Lobivia Britton & Rose, a cytogenetic and phylogenetic analysis of species from Argentina.

Introduction

Cactaceae is considered a monophyletic family that includes more than 90 genera and 1400 species from South Canada to Argentina and Chile (Hunt *et al.* 2006). It is divided in four sub-families: Pereskioideae K. Schum., Opuntioideae K. Schum., Maihuenoideae P. J. Fearn y Cactoideae K. Schum (Hunt *et al.* 2006). Cactoideae is the largest one and is divided into nine tribes according to Buxbaum (1958) classification with slight modifications (Hunt *et al.* 2006).

One of the Cactoideae tribes is Trichocereeae, containing one of the most interesting genera: "*Echinopsis",* that nowadays, according to Hunt (2006), Anderson (2001) and other authors, includes many genera like *Lobivia* Britton & Rose*, Chamaecereus* Britton & Rose*, Helianthocereus* Backeb.*, Pseudolobivia* Backeb.¸ *Soehrensia* Backeb. *and Trichocereus*(A. Berger) Riccob; with more than 500 species.

However, some authors suggested that *Echinopsis "sensu lato"* might not be monophyletic. Hernandez & Hernandez (2011) performed a phylogenetic analysis within Cactaceae using four chloroplast (trnK-matK; matK; trnL-trnF; rpl16) and one nuclear (ppc) markers, obtaining as a result that *Echinopsis "sensu lato"* was poliphyletic. Moreover, Schlumpberger & Renner (2012) analyzed 144 species and subspecies using three chloroplast markers (trnS-trnG; trnL-trnF y rpl16), reaching the same conclusion and splitting *Echinopsis* in 10 "clades" like *Lobivia, Echinopsis sensu stricto, Helianthocereus, Harrisia* and *Trichocereus.*

Lobivia Britton & Rose comprises mostly globular plants, with short and always diurnal laterals flowers (Britton and Rose 1924, Rausch 1985). In Argentina, it is possible to find at least 30 different taxa of *Lobivia*, including species and varieties, according to Kiesling *et al* (2008) along the northwest of the country, from Tucumán to Jujuy. However, many of them are endemic with very narrow distribution areas. (Aagesen *et al.* 2012). *Lobivia* plants are very appreciated by cactus collectors from all over the world because of their beautiful colourful flowers. In fact, most data regarding distribution and collection sites is only available in Cactus-enthusiastic home pages, in sharp contrast with the sparse information available from traditional academical sources (Aagesen *et al.* 2012).

My PhD project is focused on the genus *Lobivia*, especially on the Argentinean species, and my main goal is to combine molecular, cytogenetical and morphological studies to reach a better understanding of the genus, defining its circumscription and the relationship between the known species and its varieties.

Polyploidy is considered the main evolution mechanism in angiosperm (Levin 2002) and particularly in Cactaceae (Pinkava *et al.* 1985; Arakaki *et al.* 2007; Las Peñas 2009). However, published chromosome counts in *Lobivia* show that most of the species are diploid (2n= 2x= 22) with a few exceptions like *L. tiegeliana* that presents two cytotypes, diploid and tetraploid (Schlumpberger & Renner 2012)

Chromosomes are a dynamic system that usually change during the evolutionary history of organisms, and we can certainly analyse some traits like number, shape and size, that suggest patterns of chromosomal evolution. The appropriate interpretation of the cytogenetic characteristics, based in DNA sequence phylogenies, might allow us to understand the genetic divergence between species (Guerra 2012). In Cactaceae, the basic number is x= 11 and polyploidy is the main type of variation (Guerra 2008). However, there are few karyotypes studies that cover several species (Cota & Wallace 1995;Las Peñas *et al.* 2008, 2009, 2011, 2014;Moreno *et al.* 2015 a, b).

Molecular cytogenetics seek to combine morphological and molecular chromosome evidence, allowing us to perform chromosome mapping using repetitive sequences such us rDNA, telomeric, centromeric or satellite DNA (Guerra 2004), to analyse evolutionary relationships between species and to study the genome organization (Doležel *et al.* 2007). The use of these techniques in Cactaceae has allowed establishing chromosome homologies among different taxa (Las Peñas *et al.* 2009, 2011, 2014; Moreno *et al.* 2015 a, b). The rDNA 18S–5.8S–26S location is conserved between taxa and its

number is an indicator of the ploidy level (Las Peñas *et al.* 2009, 2014; Moreno *et al.* 2015 a, b). Moreover, rDNA 5S is more variable and can be located in different linkage groups (Las Peñas *et al.* 2014; Moreno *et al.* 2015 a,b) what is useful to describe chromosome evolutionary patterns (Moreno *et al.* 2015 b). In December 2018, we collected many *Lobivia* plants, from northwest Argentina (Catamarca, Tucuman, Salta and Jujuy provinces). Among this plants, there are some that are known as varieties of the same species, such asforms of *L. densispina* (*L. densispina var. rebutioides and var. pectinifera*) forms of *L. haematantha* (*L. haematantha var. kuehnrichii, var.elongata*), *L.saltensis and L. schreiterii* and *L. formosa* group (*L. korethroides and L. bruchii*). However, many plants like *L. haematantha var. amblayensis and var. chorrillosensis, L. schreiteri var. riolarensis* and *L. stilowiana* were not found or some others did not survive once in cultivation. As we have now more data about collection sites of the missing and already collected plants, we would like to make another field trip in northwest Argentina to collect those plants for the cytogenetic analysis, and to make herbarium vouchers. It would be also interesting to visit the herbarium of La Paz (Bolivia) to analyze the *Lobivia* specimens and to begin an interaction with Bolivian specialists that would be very important for future and broader studies of the group.

Budget: I would be very grateful to receive the financial support of an IAPT grant in order to be able to carry out my studies. I have estimated a budget of U$S 2000, that includes expenses for fieldwork (U$S 1500) and for sequencing (U$S 500). 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, other grants provided by my supervisor or with my own scholarship.

Materials and Methods

Phylogenetic analysis: Samples will be collected from floral or vegetative tissue and silica dried. Total genomic DNA will be extracted using the CTAB II protocol (Hoisington *et al.* 1994), We are going to amplify three chloroplast regions: rpl16 (like in Schlumberger & Renner 2012), petL-psbE; psbA-trnH and one nuclear: nhx1 (Franck 2012). PCR reactions will be performed following Shaw protocol (Shaw 2005, 2007). Products will be sent to Macrogen INC for sequencing. Sequences will be aligned using Muscle algorithm in MEGA 7 (Kumar *et al.* 2016). The Best model of nucleotide substitution will be estimated using Modeltest v. 2.3 (Nylander *et al.* 2004). The phylogenetic hypothesis will be built by Bayesian Inference using Mr Bayes v.3.1.2 (Ronquist & Huelsenbeck 2003).

Cytogenetic Analysis. Root tips will be collected and immediately transferred to 2 mmol/L 8-hydroxyquinoline to arrest metaphases, then fixed in an ethanol–acetic acid (3:1) solutionand stored until use at −20 °C. For conventional staining, Guerra (1983) protocols will be followed. For chromosome CMA-DAPI banding, protocols descripted by Schweizer & Ambros (1994) with some modifications (Las Peñas *et al.* 2008); and for Fluorescent hybridization in situ (FISH) protocols by Schwarzacher & Heslop-Harrison (2000), where two probes will be used : 18S–5.8S–26S(pTa71) (Gerlach & Bedbrook 1979) and 5S (Las Peñas *et al.* 2011). For each species, a karyotype will be made, using the measurements of at least five metaphases by ImageJ (Schindelin *et al.* 2012).

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