Comparative leaf and stem anatomy of Anarthrioideae (Restionaceae)

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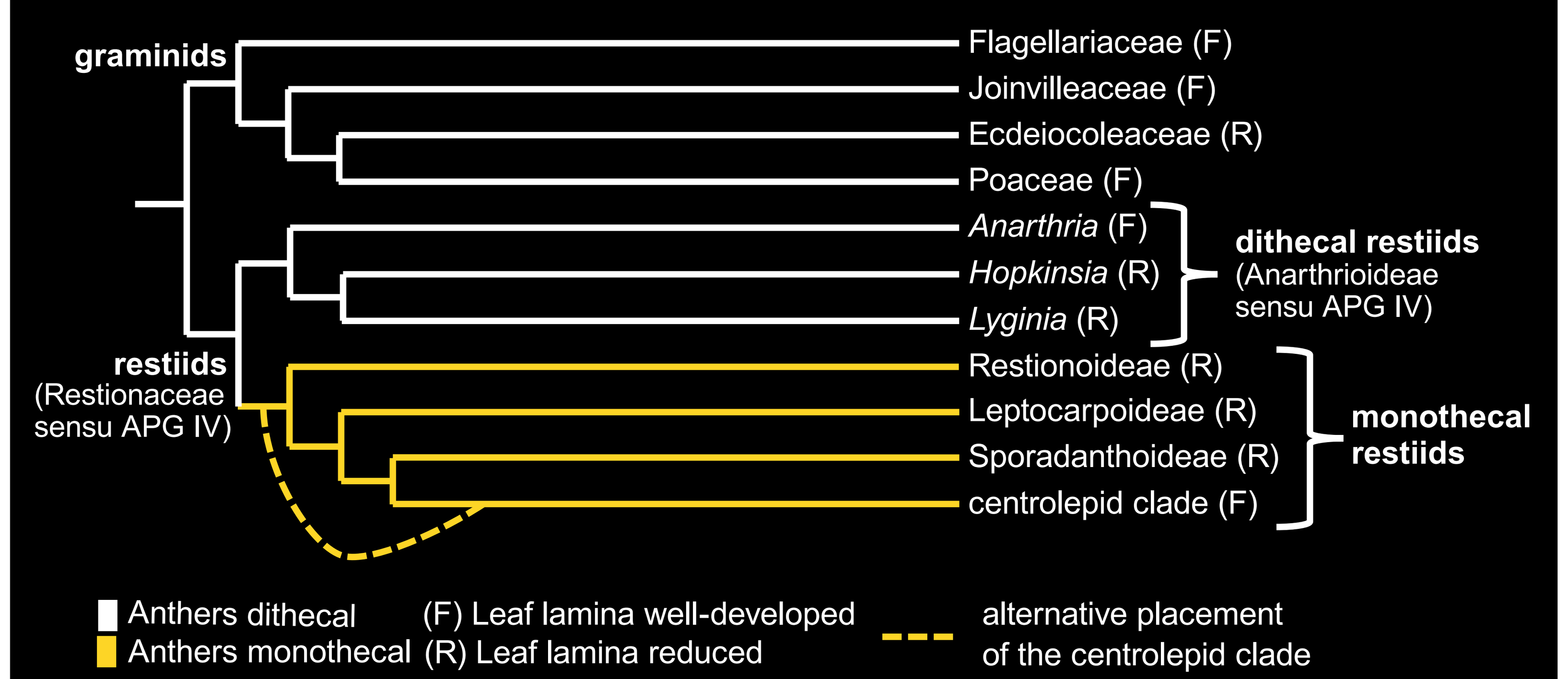
Introduction

Southwest of Australia is a unique floristic region, one of the most species-rich within the entire continent with a high proportion of endemic plant taxa, so the study of cryptic and other previously unrecognised by taxonomists species in this region is an important and relevant goal of the modern plant taxonomy. Restionaceae family closely related to grasses. It has a major center of taxonomic diversity in southwestern Australia, and its representatives play a significant role in the composition of the vegetation in this region. The subfamily Anarthrioideae is a sister group to all other Restionaceae and is endemic in southwest of Australia. The study of members of this subfamily is important for understanding the early evolution of a large clade within the order Poales, which includes grasses. Though species-rich, Restionaceae is well-known as difficult in species identification for botanists not directly specialised in the group. The three genera of Anarthrioideae comprise in total thirteen species, all endemic to the southwest of Western Australia. *Anarthria* is the largest genus of the group with eight species. The genus *Anarthria* has the most symplesiomorphies in restiids, such as dithecal anthers, trimerous flowers, long ovules and leaves with well-developed laminas.
As part of the assessment of the possibility of the existence of previously unidentified and morphologically similar species among the members of Anarthrioideae, a goal to study the micromorphological features of vegetative organs was set. Stems and leaves of representatives of all three genera of the subfamily were used in the analysis.



