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## Ecology

### Genetic diversity and population structure of the Chilean native, *Tillandsia landbeckii* (Bromeliaceae), from the Atacama Desert

#### *Diversidad genética y estructura poblacional de Tillandsia landbeckii (Bromeliaceae), nativa chilena del desierto de Atacama*

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#### Abstract

*Tillandsia landbeckii* Phil. is a typical plant of the Atacama Desert in the north of Chile. There is no genetic data available at the population level for this species, and this information is critical for developing and implementing effective conservation measures. In this study, we investigated for the first time the genetic diversity and population structure in 2 natural populations of *T. landbeckii* using AFLP markers. Seven primer combinations produced 405 bands and of them, 188 (46.42%) were polymorphic. The Pampa Dos Cruces population ( $P_p = 88.30\%$ ,  $H_e = 0.327$ , and  $I = 0.483$ ) showed a higher genetic diversity level than Pampa Camarones population ( $P_p = 71.28\%$ ,  $H_e = 0.253$ , and  $I = 0.380$ ). Analysis of molecular variance (Amova) revealed that 25.12% of the total genetic diversity resided among populations, while 74.88% within populations. A moderate-high genetic differentiation coefficient ( $F_{st} = 0.251$ ) and a moderate population gene flow ( $N_m = 1.490$ ) were also observed. Principal coordinates analysis (PCoA), Neighbor-net and STRUCTURE analysis supported the grouping of the sampled populations into 2 moderate genetic clusters. This first study provides data that will allow assist and support conservation decisions taken for this species.

**Keywords:** Molecular markers; AFLP; *Tillandsia landbeckii*

#### Resumen

*Tillandsia landbeckii* Phil. es una planta típica del desierto de Atacama en el norte de Chile. No hay datos genéticos disponibles a nivel de población para esta especie y esta información es crítica para desarrollar e implementar

medidas de conservación efectivas. En este estudio, investigamos por primera vez la diversidad genética y la estructura poblacional en 2 poblaciones naturales de *T. landbeckii* mediante marcadores del tipo AFLP. Siete combinaciones de primers produjeron 405 bandas, de las cuales, 188 (46.42%) fueron polimórficas. La población de Pampa Dos Cruces ( $P_p = 88.30\%$ ,  $H_e = 0.327$  y  $I = 0.483$ ) mostró un nivel de diversidad genética mayor que la población de Pampa Camarones ( $P_p = 71.28\%$ ,  $H_e = 0.253$  y  $I = 0.380$ ). El análisis de la varianza molecular (Amova) reveló que el 25.12% de la diversidad genética total residía entre las poblaciones, mientras que el 74.88% está dentro de las poblaciones. Se observó un coeficiente de diferenciación genética de moderado a alto ( $F_{st} = 0.251$ ) y un moderado flujo genético en la población ( $N_m = 1.490$ ). Los análisis de coordenadas principales (PCoA), neighbor-net y STRUCTURE soportaron la agrupación de las poblaciones muestreadas en 2 grupos genéticos moderados. Este primer estudio proporciona datos que permitirán asistir y apoyar decisiones de conservación que se tomen para esta especie.

*Palabras clave:* Marcadores moleculares; AFLP; *Tillandsia landbeckii*

## Introduction

Genetic diversity is a fundamental component of biological diversity; preservation of the genetic diversity of plant species can significantly affect their long-term survival and evolution in changing environments (Frankham et al., 2010). Additionally, the knowledge of the genetic diversity and population structure of threatened plant species is essential for their protection and management (Frankham et al., 2003; Gordon et al., 2011; Peng et al., 2018).

The subfamily Tillandsioideae is the largest in the Bromeliaceae, with approximately 1,100 species, included into 9 genera: *Alcantarea* (16 spp.), *Catopsis* (21 spp.), *Glomeropitcairnia* (2 spp.), *Guzmania* (176 spp.), *Mezobromelia* (9 spp.), *Racinaea* (56 spp.), *Tillandsia* (551 spp.), *Vriesea* (188 spp.), and *Werauhia* (73 spp.) (Barfuss et al., 2005). *Tillandsia* is a predominantly epiphytic genus, with some lithophytic, and a few terrestrial species that is mainly confined to tropical and subtropical regions of North and South America (Soltis et al., 1987). Some members of this genus have developed strategies to survive in the arid coastal zones of the Atacama Desert in Chile and Peru, where they are dominant and provide essential biomass to these ecosystems (Rundel & Dillon, 1998). Three, out of 6 species of *Tillandsia* that are grown in Chile, have been reported in its northernmost region: *T. marconae* W. Till & Vitek, *T. landbeckii* Phil., and *T. virescens* Ruiz et Pav. (Pinto, 2005; Pinto et al., 2006; Rodríguez et al., 2018). *T. landbeckii* is a perennial herbaceous plant, which displays a fascinating ecology and has evolved a highly specialized growth habit, unrooted on sand, and requires the regular fog humidity to survive in the hyper-arid coastal zones of the Atacama Desert (Pinto, 2005; Pinto et al., 2006; Rundel et al., 1997). This species forms perpendicular bands to fog penetration (Pinto et al., 2006). Fog is the most important source of humidity in the Atacama Desert and the species that occur along the coastal zones are

considered as "vulnerable species" due to their extreme specialization and dependence on fog humidity (Zizka et al., 2009).

