

CHEMOTAXONOMIC AND PHYLOGENETIC RELATIONSHIPS OF ESPELETIINAE (ASTERACEAE) AND ITS CLOSEST RELATIVES

Guillermo F. Padilla-González, Fernando B. Da Costa

*School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Av. do Café s/n, 14040-903,
Ribeirão Preto, SP, Brazil, padillaf@fcfrp.usp.br*

Abstract: The subtribe Espeletiinae (Asteraceae) comprises *ca.* 143 species endemic to the Páramos of Venezuela, Colombia and Ecuador. This group is considered a classic model to study rapid adaptive radiations in plants; therefore, the taxonomy, morphology and ecophysiology of the subtribe have been well documented in recent years. However, attempts to reconstruct its evolutionary history and the relationships of the subtribe with other taxa are scarce, mainly due to problematic results regarding the low variation of conventional DNA markers. Thus, we evaluated the application of secondary metabolites as taxonomic markers, and determined if chemical data can be used in combination with DNA sequences to obtain more resolved and/or better-supported phylogenies. All secondary metabolites reported from the eight genera of Espeletiinae and from the genera *Smallanthus* and *Ichthyothere* were retrieved from the literature as well as sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. Chemical data was used to perform hierarchical clustering analysis with bootstrap resampling (HCAbp) and orthogonal partial least squares discriminant analysis (OPLS-DA) to determine chemotaxonomical relationships within these three taxa. Phylogenetic analyses based on Bayesian inference were performed with a concatenated matrix containing ITS sequences and chemical data. A database containing 1551 reports of secondary metabolites in Espeletiinae, *Smallanthus* and *Ichthyothere* was built including chemical, biological and geographical information. Additionally, the metabolomic fingerprint of twelve species of Espeletiinae was obtained by UHPLC-UV-FTMS in quest for chemotaxonomical valuable compounds. The HCAbp demonstrated that secondary metabolites can be used to segregate the subtribe Espeletiinae from its closest relatives (*Smallanthus* and *Ichthyothere*). Moreover, it showed that species within the Espeletiinae group among each other according to their country of origin and that each taxa (Espeletiinae, *Smallanthus* and *Ichthyothere*) present specific metabolites that allow their separation, as confirmed by the OPLS-DA. Phylogenetic analyses based on ITS sequences and chemical data demonstrated that the addition of secondary metabolites to the DNA-based phylogenies led to a better-supported monophyly of the subtribe Espeletiinae and to more resolved tree topologies within the *Ichthyothere* clade. The metabolomic analyses allowed the identification of previously unreported compounds with high chemotaxonomic value such as flavonoids, a sesquiterpene lactone and caffeic acid esters derivatives. These results reveal the potential of secondary metabolites in Espeletiinae as taxonomical markers and offer the first report of the application of secondary metabolites in combination with DNA sequences to reconstruct the evolutionary history of a taxon by Bayesian inference methods.
