A Phylogenetic and Geographic Analysis of Leaflet Anatomy in *Zamia* (Cycadales: Zamiaceae)

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- Leaflet anatomy in the cycad genus *Zamia* displays **striking variation** within a **consistent core bauplan.**
- Most of the anatomical characters examined are **not phylogenetically informative**, but the presence of **sclereid-like cells** may prove diagnostic for one clade.

Premise

Cycads in the genus *Zamia* (81 spp.) display a high level of morphological convergence that has historically frustrated efforts to elucidate fine-scale species relationships. As such, these relationships have only recently been resolved through phylogenetic analyses of DNA-based character data. However, it remains unknown whether leaflet *anatomical* variation corresponds to phylogeny and geography, as has been investigated in other major cycad genera. **This project presents a broad comparative survey of leaflet anatomy across 20 *Zamia* species with the goals of defining diagnostic characters and understanding character evolution.**

Significance

Cycads are among the most ancient lineages of extant seed-bearing plants and are the most threatened plant order on Earth, with circa 75% of the 355 accepted species threatened with extinction. The knowledge obtained through this project improves our understanding of micromorphological character evolution, anatomical diversity, and phylogenetic relationships within the second-largest cycad genus. With this information, we may better understand phenotypic evolution and facilitate the identification, classification, and conservation of these highly vulnerable plants.

Methods

Plant material was sampled form the living collections at Montgomery Botanical Center and Fairchild Tropical Botanical Garden. Leaflets from 20 *Zamia* species covering all major clades and spanning the geographic distribution of the genus were chosen for sectioning. Transverse and longitudinal preparations were stained to elucidate structures, observed under light microscopy, and scored for a range of anatomical characters. Selected characters were analyzed in the context of published phylogenetic relationships and geographic distributions.

Key Findings

Zamia leaflet anatomy shows widespread anatomical variation within a consistent core bauplan. Most anatomical characters have a high degree of homoplasy consistent with variation seen in gross leaflet morphology. A newly reported mesophyll cell type with thick walls and conspicuous pitting ("flat tire" cells) may prove diagnostic for the clade consisting of species from South America and the Isthmus of Panama plus *Zamia soconuscensis*.

Next Steps

The incongruence between anatomy and phylogeny indicates that we must look elsewhere to enhance our understanding of the distribution of anatomical traits in *Zamia*. Future studies comparing specific anatomical characters to habitat or climatic variables may reveal patterns not apparent in the phylogeny. Additional analysis is also required to determine the composition, function, and distribution of the sclereid-like "flat tire" cells across the entire genus.

Acknowledgments

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Full text and references:

1 Leaflet morphology is highly variable, but most whole-leaflet traits are only diagnostic in combination with other characters.