Conservation of genetic diversity has become the goal of many conservation programs; knowledge of the distribution of this diversity within and among natural populations is the first step in this process (Holsinger & Gottlieb, 1989). Research on population genetic diversity is also essential to provide information to design conservation strategies in species with extinction risk and, in this context, DNA-based molecular markers are widely used for studying genetic variability (Neel & Ellstrand, 2001). Several molecular markers have been developed for Bromeliaceae; however, only 3 studies have been performed to estimate the genetic variability specifically in Mexican endemic epiphyte *Tillandsia* species, including: isozymes in *T. achyrostachys* É. Morren ex Baker (González-Astorga et al., 2004), *T. ionantha* Planch. (Soltis et al., 1987), and microsatellites (SSR) in *T. recurvata* (Gaudich.) Baker (Solórzano et al., 2010; Soltis et al., 1987). There is no study of this type in any terrestrial member of this genus. Amplified fragment length polymorphism (AFLP) is a PCR-based marker for the rapid screening of genetic diversity and intraspecific variation without the need for prior sequence knowledge. AFLP markers have been widely used for diversity, phylogenetic and population genetics studies and are very efficient at revealing polymorphisms even between closely related individuals (Peng et al., 2018).

There is very detailed knowledge of the distribution of *T. landbeckii* in northern Chile (Pinto, 2005). However, despite its ecological importance in the region, there is no information on the genetic structure and variability of its natural populations. In this study, we investigated for the first time the genetic diversity and genetic structure of 2 populations of *T. landbeckii* from northernmost Chile by AFLP analysis, to reveal the level of genetic diversity, to explore the distribution of genetic variation within and

between populations, and to discuss possible implications of genetic data for its management and protection.

## Materials and methods

*Tillandsia landbeckii* is a terrestrial perennial herbaceous species which grows over 930 m elevation, with pale greenish-grey, lanceolate, basal leaves, 30 cm tall at flowering, and roots are present in small specimens and absent in later stages, inflorescences terminal with 1-3 flowers with several colors (yellow, orange, purple and brown) at the tip of a thin stalk (Fig. 1). The characteristics growth pattern are mounds distributed as horizontally extended bands across the hillsides or flat surface of the desert, which normally reach 20-40 cm in width, and 2-4 m in length and each individual mound may be produced by cloned ramets from a single colonizing genet (Rundel et al., 1997). Its distribution extends from south Peru (13°50' S) to north Chile (31°50' S) (Pinto, 2005). Two areas of the Arica and Parinacota region, Pampa Dos Cruces (18°28'43.48" S, 70°5'16.69" W) and Pampa Camarones (18°52'29.66" S, 70°7'10.85" W) located 23 and 22 Km from the coast, respectively, and separated geographically by around 45 km and 3 transversal coastal valleys, were sampled (Fig. 2). This region is located in the northernmost portion of Chile, which is part of the Atacama Desert, with an average annual temperature of 18.6 °C and an annual mean precipitation of 1.6 mm (Sarricolea et al., 2017). In this study, we considered 1 mound as 1 individual sample. Fresh young leaves of 10 randomly selected mounds from each of the 2 populations of *T. landbeckii* were collected and stored at -80 °C until DNA extraction. Detailed information regarding locations and codes of the study samples is shown in Table 1.

Total genomic DNA was extracted from 100 mg of fresh leaves of individual plants according to Huanca-Mamani et al. (2015). The quality and purity of the extracted DNA were verified by agarose gel electrophoresis. DNA concentration was determined spectrophotometrically and adjusted to a final concentration of 50 ng/μl and stored at -20 °C.

AFLP analyses were performed in accordance with the methodology described by Vos et al. (1995). In short, 200 ng of genomic DNA were double-digested in a final volume of 15 μl at 37 °C with EcoRI and MseI (New England Biolabs), followed by ligation of EcoRI and MseI adapters in the same reaction. Pre-selective PCR amplifications were performed using the primer pair EcoRI + 0 and MseI + 0. For selective PCR amplification, 7 EcoRI + 3 and MseI + 3 primer combinations were chosen from the 16 combinations tested. Final amplifications products were separated on 6% polyacrylamide gels electrophoresis in 0.5

Table 1

Plant materials collected in 2 populations of *T. landbeckii* used in this study.

