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# Genetic diversity of *Geoffroea decorticans*, a native woody leguminous species from Atacama Desert in Chile

## Diversidad genética de *Geoffroea decorticans*, una especie de leguminosa leñosa nativa del Desierto de Atacama en Chile

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SUMMARY

*Geoffroea decorticans* (Fabaceae), chañar, is one of the few native tree species that are adapted to the ecologically limiting conditions from Atacama Desert. Despite its considerable value as a crop species, and important medicinal and food properties, the genetic variability of this plant has not yet been evaluated. The aim of this study was to analyze genetic diversity and degree of fragmentation in *G. decorticans* populations from six provinces in northern Chile. Genetic variability was assessed using eight ISSR and three RAPD primers, generating 97 % and 81 % polymorphic bands, respectively. AMOVA results based on nine populations showed high genetic diversity within populations (65 %) and moderate levels among populations (35 %). Moreover, genetic relationships among individuals provided evidence for the existence of two well defined clusters in the northern and southern regions of the Atacama Desert. A Mantel test showed a significant positive correlation between geographic and genetic distances (r = 0.58) in seven populations, indicating significant isolation-by-distance. Average Shannon Weaver diversity indices showed significantly lower values associated with Pachica, Copiapó river-mouth, Alto del Carmen and Vicuña populations relative to populations from San Pedro, Calama, Azapa and Copiapó. In this work, a combination of ISSR and RAPD methods provided high quality information to evaluate genetic variability, and could be used as an alternative method to identify and distinguish *G. decorticans* genotypes of interest. This work represents the first attempt to molecular characterization of *G. decorticans* in Chile, and its significance to future genetic research is discussed.

*Key words:* RAPD, ISSR, genetic diversity, forest fragmentation, Atacama Desert.

RESUMEN

*Geoffroea decorticans* (Fabaceae), chañar, es una de las pocas especies nativas de árboles adaptadas a las condiciones ecológicamente limitantes del Desierto de Atacama. No obstante su valor como especie de cultivo y de sus propiedades alimenticias y medicinales, la variabilidad genética de esta especie aún no se ha evaluado. El objetivo del estudio fue analizar la diversidad genética y el grado de fragmentación de poblaciones de *G. decorticans* de seis provincias de Chile. La variabilidad genética se evaluó utilizando ocho ISSR y tres cebadores RAPD, generando 97 % y 81 % de bandas polimórficas, respectivamente. Los resultados AMOVA basados en nueve poblaciones mostraron gran diversidad genética dentro de las poblaciones (65 %) y niveles moderados entre poblaciones (35 %). Las relaciones genéticas proporcionaron evidencia de dos grupos bien definidos en el norte y sur del Desierto de Atacama. Una prueba de Mantel mostró una correlación positiva significativa entre las distancias geográficas y genéticas (r = 0,58) para siete poblaciones, lo que indica un aislamiento por distancia. El índice de diversidad promedio de Shannon Weaver mostró valores significativamente bajos asociados con las poblaciones de Pachica, Copiapó, Desembocadura del río, Alto del Carmen y Vicuña en relación con las poblaciones de San Pedro, Calama, Azapa y Copiapó. Una combinación de métodos ISSR y RAPD proporcionó información para evaluar la variabilidad genética y como método para identificar y distinguir genotipos de *G. decorticans*. Se presenta el primer intento de caracterización molecular de *G. decorticans* en Chile, y se discute su importancia para futuras investigaciones genéticas.

*Palabras clave:* RAPD, ISSR, diversidad genética, fragmentación de bosques, Desierto de Atacama.