Material & Methods

Fixed material was dissected in 70% ethanol and then transferred to 100% acetone using the following series: 96% ethanol (twice for 30 min), 96% ethanol:100% acetone (1:1 v/v, 30 min), 100% acetone (three times for 30 min). The material was critical point dried in liquid carbon dioxide using a Hitachi HCP-2 critical point dryer (Hitachi, Tokyo, Japan), coated with gold and palladium using a Giko IB-3 ion-coater (Tokyo, Japan), and imaged using a CamScan S-2 (Cambridge Instruments, Cambridge, Great Britain) at the Laboratory of Electron Microscopy at the Biological Faculty of Moscow University.

Results & Discussion

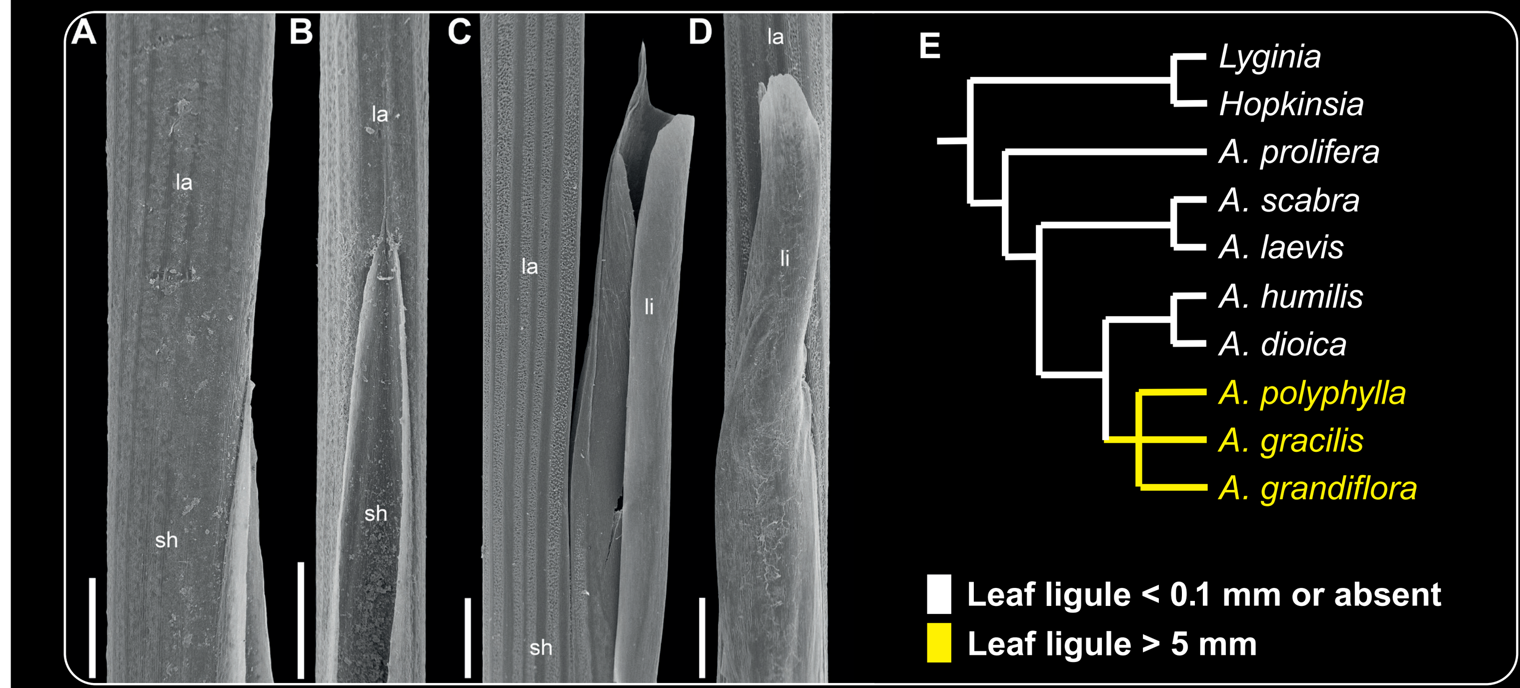


Figure 1: Leaf sheath to lamina transition in some species of *Anarthria* (SEM). A conspicuous ligule is present in *A. polyphylla*, *A. gracilis* and *A. grandiflora*, which comprise a clade in the trees inferred from molecular data. Other species of *Anarthria* possess a short, barely visible ligule. In *Lyginia* and *Hopkinsia*, the laminas are reduced to short cylindrical appendages and ligule is absent. (A, B) *A. dioica*. (C, D) *A. gracilis*. (A, C) side view. (B, D) view from the adaxial side. (E) Simplified phylogenetic tree of Anarthrioideae based on the plastid marker *trn*L-F. Abbreviations: la, lamina; li, ligule; sh, sheath. Scales = 1 mm.

