An integrative approach to disentangle the diversity of *Mimosa* L. genus (mimosoid clade, Fabaceae) in Pampa grasslands, South America

Introduction

Mimosa L. is mainly a neotropical genus with more than 500 species acknowledged by its sensible leaves to touch although most of the species do not have this trait (Simon *et al*., 2011). Major *Mimosa* species diversity and endemism are found in Central Brazil, subtropical South America (Paraguay, Argentina, Uruguay, and southern Brazil), and Mexico, with secondary clusters of species diversity in the Andes, the Caribbean and Madagascar (Simon *et al.*, 2011). Brazil is a center of diversity and endemism of *Mimosa* genus, which is represented by ca. 368 species (Flora do Brasil 2020 (under construction) 2020), particularly in central and southern regions (Simon *et al.* 2011). However, many gaps still remain about diversity knowledge and conservation status of Brazilian *Mimosa* species, chiefly regarding the species from the Brazilian Pampa grasslands. These grasslands belong to Río de la Plata ecoregion, one of the largest continuous grasslands of the Americas (Soriano,1992), harboring circa 2,150 higher plant species (Boldrini *et al.*, 2015).

In addition, the *Mimosa* is a morphologically variable genus with a complex taxonomy, which may be elucidated by the analyses of several lines of evidence in order to delimit robustly the species hypotheses (Pante *et al.* 2015; Dayrat, 2005). Considering this, I have been looking for new morphological features, the application of multiple perspectives such as anatomy, cytology; and ecology to delimit *Mimosa* species, especially solving species complexes, and to understand the evolution of this genus in the South America grasslands in my thesis. Some of my hypotheses are: (1) the Pampa biome is as rich in *Mimosa* species as the Cerrado grasslands; however, its diversity is underestimated due to the lack of a taxonomic revision in this biome; (2) the secretory structures such as glandular trichomes may be useful to distinguish species, mainly in the richer series *Stipellares* in the Pampas grasslands; (3) most Pampa grassland species have a restricted distribution; (4) the number of *Mimosa*'s polyploids are underestimated for high latitudes; (5) ca 50% of species will be evaluated in some IUCN threat category owing the habitat loss of these grasslands, restricted distribution and; the number of remaining populations.

Objectives

1. To provide a checklist of *Mimosa* diversity in Pampa grasslands (e.g reporting new species and occurrences).
2. To explore new morphological features to delimit Mimosa's species (e.g. secretory structures, waxes, leaflet anatomy, pollen).
3. To evaluate the contribution of ecology to delimit species (e.g. ecological niche modelling).
4. To analyze the number of chromosomes and genome size of Pampa's Mimosas in an evolutionary context.
5. To evaluate the conservation status of *Mimosa* species of Pampa grasslands according to IUCN criteria.

Material and Methods

Herbaria collections revision The Herbaria revision is a fundamental step of this thesis. Until now several herbaria's from Brazil (BHCB, BOTU, CRI, ESA, FLOR, FURB, HAS, HDCF, HPBR,HUCS, HUEM, HURG, HVAT, IAC, ICN, JOI, MBM, PACA, PEL, SMDB, SPSF, SJRP) and Uruguay (MVFA, MVJB, MVM,) were visited as well some international herbaria from online source were consulted (NY, K, E, F, MO, P, SI,W). Nevertheless, herbaria from Argentina and Paraguay still to be visited because they are not digitalized or do not loan the material. **The resource will be mainly used to review the Argentine herbaria.**

Fieldwork I have done several excursions in Pampa grasslands in RS (southern Brazil), but I still lack to perform fieldwork in Argentina and Uruguay grasslands. The fieldwork is essential not only to delineate species diversity distribution but also to evaluate the species conservation status; and obtain DNA, seeds and

observational data. **The resource will be used for the fieldwork of one week in Argentina or Uruguay grasslands.**

Anatomical approach For anatomical studies of glandular trichomes, extrafloral nectaries and comparative leaflet anatomy, leaves fixed or from herbaria of species of *Mimosa* section *Batocaulon* series *Stipellares* will be rehydrated in glycerin, serially dehydrated in ethanol and stored in 70% ethanol. Cross-sections of the leaflets will be obtained by inclusion in Historesin (Leica®) and sectioning in a rotary microtome. To study the nature of the secretion of glandular trichomes, several histochemical tests will be conducted on fresh material such as Sudan IV, NADI reagent, Vanillin hydrochloric, Ferric Chloride. Scanning electron microscopy analysis (SEM) will be conducted to study the structure of waxes of branches and also to determine the pollen morphology. The samples will be submitted to critical point-dried using CO_2, mounted on aluminium stubs and coated with gold. **The resource will be used to cover the costs with historesin and SEM analysis**.

