

Coevolution of Pooideae grasses and their *Epichloë* endophytes within a hologenome framework

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Pooideae represents a diverse subfamily of grasses with over 4000 species classified into 12 tribes and 216 genera. Over 67 million years of evolution shaped their dynamic genomes, resulting in their enormous phenotypic and ecological diversity. During this time, many members of the Pooideae have further co-evolved with fungal endophytes of *Epichloë* (family Clavicipitaceae). *Epichloë* display long-term associations with their temperate grass hosts and are naturally restricted within the Pooideae to a host genus or closely related grass genera within a tribe. *Epichloë* grow systemically within the above ground organs of their grass hosts and form a continuum of interactions from vertically transmitted mutualists (asexual species) to horizontally transmitted antagonists (sexual species). While sexual *Epichloë* species are haploid, most asexual species are allopolyploids, interspecific hybrids having ancestries involving two or more distinct *Epichloë* lineages. Comparative genomic and phylogenomic analyses of Pooideae and *Epichloë* species make these symbioses an excellent model for tracing coevolution through diversification within a hologenomic framework. We hypothesised that the predominant vertical transmission of these endophytes would favour a Pooideae-*Epichloë* coevolutionary scenario, although horizontal transmission, both sexual and asexual, of endophytes can alter this. We reconstructed the phylogeny of Pooideae host species using single-copy nuclear genes and that of *Epichloë* species using nuclear barcoding markers and available sequenced genomes. We also filtered and assembled *Epichloë* genes from host genome skimming data. Phylogenetic reconstructions

were performed using maximum likelihood and coalescence-based approaches. Potential coevolutionary trends of Pooideae grasses and their *Epichloë* fungal endophytes were analysed statistically, based on host-parasite associations, and through concordance scores of host-endophyte topological conflicts. We performed coevolutionary analyses for the main tribal and subtribal representatives of Pooideae and described *Epichloë* species and then focused on the more recently evolved Loliinae grasses (e.g., *Festuca* and *Lolium*) and their *Epichloë* symbionts. We found diffuse and lineage-specific coevolutionary trends for some groups of Pooideae and their endophytes, also explored through co-genomic approaches. The more detailed analysis of ~200 Loliinae species confirmed that at least 15% of them associate with *Epichloë* endophytes. Furthermore, we detected 20 lineage-specific relationships for temperate and tropical mountain fescues and their endophytes. Our analyses revealed significant coevolution for Loliinae and their symbionts at individual scale for two lineages, the fine-leaved *F.* sect. *ovina* clade (sheep fescues) with *E. festucae* strains, and the broad-leaved *Schedonorus-Lolium* clade (meadow and tall fescues, ryegrasses) with *E. typhina*, *E. hybrida*, *E. occultans*, *E. siegelii*, and *E. uncinata* strains. Interestingly, these two groups of Loliinae showed high intraclade hybridisation rates, but low interclade rates. Our results highlight the impact of the narrow vs. broad hybridogenous nature of the host plant in the strong versus weak lineage-specific coevolutionary trends with their respective *Epichloë* endophytes.