## INTRODUCTION

*Geoffroea decorticans* (Gillies ex Hook. *et* Arn.) is a native tree belonging to the Fabaceae family (Lavin *et al.* 2001). Locally known as "chañar", its distribution includes the semiarid regions (Atacama Desert) of northern Chile, the Paraguayan Chaco, southern Peru, Bolivia, southern Uruguay and northern to central south-east Argentina (Squeo *et al.* 2008). In Chile, this woody species inhabits valleys and oases from Arica-Parinacota to Coquimbo Regions (Squeo *et al.* 2008), and is one of the most commonly occurring tree species in Copiapó and San Pedro de

Atacama valleys. *G. decorticans* has multiple uses and is considered a valuable crop, well adapted to dryland conditions. For instance, the fruit is widely used to prepare "*arrope*" (a sweet honey-like extract), flour, cookies, jams and jelly. Medicinal studies have shown that the aqueous extract from fruit and *arrope* possesses antinociceptive, antitussive and expectorant effects (Reynoso *et al.* 2016), as well as antioxidant properties (bioactive polyphenols) against diseases associated with oxidative stress, inflammatory mediators and metabolic syndrome (Costagama *et al.* 2016, Jiménez-Aspee *et al.* 2017). Recently, *G. decorticans* has been evaluated as a multipurpose energy crop in semiarid regions due to its potential as feedstock for biodiesel and biomass pellet production (Santibañez and Vargas 2017).

Chilean *G. decorticans* genotypes are well adapted to the arid and semi-arid conditions of the Atacama Desert. They inhabit a range of geographical and environmental conditions, varying from low (Copiapó river-mouth and Azapa valley) to high altitude areas (San Pedro de Atacama) and saline soil (Copiapó valley). Forest genetic resources in Chile arid zones are fragmented and reduced in extent as a result of indiscriminate extraction and unsustainable use (Gacitúa and Villalobos 2012). According to Squeo *et al.* (2008), *G. decorticans,* in the Atacama Region, has been granted the conservation status of "Vulnerable". Considering their widespread geographic distribution, adaptive capacity to a range of environmental conditions and their value as a dry-land crop, evaluating the genetic variability of northern Chilean *G. decorticans* genotypes presents a compelling research question.

DNA markers are a powerful tool for genetic analyses, because they allow direct comparison and identification of species and plant varieties independently from environment fluctuations and/or developmental stages. Random amplified polymorphism DNA (RAPD) markers (Williams *et al.* 1990) are a promising marker system widely used in plant research ranging from phylogenetic studies to genetic mapping, DNA fingerprinting, plant breeding and germplasm management. Both simple and efficient, RAPD is useful for variety and cultivar analyses, and is capable of detecting relatively small amounts of genetic variation without the need for prior information on the genome. Similar to RAPD, the ISSR (Inter-simple sequence repeat) genotyping technique, based on variations occurring in the inter-microsatellite regions, has a wide range of applications in genetic analyses (Zietkiewicz *et al.* 1994).

Based on the capacity of the molecular markers ISSR and RAPD to estimate the genetic variability in plant species populations, we focused the genetic analysis of *G. decorticans* using these markers to (i) assess degree of fragmentation from *G. decorticans* populations in Northern Chile and (ii) evaluate genetic diversity of nine *G. decorticans* populations. We assume that *G. decorticans* populations in Atacama Desert are highly fragmented, with low genetic diversity within populations, and a high correlation between genetic and spatial distances among populations.

## METHODS

*Plant material.* Thirty-eight *G. decorticans* individuals were randomly selected for sampling in the following provinces in northern Chile: Arica, Tamarugal, Loa, Copiapó, Huasco and Elqui. Sampling was conducted in March of 2016. Young leaves from only one season of the year were collected. According to Gutiérrez *et al.* (2015), an empiric estimation of 50 unrelated trees can represent 99 % of the adaptive genetic variation of a population that can vary between 50 and 5,000 trees. The size of the *G. decorticans* populations in the localities of northern Chile is relative; this can vary from 50 to more than 4,000 individuals. In some valleys, these trees can be widely distributed from the mountain to the sea or reduced to a few tens in other valleys. For the study, we determined to collect leaves of approximately 30 *G. decorticans* individuals for each province. The randomly selected trees were sampled at a distance > 30 meters (Gutiérrez *et al.* 2015), to avoid kinship relationship among trees. Fifty grams of young leaves were collected from each specimen and placed in polyethylene bags, and immediately transported in a cooler at 4 °C to the laboratory, where they were stored at -80 °C. The subsequent selection of samples collected for genetic analyses will depend on their optimum DNA quality (table 1). GPS coordinates of the selected samples are listed in table 2.

