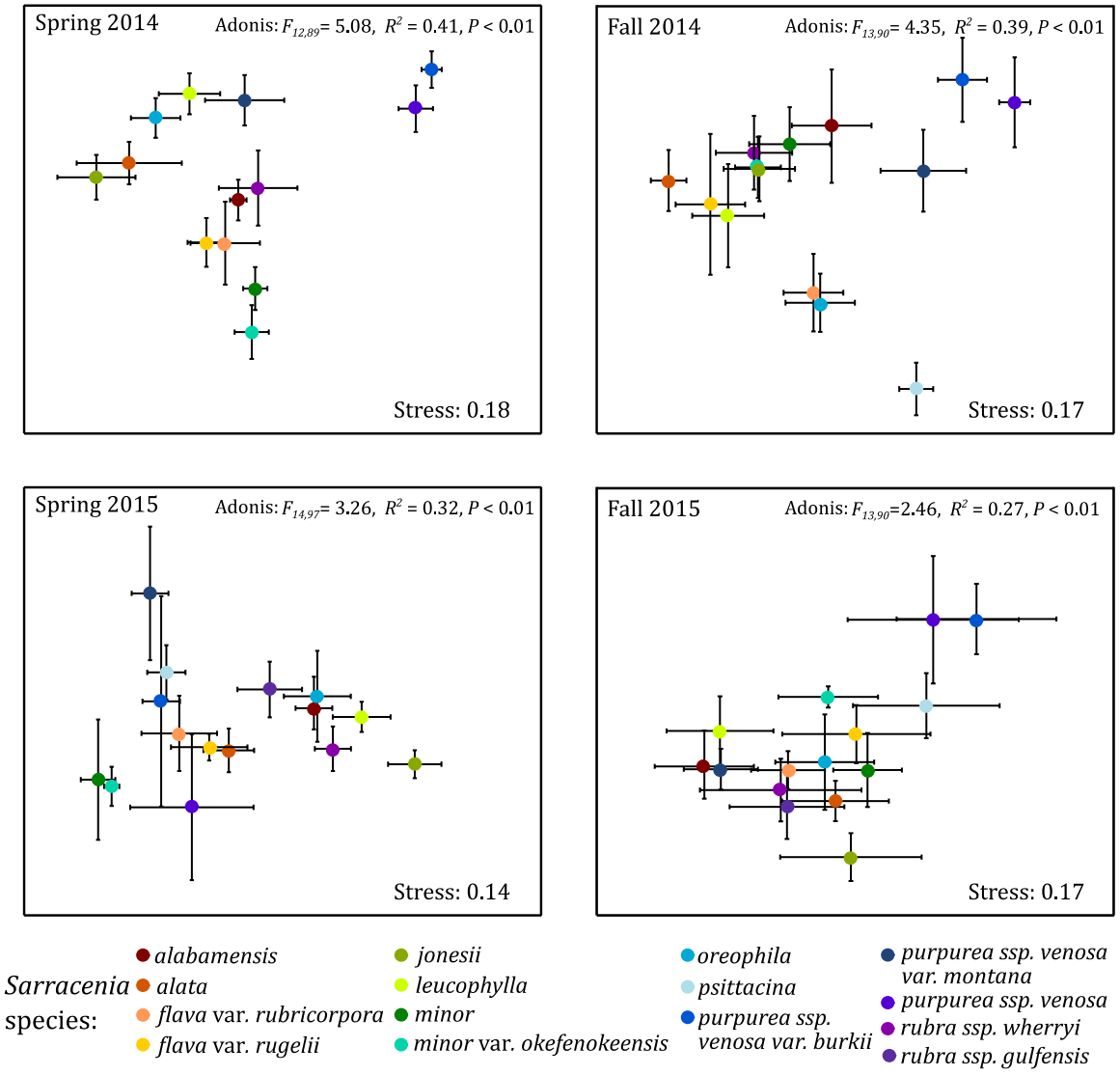

Figure S5.4. Non-metric multidimensional scaling (NMDS) plots at the 97% OTU level for weighted UniFrac bacterial communities with standard error measures across sampling periods.