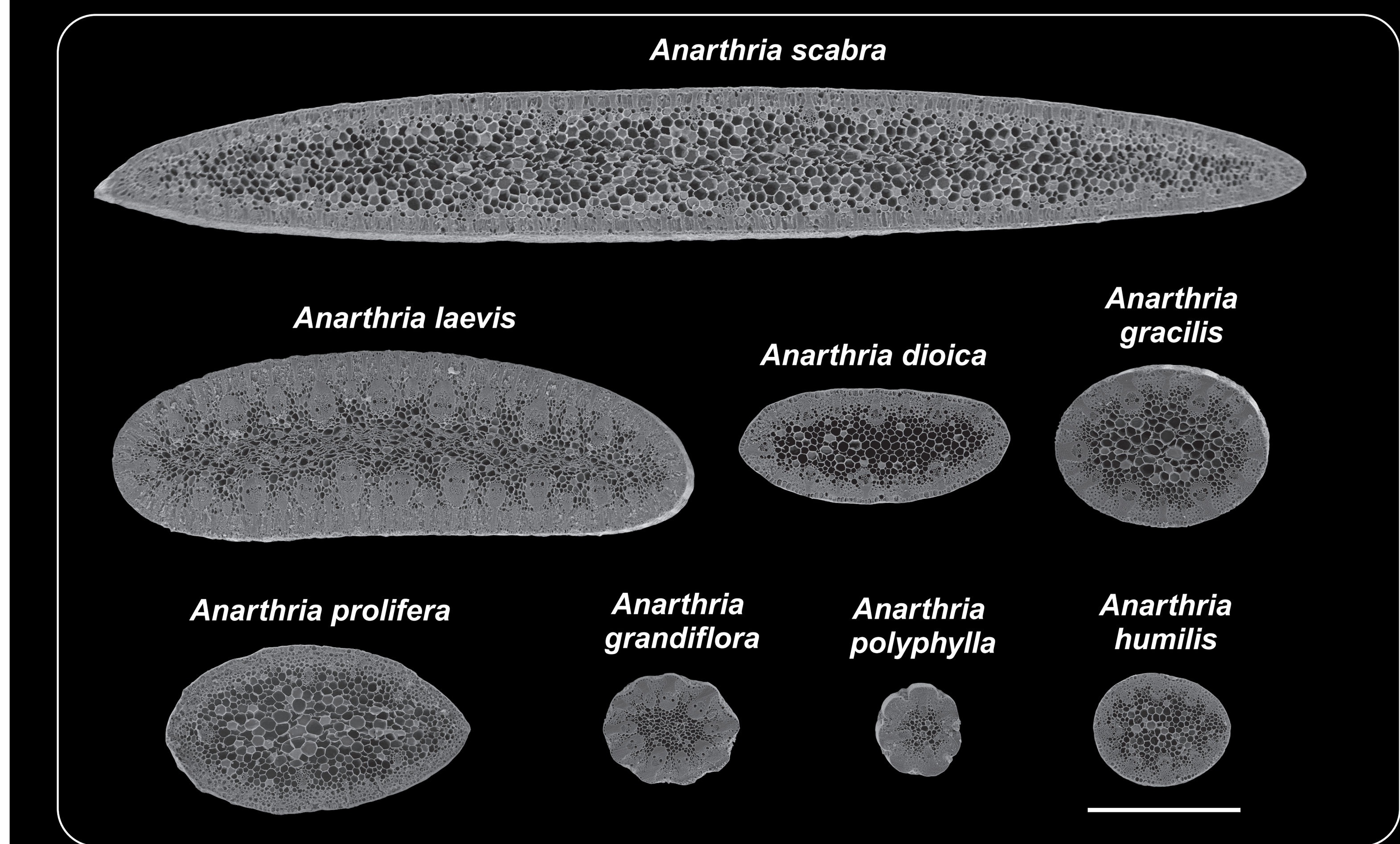


Figure 2: Cross-sections of lamina in *Anarthria*. A great diversity of lamina types is observed in *Anarthria*. Flattened laminas are common in *A. laevis* and *A. scabra*, elliptic in *A. dioica*, *A. gracilis* and *A. prolifera* and rounded in *A. humilis*, *A. grandiflora* and *A. polyphylla*. In *A. laevis* and three ligulate species, the epidermis surface forms longitudinal stripes of cell rows with smooth outer cell walls and rows with papillose cells, whereas in the non-ligulate plants, the leaf surface possesses no papillose stripes. Scale bar common to all images = 1 mm.

