GRANT APPLICATION FORM

"Diversity of *Gymnocalycium monvillei* (Lem.) Britton & Rose (Cactaceae) along its altitudinal range."

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Project proposal

Introduction

Gymnocalycium monvillei (Lem.) Britton & Rose (Cactaceae) is a globose cactus species endemic to the Córdoba and San Luis Mountains**,** Argentina. It belongs to the subgenus Michrosemineum, characterised by plants of rather big size and small seeds (Charles 2009). *G. monvillei* inhabits rocky outcrops from 880 to 2200 m a.s.l. (Demaio *et al*. 2011; Gurvich *et al*. 2014). This characteristic makes the species an ideal model to study genetic diversity patterns in mountain environments, and the possible processes involved in the diversification and speciation of the specie in their habitat. The main centers of cacti diversity in the Americas (Mexico & SW EEUU, North East Brazil and NW Argentina-Bolivia-Peru) are mountain environments (Ortega Baes & Godínez Alvarez 2006). Surprisingly, few studies had analysed biological or ecological patterns of species along these gradients (Gurvich *et al*. 2014, Bauk *et al*. 2015). The understanding of these patterns is necessary not only to understand how species cope with environmental factors, and hence present wide altitudinal ranges, but also to predict species responses to climate change (Alsos *et al*. 2012).

Genetic diversity is one of the three levels of diversity that the IUCN has encouraged to preserve, and is important since gives natural populations to variability to support disturbances, and is the basis of evolution process (Frankel & Soulé 1981, Reed *et al*. 2003). However, at the moment is poorly known the levels of genetic diversity in plants, and how are related to environmental factors as elevation (Leffler *et al*. 2012). Particularly in Cactaceae very few studies analysed genetic diversity (Solórzano *et al*. 2014, Rodrigues Monteiro *et al*. 2015, Bustamente *et al*. 2016). Genetic diversity could depends in many factors, as population size, geographical distribution, pollination and dispersal mode and isolation among populations (Lammi *et al.* 1999, Premoli *et al*. 2011). Sheng *et al.* (2014) found a high gene flow among altitudinal populations, and attribute this pattern to the pollination and dispersion mode of the species. In species with less efficient pollination and dispersal mode it would be expected a different genetic pattern (Premoli *et al.* 2011). Cacti species of the genus *Gymnocalycium* are dispersed by ants, which are very bad in long distance dispersal.

Different aspects of genetic diversity, such as DNA content and ploidy level may be distinct along environmental gradients (Knight & Ackerly 2002); the main implication of the level of ploidy is in the reproductive isolation of organisms of one species, and thus contributing to speciation (Ramsey & Ramsey 2014). In *G. monvillei* the level of ploidy is 2n=22, 44 (Till & Lambrou 1998) but in my thesis of degree, studying different populations in an altitudinal gradient, all the populations were tetraploids, that is why we would like to investigate how the population is structured genetically at different altitudes, because biophysical changes occurring along altitudinal gradients impose potential barriers to gene flow among populations (Mathiansen & Premoli 2013), such as differences in floral phenology imposed by altitude. These barriers to gene flow can affect the genetic structure of populations. Taking into account that in *G. monvillei*, more noticeable differences were found in ecological characters (population size, fruit and seed production, percentage of germination and seedling size), than genetic differences (DNA content and ploidy level) along of an altitudinal gradient (Bauk, 2014; Bauk *et al*., 2015), it is intended to use population genetics techniques to

analyze the genetic structure of this species. Results will be important not only to understand basic biological processes but to predict species response to disturbances (climate change, illegal collections) and will be useful to design conservation strategies. Taxonomy is a very important science and a tool that helps botany to sort species. In the case of *G. monvillei*, a species with different degrees of ploidy, would be very interesting to know how they are distributed and from there, to understand if the species is suffering from speciation and, if necessary, to suggest the separation into different species and from there it would be important to review gender systematic.

Objective

The aims of this study are to analyse the genetic diversity of *G. monvillei* in different localities encompassing is entire elevation and geographical range. We also want to analyse how the genetic diversity is related to the population size of the different localities, and examine the possible processes involved in the diversification and speciation. We would like to perform a multidisciplinary analysis that includes ecological and genetic studies, to evaluate the conservation status of this endemic species and to characterize genetic and ecologically throughout its distribution.

Materials and Methods

Study species and area. The study will be carried out in the Sierras Grandes range (Córdoba Mountains, Central Argentina), at an altitude between 800 and 2300 m a.s.l. Mean annual temperature and precipitation varied from 16.5 °C and 680mm to 10.3°C and 790mm, between the lowest and the highest sites of this altitudinal gradient, respectively (De Fina 1992). We will sample sites that have been previously investigated (Bauk *et al*. 2015), but also new sites, trying to encompass a wider latitudinal range (e.g. sites north and south of the previously studied area) where the species is present (Charles 2009).

Genetic measurements. The DNA extraction will be performed according to the methodology described by Hoisington *et al.* (1994) from stem samples obtained in the field and will be kept in dry silica gel. DNA extraction will be from ground material using a mortar and liquid nitrogen. Thirty individuals from each population will be sampled. The plant material will be placed in buffer CTAB II. This is followed by two extractions with chloroform: isoamyl alcohol (24: 1) and a DNA precipitation in 100% isopropanol. For each marker the expected discriminatory power (Dj) and observed (Do) will be estimated (Tessier *et al*. 1999). The Polymorphic Information Content (PIC) will be calculated according to Anderson *et al*. (1993). To establish the best combinations of markers SSRs, to differentiate the samples tested, we will follow the methodology of Tessier *et al*. (1999). Data analysis will be performed in the Polysaten R program (Clark *et al.* 2011). For cytogenetic techniques, mitotic chromosomes will be analyzed, using Giemmsa stain, CMA/DAPI fluorescent chromosome banding and fluorescence in situ hybridization (FISH, probes of 5S rDNA and pTa71 for 18-5.8-26S rDNA), and we will build the karyotypes for each population.

Population density. In each of the sites we will establish 100 1x1m plots in which all individuals will be registered and measured its diameter with a calliper.

Statistical analysis. The statistical analyzes will be performed with the Arlequin program (Excoffier *et al*. 2005). The genetic estimators that will be used to describe the genetic diversity and population structure and entire species will be: 1) Observed heterozygosity (Ho, Nei 1977). 2) Expected heterozygosity (HE, Nei 1977). 3) Allelic diversity (Na, Frankham *et al*. 2002). 4) Inbreeding coefficient for each population (FIS, Wrigh 1951). 5) Coefficient of genetic differentiation between populations (F_{ST}). 6) Genetic differentiation. This parameter will be estimated between each pair of populations, as well as the average for all species (index of global differentiation) with the RST coefficient adjusted for microsatellite markers (Michalakis & Excoffier 1996).

Budget. I would be very grateful to receive the financial support of an IAPT grant in order to be able to carry out these studies. I have estimated a budget of U$S 2000, that includes expenses for transport to the field (U$S 600) and laboratory investigations (genetic analyses) (U$S 1400). It is most likely that the grant will not cover the total expenses but the rest would be covered by other grants that I am requesting.

Additional Funding. In the previous projects we received equipment (GPS and digital camera) that will be used in the present one. The Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET) have a four-wheel truck necessary for the fieldwork and we also dispose a molecular biology lab where basic genetic techniques can be performed. We also have another financial support of CSSA (The Cactus And Succulent Society Of America) of U$S 3000.

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