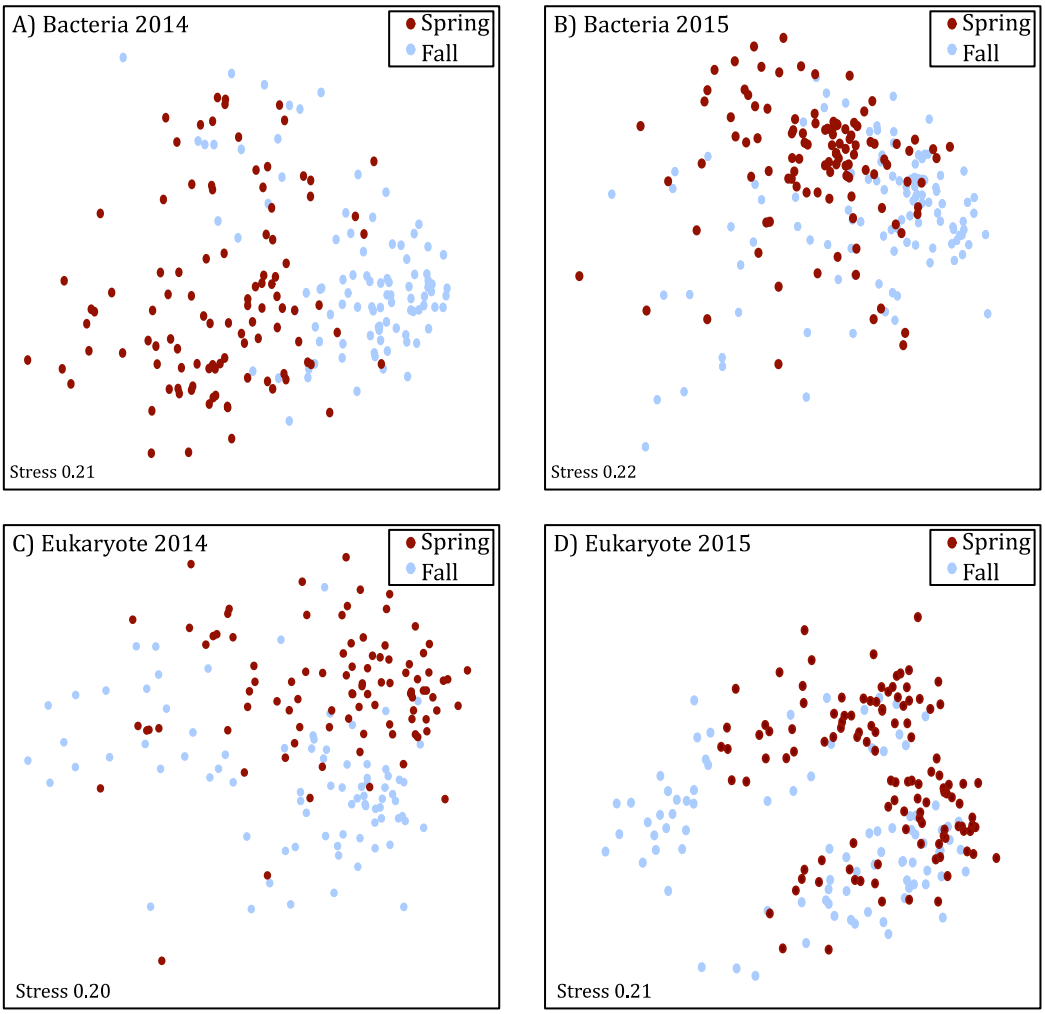

Figure S5.5. Non-metric multidimensional scaling (NMDS) plots at the 97% OTU level for unweighted UniFrac bacteria (A, B) and eukaryote (C, D) communities across seasons.