Sample code	Location	Coordinates (UTM)		Elevation (m asl)
		W	S	
1	Pampa Dos Cruces	385759	7956833	1,015
2	Pampa Dos Cruces	385745	7956756	1,017
3	Pampa Dos Cruces	385989	7956625	1,028
4	Pampa Dos Cruces	386022	7956518	1,035
5	Pampa Dos Cruces	385367	7957200	993
6	Pampa Dos Cruces	385405	7957215	998
7	Pampa Dos Cruces	385400	7957151	1,001
8	Pampa Dos Cruces	385376	7957169	997
9	Pampa Dos Cruces	385456	7957053	1,005
10	Pampa Dos Cruces	385371	7957067	1,002
11	Pampa Camarones	383188	7912595	1,054
12	Pampa Camarones	383272	7912740	1,052
13	Pampa Camarones	383266	7912823	1,047
14	Pampa Camarones	383405	7912645	1,058
15	Pampa Camarones	383520	7912511	1,066
16	Pampa Camarones	382804	7912371	1,046
17	Pampa Camarones	382102	7912698	1,027
18	Pampa Camarones	382134	7912766	1,024
19	Pampa Camarones	382260	7912743	1,028
20	Pampa Camarones	382240	7912672	1,029

X Tris-borate-EDTA buffer using a 1,000 bp DNA marker (BioLabs); 0.2% silver nitrate was used for staining.

Each band (monomorphic and polymorphic) was scored manually as a dominant marker and transformed into a 1 (presence) or 0 (absence) binary matrix. Only bands that could be scored unequivocally were included in the analysis. The band size range between 80 and 1,000 bp was considered for the analysis. The binary matrix was edited using Microsoft Excel 2015. The percentage of polymorphic loci (Pp), observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), expected heterozygosity (He), number of private bands (NPB), and number of locally commons bands (NBLC) were calculated using GenAlex 6 (Peakall & Smouse, 2012). Analysis of molecular variance (Amova) was estimated using Arlequin v 3.5 software and GenALEX6 (Excoffier & Lischer, 2010; Peakall & Smouse, 2012),





Figure 1. *Tillandsia landbeckii* in the Atacama Desert of northern most Chile. Typical *T. landbeckii* mound distributed as horizontally extended bands over surface of the desert.

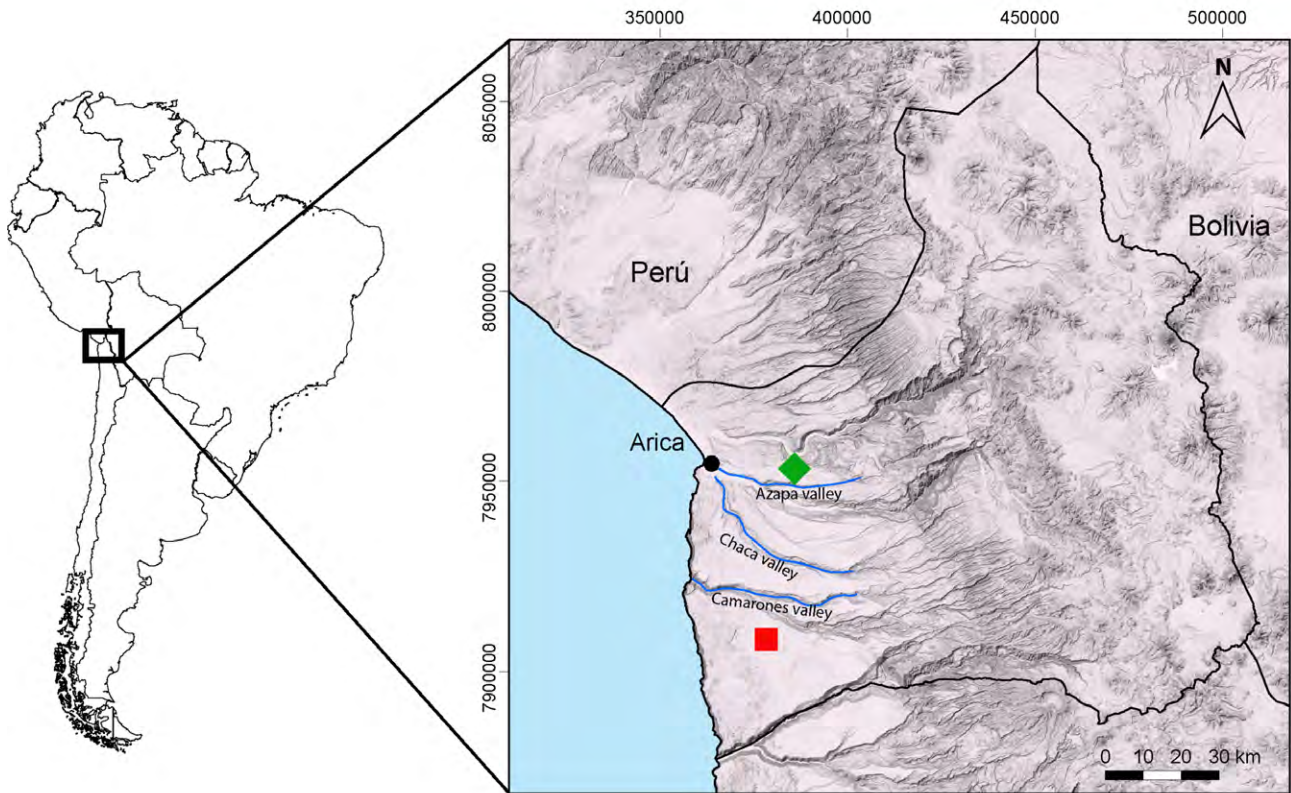


Figure 2. Study area in South America (left) and locations of collection sites of the 2 populations (right) of *Tillandsia landbeckii* in the Atacama Desert of northernmost Chile: Pampa Dos Cruces (diamond green) and Pampa Camarones (red square).

based on 1,023 permutations to evaluate the distribution of genetic variation within and among populations, as well as to estimate the genetic differentiation index ( $F_{st}$ ). The amount of gene flow between populations was calculated using population differentiation  $N_m = 0.5 ((1-F_{st}) / F_{st})$  using Arlequin v 3.5 software (Excoffier & Lischer, 2010).

