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Morphological and phylogenetic evidence that the novel leaf structures of multivein *Selaginella schaffneri* are derived traits

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ABSTRACT

Microphylls, simple leaves with a single vein and no leaf gap, are the typical lycophyte leaves. However, *Selaginella schaffneri* has complex veins. Structural features and phylogeny associated with this unusual venation have remained unknown. We studied the leaf, venation, spore structures, and phylogeny of *S. schaffneri*, with *S. erythropus* as a typical *Selaginella* for comparison. Leaf veins of both *S. schaffneri* and *S. erythropus* originate from a single vascular strand in the stem and have no leaf gaps. In *S. schaffneri,* this single vascular strand prominently enlarges as a hub-like vein node at the leaf base and then divides multiply in the leaf blade. Unusual structures, more commonly found in angiosperms, are revealed, including vessels, bundle sheath cells, three stomatal types, and differentiated mesophyll tissue. Other unusual structures include transparent zones on the leaf margin and a complex open hexagonal three-dimensional structure on the megaspore walls. Fifty one concatenated protein-coding genes from plastomes were used to construct the phylogeny of *S. schaffneri* within Selaginellaceae, which shows that *S. schaffneri*, together with the *sanguinolenta* group, is the earliest-diverging lineage of subgenus *Stachygynandrum*. The unusual structures of *S. schaffneri* are consistent with drought resistance. However, these structures are not known in more basal members of *Selaginella* and appear to be derived in *S. schaffneri*. The leaf veins of *S. schaffneri*, originating from the branching of a single vein, imply a variation on a microphyll. Despite the general simplicity of structure in *Selaginella, S. schaffneri* shows unusual structural homoplasy with angiosperms in these traits.

1. Introduction

As the major organ for photosynthesis of vascular plants, leaves ought to be subject to strong evolutionary pressures (Taiz and Zeiger, 2006). Leaf morphological traits, including phyllotaxy, leaf size, shape and venation, vary greatly between different plant groups (Simpson, 2006). At the coarsest level, comparative morphology distinguishes two fundamental categories of leaves in vascular plants: microphylls and megaphylls (Bold et al., 1987). Microphylls are distinctive of lycophytes (Lycopodiophyta) dividing them from all other vascular plant groups, including ferns, gymnosperms and angiosperms, all of whose leaves are classified as megaphylls. Extant lycophytes include three families only: Lycopodiaceae, Isoetaceae and Selaginellaceae. Because they are characterized by microphylls, they are also termed 'Microphyllophyta' (Bold et al., 1987). Microphylls are of small size with single unbranching veins, and their leaf traces do not leave leaf gaps in the stem (Bold et al., 1987; Tomescu, 2009). In contrast, megaphylls are commonly much larger, with highly branched veins, and have leaf gaps (Bold et al., 1987; Tomescu, 2009).

These features of microphylls and megaphylls are generally accepted for vascular plants, but some inconsistent cases have been reported (Boodle, 1901; Mukherjee and Sen, 1981; Wagner Jr et al., 1982). In Selaginellaceae, *S. adunca* A.Br. *ex* Hieron. (native to India and Nepal) and *S. schaffneri* Hieron. (native to Mexico) were observed to have

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