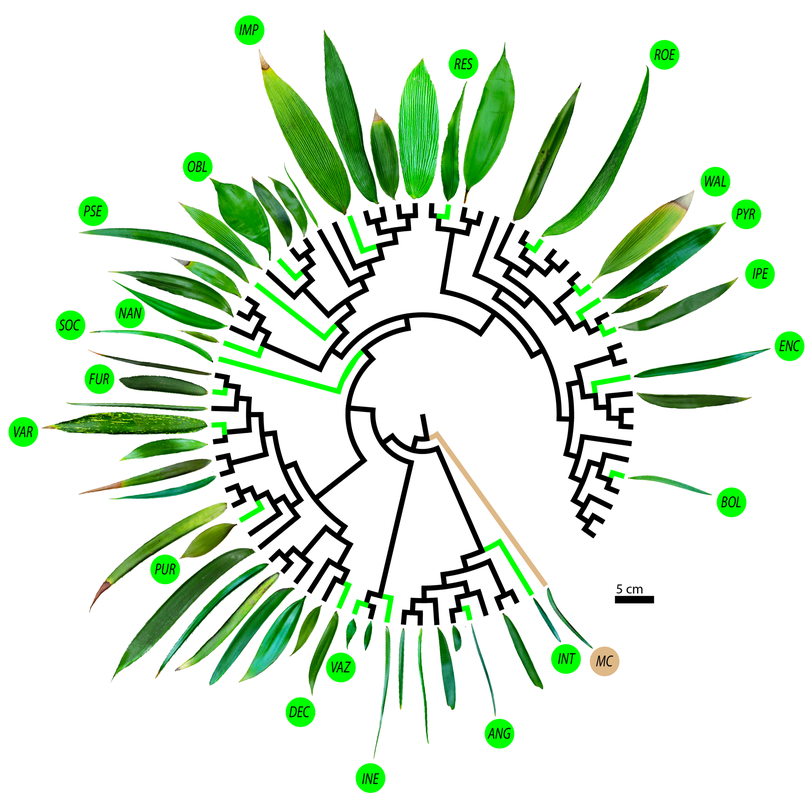

Fig. 1. Leaflet morphological variation across the genus *Zamia*. Leaflets from 52 *Zamia* species plus *Microcycas calocoma* (MC) shown alongside the published cladogram (Calonje et al. 2019). Green branches represent *Zamia* species sectioned in this study and brown branch indicates the outgroup. Abbreviations: INT: (*Zamia) integrifolia,* ANG: *angustifolia,* INE: *inermis*, VAZ: *vazquezii,* DEC: *decumbens*, PUR: *purpurea*, VAR: *variegata*, FUR: *furfuracea*, SOC: *soconuscensis,* NAN: *nana,* PSE: *pseudoparasitica,* OBLL: *obliqua*, IMP: *imperialis*, RES: *restrepoi*, ROE: *roezlii,* WAL: *wallisii,* PYR: *pyrophylla,* IPE: *ipetiensis,* ENC: *encephalartoides*, BOL: *boliviana.* Follow QR code for accession information.

2 All *Zamia* species examined follow the same general pattern in leaflet anatomy.

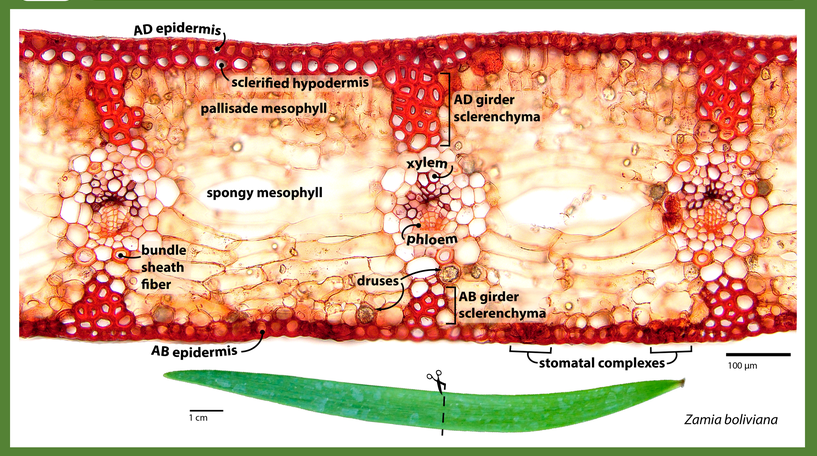

Fig. 2 General anatomical features of a *Zamia* leaflet, transverse section of *Zamia boliviana* stained with safranin. AD: adaxial, AB: abaxial. Single mature leaflet shown below with dotted line indicating approximate location of sectioning.