Principal Co-ordinate analysis (PCoA) was calculated using GenAlEx 6 (Peakall & Smouse, 2012). A NeighborNet tree was constructed using SplitsTree4 (Huson & Bryant, 2006). Neighbor-net is similar to the common Neighbor joining method, but by showing reticulations, it can represent alternative trees in the presence of distinct phylogenetic signals, which may arise, for instance, from gene flow between populations.

Bayesian analysis to assign the individual samples the probability of belonging to a homogeneous cluster (K populations) without prior population information was conducted using STRUCTURE version 2.3.4 (Pritchard et al., 2000). This program was run using the admixture model, with a burn-in period of 10,000 and 50,000 iterations and a posterior number of Markov Chain Monte Carlo (MCMC) of 50,000. A total of 20 independent runs were performed with the number of clusters in the range of 1-5. Structure harvester was used to determine the best K using the method described by Evanno et al. (2005) (Earl & von Holdt, 2011).

## Results

Among 16 primer combinations evaluated, 7 combinations amplified well-distributed fragments with good distinctness, which were highly polymorphic and with a size range of 100 – 1,000 bp. A total of 405 clear and quantifiable fragments was generated in the 2 populations, ranging from 19 to 85 fragments with an average of 57.86 per primer combination. Among 405 loci, 188 (46.42%) was polymorphic, with an average of 26.86 per primer combination. The primer combination E-ACA / M-CTC generated the highest gene polymorphism, with  $P_p = 64.71\%$ ; and primer combination E-ACG / M-CAT the lowest polymorphism, with  $P_p = 25.00\%$  (Table 2).

In the combined data matrix of all 7 primer combinations, Shannon's information index (I) was 0.483 ( $\pm 0.017$  SD) and 0.380 ( $\pm 0.019$  SD) for Pampa Dos Cruces and Pampa Camarones, respectively, with a total average of 0.432 ( $\pm 0.013$  SD). The effective number of alleles ( $N_e$ ) was 1.580 ( $\pm 0.027$  SD) and 1.429 ( $\pm 0.026$  SD) and the number of private bands (NBP) was 34 and 9, respectively (Table 3). The Pampa Dos Cruces population ( $P_p = 88.30\%$ ,  $I = 0.483$  ( $\pm 0.017$  SD) and  $H_e = 0.327$  ( $\pm 0.013$  SD)) showed greater genetic diversity than the

Table 2

Polymorphism and primer informativeness of 7 primer combinations selected.

Primer combinations	Total number of bands	Number of polymorphic bands	Pp
E-AAC/M-CTG	79	32	40.51
E-ACA/M-CTC	85	55	64.71
E-ACG/M-CTT	50	28	56.00
E-ACC/M-CGT	19	11	57.89
E-ACC/M-CTC	57	27	47.37
E-ACG/M-CAT	48	12	25.00
E-AAG/M-CTG	67	23	34.33
Total	405	188	
Mean	57.86	26.86	

Pp: percentage of polymorphic loci.

Pampa Camarones population ( $P_p = 71.28\%$ ,  $I = 0.380$  ( $\pm 0.019$  SD) and  $H_e = 0.253$  ( $\pm 0.014$  SD); Table 3).

The analysis of molecular variance (Amova) performed for estimating the partitioning of genetic variance within *T. landbeckii*, an, showing that a proportion (25.12%) of the variation was due to the difference between groups, however, most of the variation (74.88%) was due to differences among individuals within populations ( $\Phi_{pt} = 0.251$ ,  $p < 0.01$ ) (Table 4). The genetic differentiation ( $F_{st}$ ) between the populations of *T. landbeckii* was 0.2510 and the number of migrants per generation ( $N_m$ ) was estimated as 1.4904 (Table 3).

The principal coordinate analysis (PCoA) was used to study the relatedness within a matrix by converting the genetic distance into eigenvectors and values. A two-dimensional PCoA analysis in the populations of *T. landbeckii* showed that the first principal coordinates accounts for 63.64% of total variation and do not clearly separate Pampa Dos Cruces from Pampa Camarones populations (Fig. 3). The second principal coordinate accounts for 13.31% of total variation and separated most individuals from Pampa Dos Cruces from those Pampa Camarones, but there was some overlap, with 3 individuals from Pampa Dos Cruces grouping with those from Pampa Camarones and vice versa (Fig. 3). Collectively, 76.94% of the total observed variation was explained by the first 2 coordinates, showing the special separation of the majority of individuals from each population. The neighbor network analysis based on genetic distances among the 20 samples

Table 3

Genetic diversity and differentiation of *T. landbeckii*.

