Sampling

We selected thirty-six taxa from across the twelve subfamilies of the Poaceae, and four from the early-diverging Poales lineages (Flagellariaceae, Joinvilleaceae, and Ecdeiocoleaceae) to study pollen wall ultrastructure (Table). These taxa occur in a range of habitats, and represent forest grasses, margin dwellers, and open habitat species. Habitats span a range of conditions from xeric to wetland. The selected taxa and the habitats in which they reside will augment understanding about whether habitat correlates with the presence or absence of intra-exinous channels. Sampling is divided among C3 and C4 photosynthetic types. Taxonomic classifications follow the phylogenies of Soreng et al., (2015, 2017).

Previous work has confirmed the presence of intra-exinous channels in pollen from multiple members of the Pooideae (Linder and Ferguson, 1985; Peltre et al., 1987; Jewell et al., 1988; Marquez et al., 1997; Diethart et al., 2007), Panicoideae (Larson et al., 1962; Christensen and Horner, 1974; Peltre et al., 1987; Marquez et al., 1997), and Chloridoideae (Liu et al., 2004). Therefore, we focus the bulk of our sampling outside of these well-studied subfamilies. The exceptions are *Brachyelytrum erectum, Brachypodium distachyon, Diarrhena obovata, Glyceria striata*, and *Hesperostipa spartea* in the Pooideae; *Centotheca lappacea, Chasmanthium latifolium, Gynerium sagittatum, Echinochloa crusgalli, Panicum virgatum*, and *Danthoniopsis dinteri* in the Panicoideae; and *Centropodia glauca* and *Bouteloua curtipendula* in the Chloridoideae. These taxa are included to improve our understanding of pollen wall ultrastructure in the earliest-diverging members of their respective tribes and subtribes.

Twenty-three of the 36 proposed taxa are selected from subfamilies in which studies of pollen wall ultrastructure are rare or absent, or in which past work was inconclusive. The presence of intra-exinous channels has been confirmed in *Pariana stenolemma* pollen (Salgado-Labouriau et al., 1993), but no other bamboos have been sampled to date. We propose to study three additional members of the Bambusoideae for which pollen is readily available: *Arundinaria gigantea*, *Guadua angustifolia*, and *Lithachne pauciflora*.

Seminal work by Chanda and Rowley (1967) in the Flagellariaceae, Ecdeicoleaceae, and what is now known as the Joinvilleaceae, on pollen aperture morphology was inconclusive as to the presence of intra-exinous channels. At the time of their writing, *Joinvillea* was included in the Flagellariaceae, but the genus was subsequently separated into its own family, the Joinvilleaceae (Tomlinson and Smith, 1970). One species in the Joinvilleaceae (*Joinvillea ascendens*), one species in the Flagellariaceae (*Flagellaria indica*), and two species in the Ecdeicoleaceae (*Ecdeiocolea monostachya* and *Georgeantha hexandra*) will be sampled. Improved TEM technology will allow us to capture images in greater detail than was possible in the 1960s. The early-diverging Poales lineages have much to reveal about the evolution of pollen wall ultrastructure.

We propose to sample sixteen taxa in the remaining subfamilies of the Poaceae. This includes two each in the Anomochlooideae (*Anomochloa marantoidea* and *Streptochaeta spicata*), Aristidoideae (*Aristida purpurea* and *Stipagrostis hirtiglumis*), Arundinoideae (*Arundo donax* and *Phragmites australis*), Micrairoideae (*Eriachne sp.* and *Micraira sp.*), Pharoideae (*Leptaspis zeylanica* and *Pharus latifolius*), and Puelioideae (*Puelia sp.* and *Guaduella sp.*); three in the Oryzoideae (*Ehrharta erecta, Zizania palustris,* and *Streptogyna americana*); and one in the Danthonioideae (*Danthonia spicata*). This sampling scheme ensures that pollen wall ultrastructure of at least two members of each subfamily, distributed more or less evenly across tribes and subtribes, will be imaged either by our efforts or those of previous researchers.

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