Cytogenetic approach Somatic chromosome numbers are determined in root tip cells pre-treated with 2 mM 8-hydroxyquinoline solution for 24 h at 4 °C and subsequently fixed in fresh 3:1 (v/v) ethanol-acetic acid solution, stored at −20 °C. For the chromosome preparations, root tips are hydrolysed in 5 M HCl for 20 min, squashed in a drop of 45% acetic acid and stained with 2% Giemsa solution for 15–20 min. Total DNA content will be assessed by flow cytometry according to Marie & Brown (1993) and Dolezel *et al.* (2003), using as internal standards *Petunia hybrida* Vilm., *Solanum lycopersicum* L. and *Pisum sativum*. The DNA content of 5,000–10,000 stained nuclei will be determined for each sample using a flow cytometer. The total 2C DNA value will be calculated as: sample peak mean / standard peak mean × 2C DNA content of standard (pg). **The source will be used to perform genome size estimations.**

Ecological approach Georeferenced collection sites of *Mimosa's* Pampa grasslands were acquired through direct field observation, SpeciesLink, and the Global Biodiversity Information Facility. Explanatory variables included a set of 19 bioclimatic Raster layers at a 30 arc-second resolution (ca. 1 km^2 at the equator) from the WorldClim website. The ecological niche modelling (ENM) will be modeled using at least three algorithms commonly used in species distribution models in the literature: Bioclimatic Envelop (BIOCLIM), Maximum Entropy (MAXENT), Generalized linear models (GLM) because SDM predictions greatly depend on the method used to model the species niche. **No resource is required.**

Conservation status The conservation status of *Mimosa* species from Pampa biome will be determined by the IUCN criteria at regional and global level (IUCN, 2001) using the GeoCAT tool (Bachman *et al.*, 2011). This tool enables to calculate the Area of Occupancy (AOO) and the Extent of Occurrence (EOO), which allow the evaluation of conservations status according to the criteria B (1 and 2) of IUCN. **No resource is required.**

Relevant Information

Since this study began I have reported new endemic species of *Mimosa* for Pampa grasslands such as *M. terribilis* (Schmidt-Silveira *et al.*, 2016), *M. cerifera* (Schmidt-Silveira *et al.*, 2019*), M. baptistae* (Schmidt-Silveira *et al.*,2019); or rediscovered species after 150 years (*M. lasiocephala* and *M. diffusa*, in preparing); re-established species considered synonyms (*M. subinermis*, Schmidt-Silveira *et al.* 2018) and indicated new records for this biome (Schmidt-Silveira,2015). Furthermore, during my dissertation, in which a revised one section of *Mimosa* for the Rio Grande do Sul state (southernmost state of Brazil), it was estimated only 30 species for this section. However, I have reported 45 species, 14 and eight new occurrences, respectively for RS state and Brazil. These findings highlighted that the diversity of this genus is underestimated in Pampa grasslands, corroborating the need of our taxonomic study. Currently, I have been performing the anatomical analyses and cultivated plants to determine genome size. I expect to publish the results of this thesis in international journals such as Systematic Botany, Flora, Botanical Journal of the Linnean Society, Perspectives in Plant Ecology, Evolution and Systematics, Taxon.

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IAPT will be very helpful to review herbaria in Argentina, conduct fieldwork in Uruguay, to buy some anatomical materials and to perform SEM and genome size analyses.

Literature cited

Bachman, S., J. Moat, A. W. Hill, J. de Torres, and B. Scott. 2011. Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 126: 117–126.

Boldrini,I. I , Overbeck, G. & Trevisan, R. 2015. Biodiversidade de Plantas. In: Pillar, V.D.; Lange, O. (Org.). Campos do Sul. 1ed.Porto Alegre: Rede Campos Sulinos, 43–50pp.

Dayrat, B. 2005. Towards integrative taxonomy. Biological Journal of the Linnean Society 85, 407–415.

Dolezel J, Bartos J, Voglmayr H and Greilhuber J. 2003.Nuclear DNA content and genome size of trout and human. Cytometry A 51A:127-128.

Flora do Brasil 2020 (under construction). 2020. *Mimosa* in Flora do Brasil 2020 em construçao. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br/reflora/listaBrasil/PrincipalUC/PrincipalUC.do;jsessionid=DE1AE89978D379436393B3BD81BBFB3D#CondicaoTaxonCP , Acessed 20 January. 2020.

IUCN. 2001. IUCN red list categories and criteria version 3.1. Gland, Switzerland and Cambridge, UK:

Marie, D & Brown, SC .1993. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. Cell 78:41-51.

Pante, E. C. Schoelinck,& Puillandre, N.2015. From Integrative Taxonomy to Species Description: One Step Beyond. Syst. Biol. 64(1):152–160.

Simon, M. F., Grether, R., Queiroz, L. P., Särkinen, T. E., Dutra, V. F., Hughes, C. E. 2011. The evolutionary history of *Mimosa* (Leguminosae): toward a phylogeny of the sensitive plants. Am. J. Bot. 98, 1201-1221.

Soriano A. 1992. Río de la Plata grasslands. In Coupland RT, ed. Ecosystems of the world 8A. Amsterdam: Elsevier, 367–407.