Source	N	P	Pp	Na	Ne	I	He	NPB	NBLC	NGD	F <sub>st</sub>	Nm
Pampa Dos Cruces	10	179	88.30	1.835 (0.035)	1.580 (0.027)	0.483 (0.017)	0.327 (0.013)	34	0			
Pampa Camarones	10	155	71.28	1.532 (0.057)	1.429 (0.026)	0.380 (0.019)	0.253 (0.014)	9	0			
Among populations		188	79.79	1.688 (0.034)	1.504 (0.019)	0.432 (0.013)	0.290 (0.010)			0.177	0.251	1.490

N: Number of plant analyzed; P: number of polymorphic loci; Pp: percentage of polymorphic loci; Na: observed number of alleles; Ne: effective number of alleles; I: Shannon's information Index; He: expected heterozygosity; NPB: number of private bands; NBLC: number of locally commons bands; NGD: Nei genetic distance; F<sub>st</sub>: coefficient of genetic fixation; Nm: Estimate of gene flow from F<sub>st</sub>.

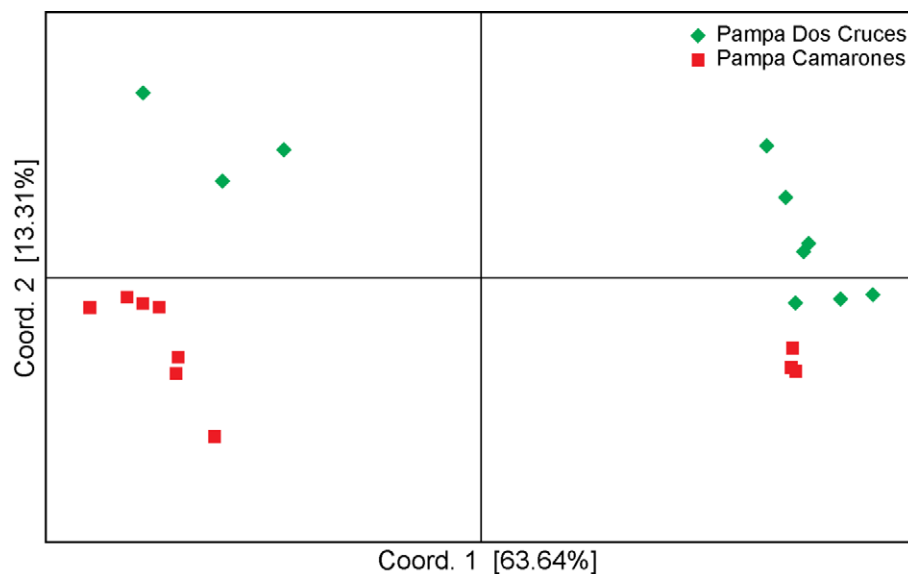


Figure 3. Two-dimensional Principal Coordinates Analysis (PCoA) using AFLP markers of 20 individuals from 2 populations of *Tillandsia landbeckii*.

formed a network with a clear geographic structure, showing a clear pattern related to their origins (Fig. 4-A), except for the overlapping individuals identified in the PCoA analysis previously mentioned. The STRUCTURE analysis for all samples, suggested that the samples should be split into 2 populations with K = 2 ( $\Delta K = 1000$ ) (Fig. 4B). Structure analysis run with K = 2 showed a moderate genetic separation between Pampa Dos Cruces and Pampa Camarones populations (Fig. 4C). This result is in line with the results of the PCoA and neighbor network analysis (Figs. 3, 4), giving support to the STRUCTURE analysis and showing a moderate separation of the populations in 2 clusters.

Table 4

Summary of Amova analysis on the basis of matrix of genetic distances of 20 individual *T. landbeckii* samples comprising 2 different population.

Source of variation	df	SSD	CV	% Total	Fixation index
Among populations	1	138.750	10.689	25.12	$\Phi_{PT}=0.251^{**}$
Within populations	18	573.500	31.861	74.88	
Total	19	712.250		100	

df: degrees of freedom; SSD: sum of squares; CV: variance component estimates; % Total: percentage of total variance contributes by each component.

$^{**}p < 0.01$  (significance test after 999 permutations)