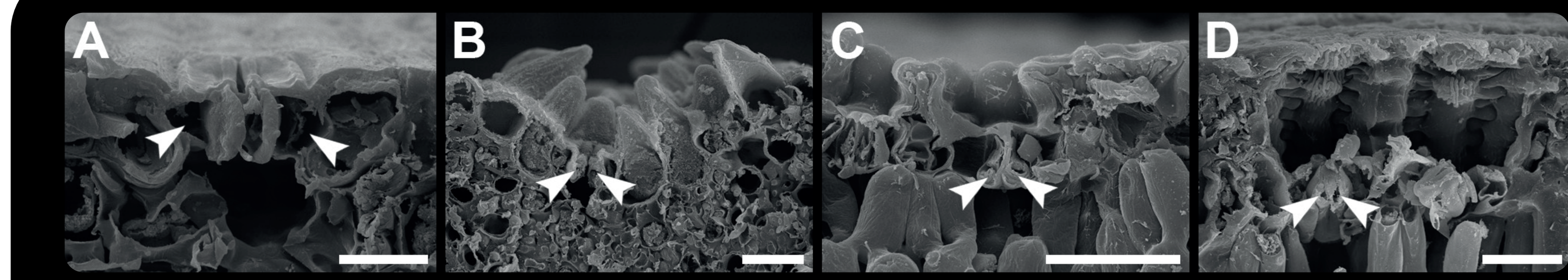


Figure 3: Types of stomata location in Anarthrioideae. (A) *A. prolifera*, stomata superficial. (B) *A. polyphylla*, stomata hidden by the papillae in the longitudinal rows. In (C) *H. anoectocolea* and. (D) *L. barbata*, stomata are located in the crypts. Arrows indicate guard cells. Scales = 30 µm.

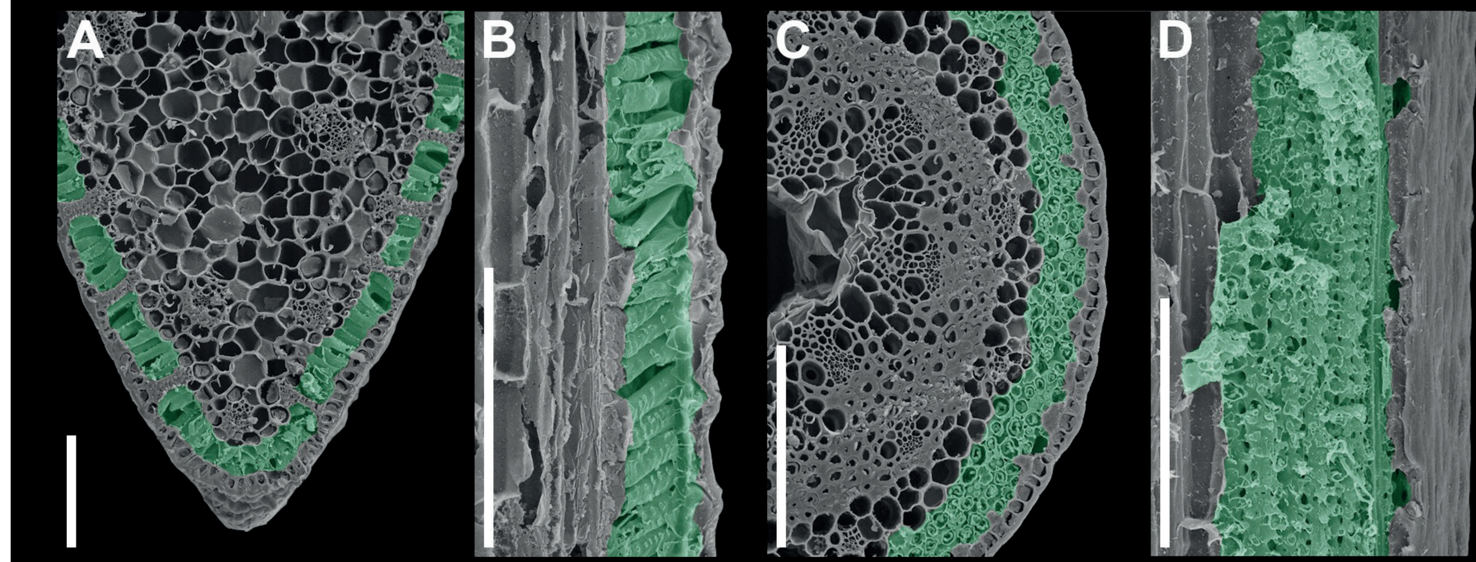


Figure 4: Chlorenchyma organisation in internode cross-sections of *Anarthria*. Cells are of a cylindrical shape, forming palisade layers in *A. scabra* (A, B), or chlorenchyma cells possess an irregular shape in *A. prolifera* (C, D). (A, C) cross section. (B, D) longitudinal section. Scales = 300 µm.