*Plant DNA extraction.* Genomic leaf DNA was extracted using the modified CTAB method (Doyle and Doyle 1987), including silica columns. The young leaves were transferred to sterilized mortars and treated with liquid nitrogen to solidify them, which permits homogenization via firm pressure of the tissue until a light powder was obtained; 100 mg of the powder were weighted in 2 mL microcentrifuge tube, using a spatula for the transfer. Afterwards, 7 µL of beta-mercaptoethanol, 4 µL of 10 mg/mL Proteinase K, 10 µL of 5 % Sarkosyl and 700 µL of CTAB preheated to 65 °C for 15 min (4 % p/v PVP-40, 100 mM Tris-HCl pH 8; 1,2 M NaCl; 20 mM EDTA, 2 % CTAB) were added to each tube. They were stirred with a vortex (Scilogex) and incubated in a water bath for 60 min at 65 °C, inverting the tubes every 15 min. The tubes were centrifuged at 14,000 rpm for 10 min at 4 °C (Universal Centrifuge 320 R Hettich), and the top aqueous phase was taken for further processing (~700 µL) in a new tube. Subsequently, 800 µL of phenol/chloroform/UltraPure TM isoamyl alcohol (25:24:1) solution were added to each tube and mixed for 10 min at 120 rpm in a shaker-incubator (Zhichengzhy-100B) at 20 °C. These tubes were then centrifuged at 14,000 rpm for 10 min at 4 °C and the top phase was recovered for further processing (~550 µL), trying not to perturb the lower fraction of

**Table 1.** Geographic location of the samples by region, province, name of population and code, and size of the sample of *Geoffroea decorticans* trees, estimated area of sampling and samples with optimal DNA quality.

Ubicación geográfica de las muestras por región, provincia, nombre de población y código, y tamaño de la muestra de árboles de *Geoffroea decorticans*, área estimada de muestreo y muestras con calidad óptima de ADN.

| Region | Province | Population and code | Estimated sampling area (km$^2$) | Sample size (*n*) | Samples with optimum DNA quality (*) |
|---|---|---|---|---|---|
| Arica-Parinacota | Arica | Azapa (AZ) | 8 | 25 | 4 |
| | Arica | Chaca (CH) | 0.1 | 10 | 3 |
| Tarapacá | Tamarugal | Pachica (PA) | 0.3 | 30 | 5 |
| Antofagasta | Loa | San Pedro (SP) | 0.23 | 20 | 4 |
| | Loa | Calama (CA) | 0.06 | 10 | 5 |
| Atacama | Copiapó | Copiapó (CO) | 46 | 25 | 10 |
| | Copiapó | Río Copiapó (DES) | 7 | 6 | 2 |
| | Huasco | Alto del Carmen (AC) | 0.2 | 15 | 3 |
| Coquimbo | Elqui | Vicuña/El Molle (VIC) | 20 | 23 | 2 |
| Total | - | - | - | - | 38 |

(*) Samples with high quality and low protein contamination from DNA extract. With $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratio >1.9 and >1.6, respectively.

**Table 2.** Geographic coordinates of *Geoffroea decorticans* samples collected in northern Chile.

Ubicación de muestras de *Geoffroea decorticans* recolectadas del norte de Chile.