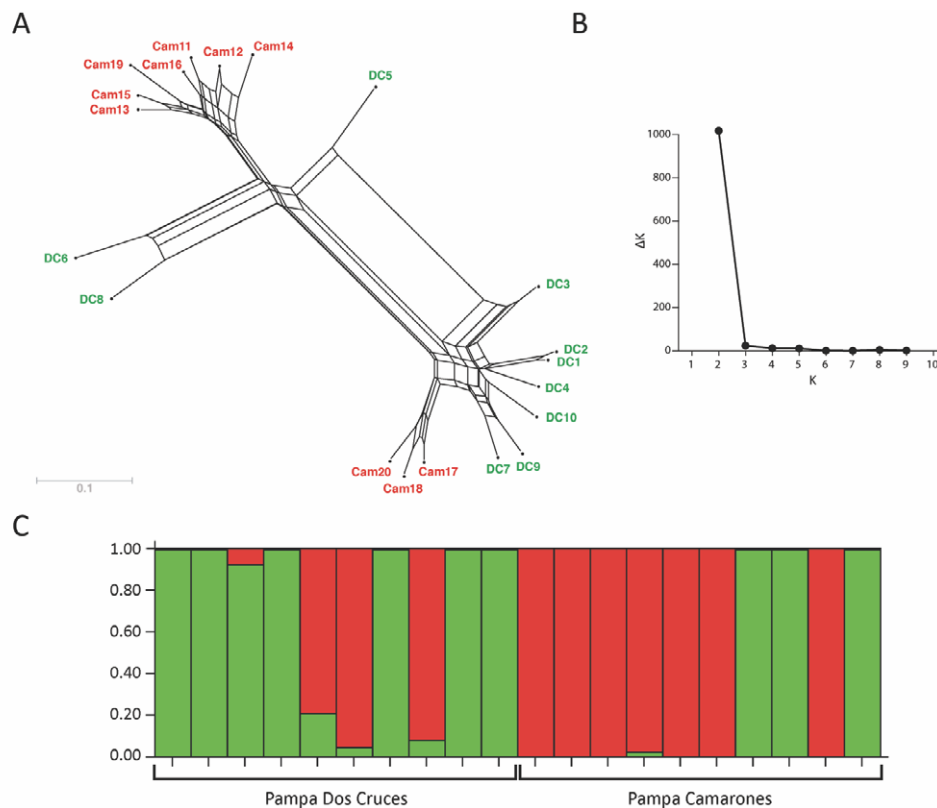


Figure 4. Genetic relationship and structure of *Tillandsia landbeckii* estimated from AFLP analysis. (A) Neighbour-Net presenting the genetic relationship between individuals of this species was calculated by SplitsTree 4. (B) Results of the Bayesian analysis using the program STRUCTURE 4.3.4. The ad hoc statistic  $\Delta K$  (Evanno et al., 2005) was plotted against various values of  $K$ , 435 suggesting  $K = 2$  as the most likely number of clusters. (C) Bayesian model-based clustering STRUCTURE analysis as inferred at  $K = 2$ . The left scale indicates the association coefficient ( $Q$ ) for the assignment of genotypes into groups. DC: Samples collected from Dos Cruces population; Cam: Samples collected from Camarones population.

## Discussion

In this study, we evaluated the diversity and structure genetic in 2 populations of *T. landbeckii*, a Bromeliaceae adapted to the hyper-arid condition of the Atacama Desert in northern of Chile, by AFLP. These populations are located about 1,000 m elevation, approximately 23 km from the coast and separated by 45 km and 3 transversal coastal valleys among them. The ecological characteristic in these population are very similar and due to the hyper-arid environmental conditions which characterize the Atacama Desert (high radiation during day, low temperature during night, and 1.6 mm of annual precipitation (Sarricolea et al., 2017). The tillandsias are exceptional organisms because they thrive under these extreme conditions, which are beyond the tolerance limits of any other vascular plant (Rundel et al., 1997). To achieve this, they absorb fog moisture and nutrients through specialized scales on

the surface of their leaves, and use crassulacean acid metabolism (CAM) as a morphological and physiological adaptation, respectively (Rundel & Dillon, 1998).

Genetic diversity is considered the consequence of long-term evolution and represents the evolutionary potential of a species to survive in various environments. High levels of genetic diversity are known to enhance the resilience of species and persistence in the wild, so these results are hopeful about the future ability of this species to survive in the future in the face of climate change and encroaching human-induced habitat destruction (Sheidai et al., 2014). AFLP with high levels of polymorphism represents a powerful tool for assessing genetic diversity in many species (Zhang et al., 2017). Thus, in this study AFLP analysis was selected as technique for studying for the first time the genetic variation present in 2 populations of *T. landbeckii*, a terrestrial and native species of *Tillandsia* genus found in the northernmost region of Chile.



The current study has shown that the genetic diversity value obtained for *T. landbeckii* was 0.43. This value is higher than the mean gene diversity estimates for native species (0.22) and long-lived perennials (0.25) (Nybom, 2004). Interestingly, the more widespread Pampa Camarones population actually had lower genetic diversity than Pampa Dos Cruces, which is the smaller and more restricted population.

Up to now, genetic diversity has been evaluated in natural populations of only 3 epiphytic species of the *Tillandsia* genus, through allozymes or SSR molecular markers (González-Astorga et al., 2004; Solórzano et al., 2010; Soltis et al., 1987). These studies revealed several levels of genetic variability, depending on the marker system and species analyzed; for this reason, it is difficult to compare directly with our results, and in addition, we studied a terrestrial species of this genus. The level of diversity found for *T. landbeckii* is similar compared with studies carried out with populations of *Tillandsia recurvata* on 2 host tree species from central Mexico using 5 SSR loci ( $H_o$ : 0.34–0.42) (Solórzano et al., 2010). In comparison with studies in other epiphytic species from the Tillandsioideae subfamily, as *Vriesea reitzii*, which showed high levels of genetic diversity using SSR ( $H_o$ : 0.360–0.499) (Soares et al., 2018). However, in *Guzmania monostachia*, SSR revealed low levels of genetic diversity ( $H_o$ : 0.031) (Cascante-Marín et al., 2014). The level of genetic diversity detected through AFLP markers in the present study suggests that natural populations of *T. landbeckii* present high genetic diversity. Even though, in this study, a small number of populations of *T. landbeckii* were analyzed, it has been reported that few populations often represent well the genetic structure for an entire species (Soltis et al., 1987). Maintenance of such diversity has to be the focus of programs of species conservation and AFLP markers proved to be a very useful tool in assessing the genetic diversity of these populations.

