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Diversity of *Epichloë* in native grasses from Uruguay

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Studies on endophyte diversity have allowed to describe at least 43 taxa that belong to the genus *Epichloë*, including distinct species, subspecies, and varieties^[1]. Screenings for grasses associated with *Epichloë* are continuously being done worldwide due to the agronomically important beneficial effects they provide to their hosts. In spite of this, *Epichloë* presence and diversity in South America has been poorly studied, with most works focused in Argentina, although preliminary studies revealed the presence of *Epichloë* in grasses from Uruguay. Our objective was to assess the diversity of *Epichloë* associated with native grasses from Uruguay, South America.

We sampled native grasses from Uruguay and inspected under microscope for typical *Epichloë* mycelia the parenchymal tissue from within the culm pith or sheaths stained with aniline blue^[2]. Fragments of leaves and culms of endophyte-infected plants were surface-sterilized and plated in Potatoe Dextrose Agar to isolate *Epichloë*. Subsequently, we obtained single spore cultures for morphological and molecular characterization by phylogenies of *calM* gene and detection by PCR multiplex of alkaloid genes^[3, 4].

Specimens of *Bromus auleticus*, *Bromus brachyanthera*, *Calamagrostis alba*, *Festuca fimbriata*, *Poa lanigera* and *Polypogon elongatus* were detected associated with *Epichloë*. Morphological differences in colony and microscopic characteristics were observed among isolates from different host species, but also among isolates from the same host species (*B. auleticus* and *P. lanigera*). Phylogenetic analyzes based on calmodulin gene (*calM*) sequences allowed us to infer at least 5 interspecific hybrid lineages. The *E. typhina* x *E. festucae* hybrid, *Epichloë tembladerae*, was detected in all of the studied hosts except in *F. fimbriata*. Some isolates from *B. auleticus* and *F. fimbriata* were also hybrids between *E. typhina* and *E. festucae* but clustered in different clades with endophytes of these hosts from Argentina, different from any previously described species. A hybrid isolate (*E. baconii* x *E. typhina*) was detected in *B. auleticus*. One isolate from *Bromus auleticus* presented three copies of *calM* indicating a triple hybrid origin (*E. festucae* x *E. typhina* x *E. elymi*) and grouped in a clade that also included isolates from Argentina. Alkaloid gene profiling indicated that all of the endophytes have *perA* gene. The endophytes from *F. fimbriata* and the triple hybrid were *dmaW* positive. *LolC* gene was only detected in some endophytes from *B. auleticus*. Indole di-terpene gene profiling allowed us to detect variability among isolates considered as *E. tembladerae*, and only some endophytes of *B. auleticus* were negative for the screened *idt* genes.

These results suggest a great diversity of endophytes in Uruguay and, although most of the endophytes have been also detected in Argentina, some lineages seem to be endemic to Uruguay. Taking into account the diversity of grasses in this country and that we only studied a small amount of isolates of each host, it is likely that more hosts and endophytes will be discovered in Uruguay.

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