3 There are many variations on the theme, but most of this variation is not phylogenetically informative.

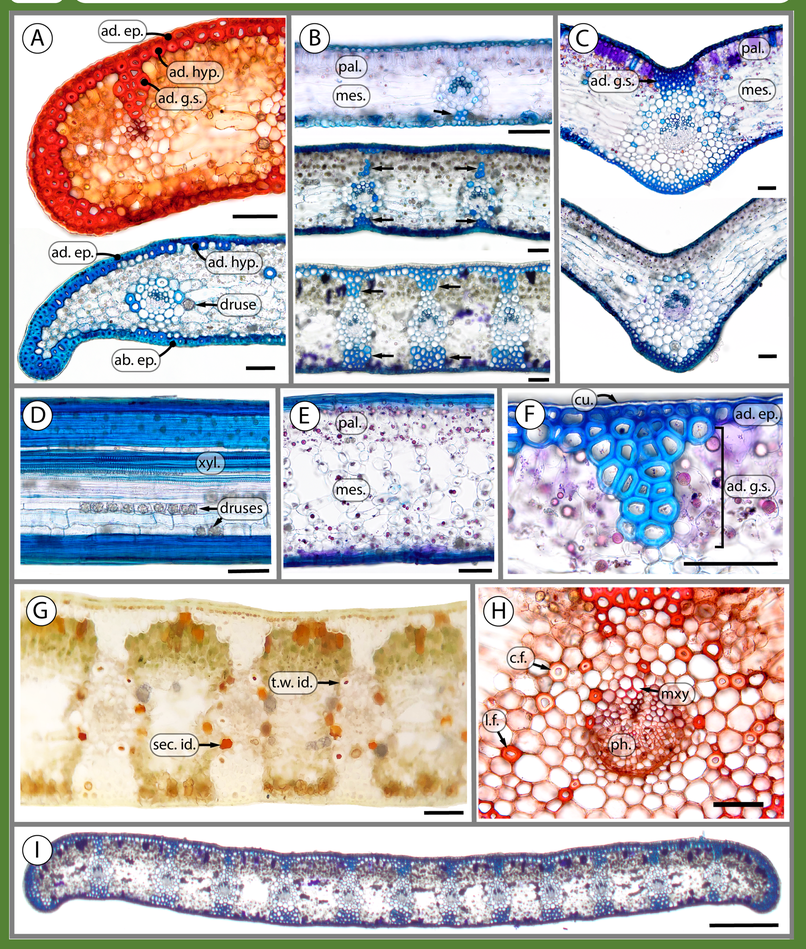

Fig. 3 *Zamia* spp. Various leaflet anatomical features. D, E, are longitudinal sections, all others are transverse sections. **A**, *Zamia boliviana*, safranin (top) and *Zamia purpurea,* toluidine blue. **B**, *Zamia vazquezii* (top), *Zamia purpurea* (middle), *Zamia angustifolia,* adaxial and abaxial girder sclerenchyma indicated by arrows. Note variation in presence/absence and thickness of girder sclerenchyma. **C,** *Zamia wallisii* (top) and *Zamia imperialis,* toluidine blue. Note the large parenchymatous cells that form the bulk of the abaxial bundle sheath in these plicate-leafleted taxa. **D**, *Zamia* decumbens, toluidine blue. **E**, **F,** *Zamia boliviana,* toluidine blue. **G,** *Zamia angustifolia,* unstained. **H,** *Zamia wallisii,* safranin. **I,** *Zamia angustifolia,* toluidine blue. Abbreviations: ad. ep. = adaxial epidermis, ad. hyp = adaxial hypodermis, druse = crystal, ab. ep. = abaxial epidermis, toluidine blue, pal = palisade, mes = mesophyll, xyl = xylem, cu = cuticle, t.w. id. = thick-walled idioblast, sec. id. = secretory idioblast, c.f. = cellulosic fiber, l.f. = lignified fiber, mxy = metaxylem, ph. = phloem. Scale bars: A-H = 100 µm, I = 500 µm.

4 However, these anatomical traits were only found in one geographically and phylogenetically-defined clade.

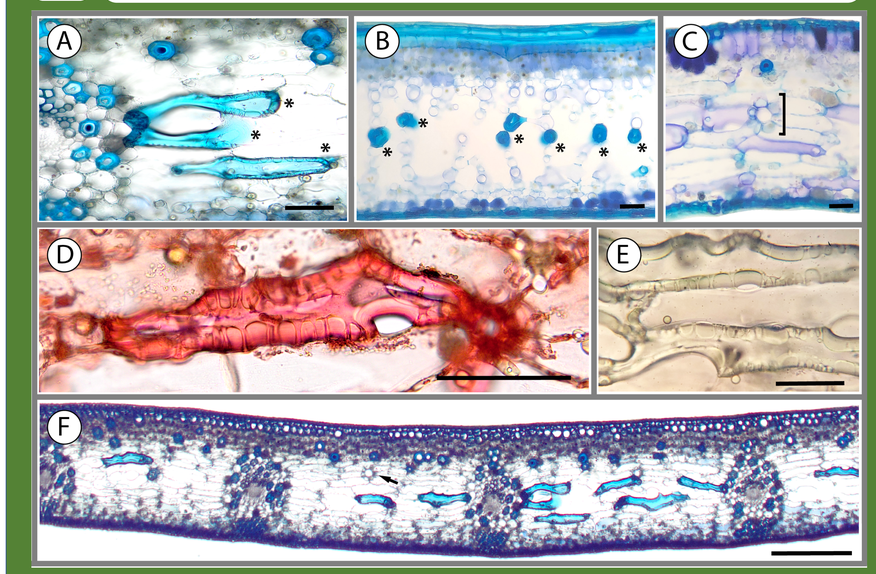

Fig. 4 *Zamia* spp. Select characters of interest in transverse (A, C-F), and longitudinal section (B) with stains used. **A, B,** *Zamia pseudoparasitica*, toluidine blue, "flat tire" cells marked with asterisks. **C,** *Zamia roezlii*, toluidine blue, bracket indicates "flower" formation. **D,** *Zamia wallisii*, safranin, single "flat tire" cell. **E,** *Zamia pseudoparasitica*, bleached, unstained, note plasmodesmata between "flat tires" and adjacent cells. **F,** *Zamia pseudoparasitica*, toluidine blue, "flower" formation indicated by arrow, "flat tires" staining bright blue. Scale bars: A-E = 100 µm, F = 500 µm.