The highly specific habitat in an area of extreme aridity and its dependence of fog moisture to survival, suggest that *T. landbeckii* distribution could be highly sensitive to small changes in environmental conditions (Rundel et al., 1997). In Pampa Camarones only lives *T. landbeckii*, while Pampa dos Cruces is possible to find *T. landbeckii* and *T. marconae*. It has been reported a gradual decline in abundance of *T. landbeckii* in populations located south of Pampa Camarones, which showed dead mounds, with no indication of recolonization (Rundel et al., 1997). Additionally, Pampa Camarones mounds are irregular in shape and poorly formed compared with other populations (Pinto et al., 2006). Field observation allows us to identify the presence of the high number of dead *Tillandsia* buried under the sand in the Pampa Camarones population

compared with Pampa Dos Cruces population. This effect is unusual because the most common response shown by all sand dune species when are partially or completely buried is stimulation of growth and the relative amounts of burial for growth stimulation in different species may vary by many orders of magnitude, but the reactive growth response is similar (Maun, 1997). Since several aspects of the life history of *T. landbeckii*, such as its life spans time or flowering frequency are still undetermined, it is difficult to suggest an explanation to the high frequency rate of dead *Tillandsias* buried.

The genetic differentiation founded among *T. landbeckii* populations is characterized by a  $F_{st}$  value of 0.251, indicating that there is a moderate-high genetic differentiation among the populations (Hartl & Clark, 1997). This value is similar than the average reported for plants with similar life-history traits: endemic plants ( $F_{st}$  = 0.26), it is higher than long-lived perennial plants ( $F_{st}$  = 0.19) (Nybom, 2004), and it is low to high as compared against the range of values obtained for member of the Bromeliace family using AFLP or SSR marker: for example, *T. recurvata*,  $F_{st}$  = 0.03 (Solórzano et al., 2010), *Guzmania monostachia*,  $F_{st}$  = 0.193 (Cascante-Marín et al., 2014), *Vriesea reitzii*,  $F_{st}$  = 0.123 (Soares et al., 2018), *Vriesea simplex*,  $F_{st}$  = 0.069 (Neri et al., 2018), and *Vriesea scularis*,  $F_{st}$  = 0.416 (Neri et al., 2018).

In *T. landbeckii* our analyses detected gene flow between populations ( $N_m$  = 1.49), which is a moderate value (Hamrick & Nason, 2000; El-Bakatoushi & Ahmed, 2018). When  $N_m$  value is below 1, it means that populations began to differentiate due to genetic drift (Wright, 1969). The gene flow observed indicates that there is a moderate genetic exchange among populations of *T. landbeckii*, although there may be enough to prevent complete isolation among them.

Currently, there are no studies in relation to pollinators, seed dispersal mechanisms, and breeding systems of *T. landbeckii*, however this plant has plumose seeds, which can facilitate seed dispersal by wind over long distances. *T. landbeckii* is one of the few species that grow near the coast of the Atacama Desert, in an open environment, with few obstacles for dispersal and where the sea winds can enhance seed dispersal.

Analysis of molecular variance (Amova) was conducted to further evaluate the partitioning of genetic differentiation among and within *T. landbeckii* populations. The results of Amova were also found comparable to  $F_{st}$ , indicating that the major proportion of the total variation is present within populations (74.88%) and the minor variation is present among populations (25.12%).

The breeding system can strongly affect the extant genetic variation in the vascular plant (Schneller &

Holderegger, 1996). It is known that *T. landbeckii* propagate vegetatively through cloned ramets, and while there are no studies about its breeding system, the presence of open flowers and siliques (field observation), allow us to suggest that *T. landbeckii* presents a mixture of sexual and clonal reproduction system, as was reported for other members of this family (Pinto et al., 2005). Further studies will be essential to determine and quantify the importance of each system in the genetic diversity of this species, because plants with the capability of altering their mode of reproduction according to environmental conditions may thrive in a broader range of conditions and be more resilient in a longer term (Wang et al., 2018). Studies in species that develop under extreme conditions, suggest the presence of a higher proportion of clonal reproduction due to the low physiological cost it has for the plant (Li et al., 2018; Wang et al., 2018). Generally, higher genetic variation within populations has been noted in outcrossing, perennial plant species, whereas populations of selfing species or species with a mixed mating system are often less variable genetically (Nybo, 2004). Based on the observed genetic variation within populations in our study (74.88%), we suspect that crossing is likely an important factor maintaining the genetic properties of both populations. Species of the family of Noctuidae and Pyralidae (Lepidoptera) have been found in the *Tillandsia* population (personal communication). Perhaps, these insects could play a role as pollinators of this species. Additionally, these findings may result from particular life-history traits of *T. landbeckii*, such as both sexual and vegetative reproduction and long-life span than tend to preserve genetic variability within these populations (Smidova et al., 2011).