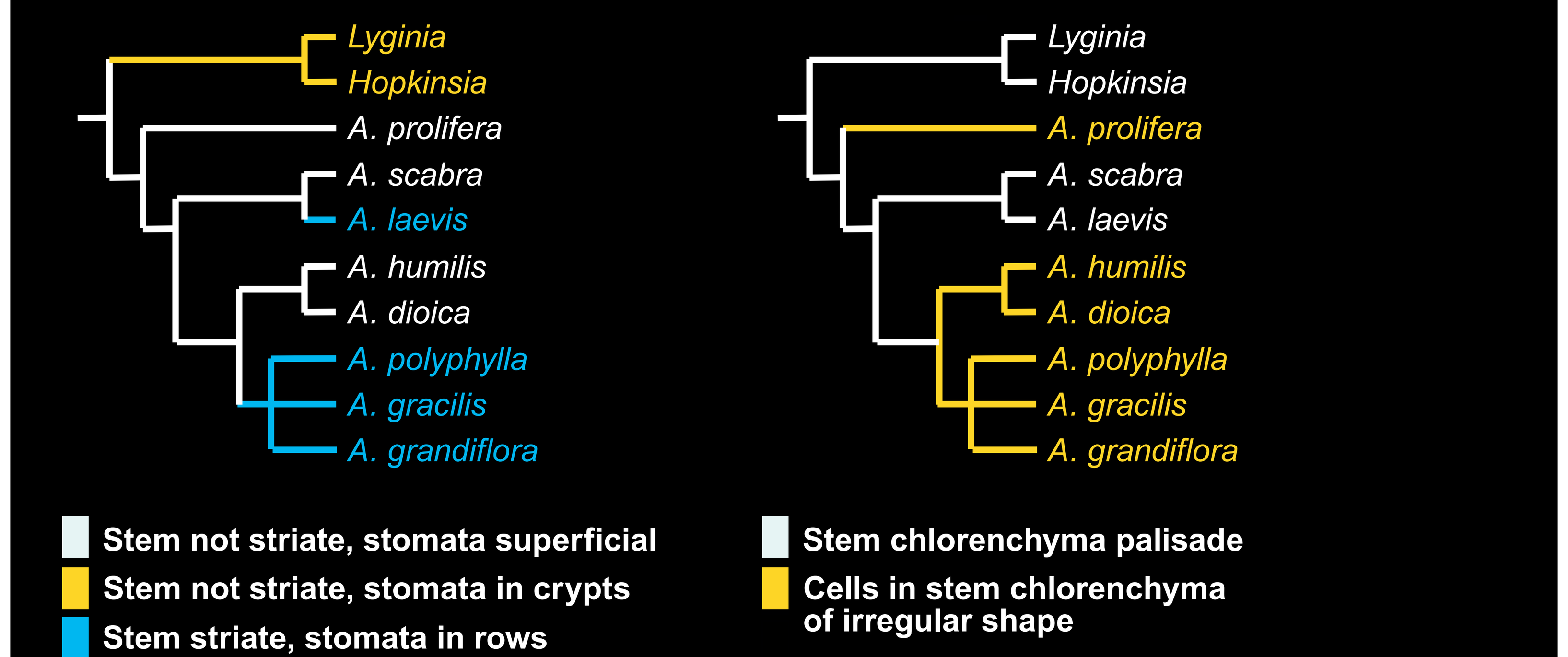


Figure 5: Character mapping of vegetative states using the Bayesian tree inferred of the concatenated nuclear (*at*103) and plastid (*trn*L-F) datasets.

Future Directions

There is much more to be done towards understanding restiid diversity and further directions of study may involve:

- Increased sampling and barcoding of restiids from across Australia
- Phylogenetic analysis using additional loci such as internal transcribed spacers 1 and 2 (ITS1 and ITS2) to produce more robust phylogeny for the group
- Ecological niche modelling of restiids to filling the gaps between habitat comditions, geographical distribution and morphological features

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Publication

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