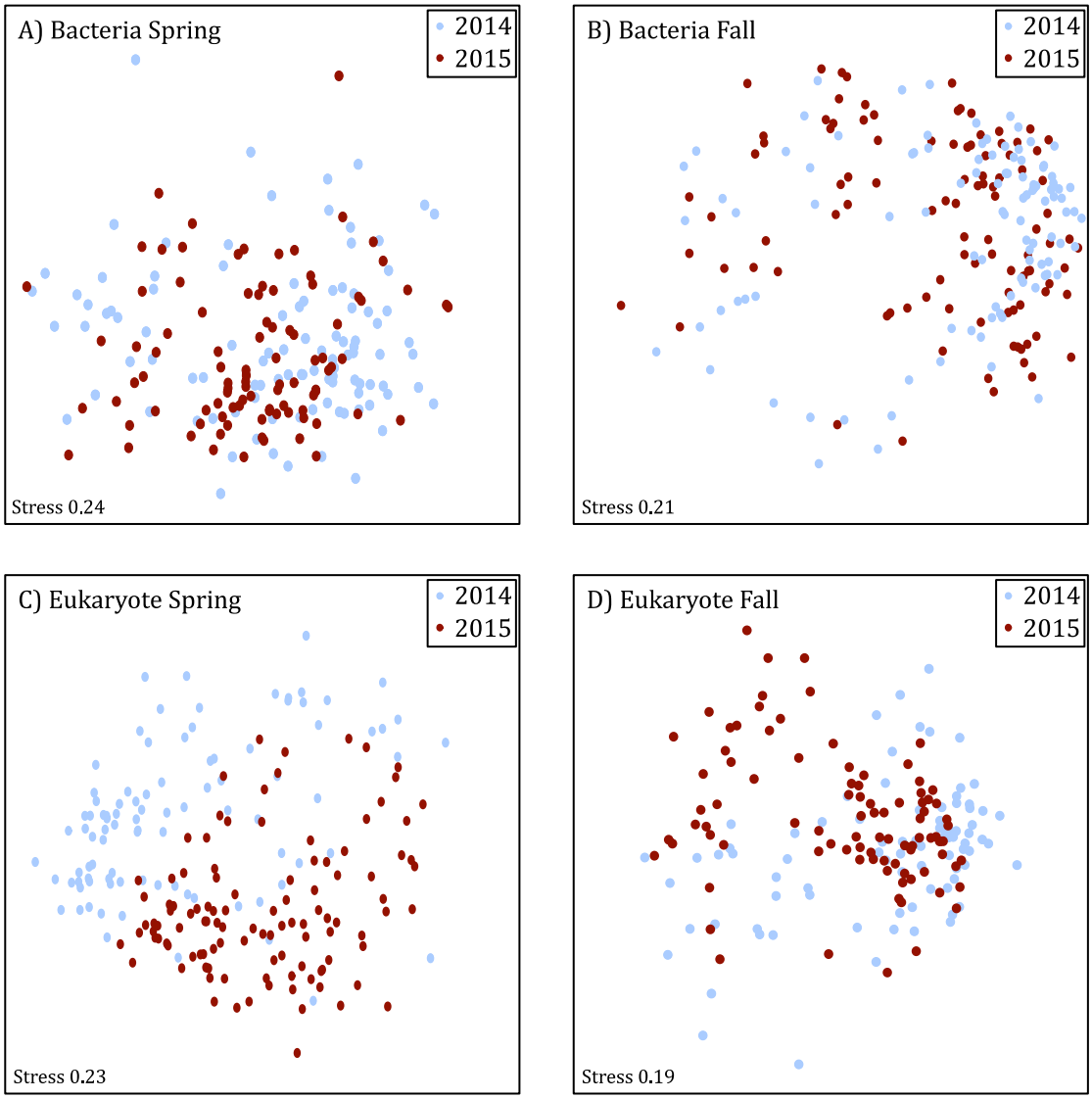

Figure S5.6. Non-metric multidimensional scaling (NMDS) plots at the 97% OTU level for unweighted UniFrac bacteria (A, B) and eukaryote (C, D) communities across years.