Neighbor-net tree showed 2 branches within the *T. landbeckii* populations that supported the grouping obtained by PCoA and revealed the presence of 2 genetic groups (Fig. 4A). The results generated from STRUCTURE for all the populations indicated a value of  $K = 2$ , indicating that it is possible to distinguish 2 genetically different groups with genotypes attributed according to each population (Fig. 4B). Three samples from Pampa Dos Cruces were identified by the Bayesian admixture analysis as similar to individuals collected from Pampa Camarones and viceversa, supporting the perceived pattern of moderate high genetic differentiation among populations. The PCoA, Neighbor-net and STRUCTURE results clearly showed a grouping and moderate genetic structure between both populations.

In plants, the population genetic structure is determined by the interaction of processes such as gene flow, mutation, selection, and mating strategy. Clustering was not clearly defined in the analyzed samples because all the individuals

in a population were not incorporated into the same group. The seed or pollen flow appears as an unlikely mechanism linking the currently, isolated populations, because both populations are separated by 45 km and the presence of 3 transversal coastal valleys between them. Small, isolated populations are particularly subject to inbreeding and genetic drift; their genetic variation is expected to be low in comparison to larger populations (Tansley & Brown, 2000). Similarly, endemic and rare species typically exhibit low levels of genetic variation (Hamrick and Godt, 1996). *T. landbeckii* maintains a high level of genetic diversity (PPB: 71.28-80.30%, I: 0.380-0.483) compared to the levels of genetic diversity reported for widespread species (Nybo, 2004). This could be explained because the distribution of genetic diversity within and between population still reflects gene flow in the past before the formerly larger populations were fragmented, maintaining in this way, a portion of the ancestral polymorphism between them. It has been suggested that a greater and unexpected contribution of the vegetative growth, coupled with a long-life span of individuals, could be producing this type of genetic distribution (Cieslak et al., 2015).

Phylogenetic reconstruction suggests that subfamily Tillandsioideae evolved just beyond of the periphery of the Guayana Shield, near the Caribbean littoral. Origin of the most highly specialized atmospheric epiphytes member of this subfamily, with a center of diversity in the Andes, began 15 million years ago (Mya). The modern *Tillandsia* genera beginning to diverge from each other ca. 8.7 Mya and later they extended to Central America, the northern littoral of South America and the Caribbean (Givnish et al., 2007). It is estimated that early-divergent bromelioids colonized the coastal Chile at ca 10.1 Mya (Givnish et al., 2011). Probably, the presence of *T. landbeckii* in northern coastal Chile occurred before of the formation of the several transversal coastal valleys, between ca. 7.5 Mya and 2.7 Mya (Kober et al., 2006), which fragmented the ancestral population and later shaped and determined its current distribution.

Population genetics studies in rare and threatened plants have become imperative for plant conservation (Holsinger & Gottlieb 1989). Diversity genetic and structure population data obtained in this study can be used for conservation decision making of *T. landbeckii*. According to our results, the populations studied showed high genetic diversity and moderate high genetic differentiation, indicating no imminent threat to this species. However, if we assume that AFLP variation obtained is a representative measure of total genetic variation, and that also there is a moderate high differentiation between the populations of Pampa Dos Cruces and Pampa Camarones, our results imply that it

is recommendable to take individuals from these natural populations for *ex situ* conservation, because the reduced number of individuals and populations under these extreme conditions makes *ex situ* conservation advisable, through the formation of a seed bank to avoid the loss of relevant alleles present in this population (León-Lobos et al., 2012). Due to most aspects of the biology and ecology of *T. landbeckii*, are unknown, it is very difficult to propose a well-founded conservation strategy. However, the lower value of genetic diversity found in Pampa Camarones population must be an aspect to consider in the future conservation strategy. Habitat preservation is usually the best strategy to keep endangered species for long-term existence (Jiménez et al., 2017). In addition, the presence of private alleles in the population of P. Dos Cruces and P. Camarones, (34 and 9, respectively), emphasizes the importance of conserving this genetic diversity and deserves special attention in any conservation measures.

The genetic diversity of a terrestrial species of the *Tillandsia* genus, adapted to survive the hyper-arid condition of the Atacama Desert in Chile, has not been explored at the molecular level. This is the first study that shows the current state of their genetic variability available in the northernmost part of Chile. However, a larger study involving more populations and samples, as well as of other *Tillandsia* species and a sequence-based analysis, are clearly needed to provide additional data, validate the findings, and provide more details about linkage and phylogeny of this species. The genetic data presented here provides a valuable baseline for future comparisons of genetic diversity to evaluate the effectiveness of protected areas and restoration in maintaining genetic diversity as well as for evaluating the consequences of further fragmentation and population loss.

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