| Samples | Latitude (ºS) | Longitude (ºW) | Altitude (m) | Samples | Latitude (ºS) | Longitude (ºW) | Altitude (m) |
|---|---|---|---|---|---|---|---|
| AZ109 | 18°31’01.4” | 70°10’53.9” | 248 | CA348 | 22°27’51.2” | 68°54’37.5” | 2,277 |
| AZ113 | 18°30’54.2” | 70°11’22.0” | 235 | CA350 | 22°27’51.1” | 68°54’37.0” | 2,276 |
| AZ128 | 18°30’07.9” | 70°14’56.1” | 125 | COM06 | 27°26’38.9” | 70°16’01.1” | 460 |
| AZ139 | 18°30’02.3” | 70°15’05.7” | 119 | COM17 | 27°20’39.3” | 70°21’46.0” | 355 |
| CH278 | 18°48’08.6” | 70°10’14.5” | 278 | COP018 | 27°20’46.8” | 70°21’36.3” | 360 |
| CH280 | 18°48’10.1” | 70°10’13.1” | 277 | COM20 | 27°20’57.1” | 70°21’22.9” | 400 |
| CH282 | 18°48’10.8” | 70°10’12.9” | 280 | COP021 | 27°21’49.3” | 70°19’42.7” | 386 |
| PA289 | 19°51’51.4” | 69°24’29.4” | 1,654 | CO477 | 27°20’13.4” | 70°35’47.2” | 198 |
| PA307 | 19°51’50.8” | 69°24’35.7” | 1,649 | CO478 | 27°20’12.8” | 70°35’47.2” | 199 |
| PA316 | 19°51’48.8” | 69°24’38.5” | 1,652 | CO479 | 27°20’12.2” | 70°35’47.2” | 201 |
| PA326 | 19°51’47.8” | 69°24’38.8” | 1,648 | CO480 | 27°20’12.3” | 70°35’45.1” | 201 |
| PA336 | 19°51’46.4” | 69°24’37.7” | 1,649 | CO481 | 27°20’12.3” | 70°35’46.6” | 201 |
| SP378 | 22°57’15.5” | 68°13’47.8” | 2,398 | DES07 | 27°19’21.2” | 70°50’39.8” | 76 |
| SP381 | 22°57’15.2” | 68°13’48.1” | 2,397 | DES35 | 27°19’02.2” | 70°55’09.0” | 6 |
| SP384 | 22°57’14.8” | 68°13’46.9” | 2,395 | ADC091 | 28°46’42.5” | 70°28’17.5” | 829 |
| SP383 | 22°57’15.1” | 68°13’46.9” | 2,396 | ADC084 | 28°46’38.3” | 70°28’22.4” | 822 |
| CA342 | 22°27’51.4” | 68°54’37.4” | 2,288 | ADC087 | 28°46’41.5” | 70°28’21.3” | 825 |
| CA343 | 22°27’51.3” | 68°54’37.9” | 2,281 | VIC618 | 29°58’31.9” | 70°58’33.1” | 331 |
| CA346 | 22°27’52.6” | 68°54’38.0” | 2,280 | VIC636 | 30°02’38.5” | 70°42’55.4” | 626 |

the tube. This supernatant was transferred to a new tube. Next, 5 µL of 10 m $mL^{-1}$ RNAse (Bio Basic) were added to every tube, followed by incubation at 37 °C for 30 min. Two thirds of isopropanol at -20 °C were added to the tubes (367 µL of isopropanol for 550 µL of solution in this case), which were then inverted 30 times at room temperature to promote mixing. The liquid was transferred to a Hi-Bind DNA mini column (Omega Bio-tek) attached to a 2 mL collection tube and left incubating for 2 min at room temperature. The mini columns were centrifuged at 14,000 rpm for 2 min and all the precipitate was discarded. They were washed once with 700 µL of 90 % ethanol at room temperature, centrifuged at 14,000 rpm for 2 min and the precipitate was discarded. This was followed by addition of 700 µL of 70 % ethanol with 10 mM NH4OAc at room temperature, centrifugation at 14,000 rpm for 2 min and discard of the precipitate. The empty mini columns were centrifuged at 14,000 rpm for 2 min to remove the remaining ethanol and the collection tube was replaced by a new 1.5 mL tube. Next, 60 µL of TE preheated to 65 °C were added to each mini column, followed by incubation at 65 °C for 5 min. Finally, the tubes were centrifuged at 14,000 rpm for 2 min, the mini column was discarded and the 1.5 ml tube with the extract was stored at -20 °C for further use. Quality and concentration of total DNA were verified by Colibri Microvolume Spectrophotometer (Titertek Berthold, Germany) at 260, 280, and 230 nm, and genomic DNA integrity checked on a 0.7 % agarose gel.

*ISSR and RAPD amplification.* Polymerase chain reaction (PCR) with eight ISSRs (UBC880, UBC823, UBC815, UBC811, UBC825, UBC807, UBC826 and UBC856) was carried out in a total volume of 24 µL containing 12 µL of SapphireAmp Fast PCR Master Mix 2X (Takara), 5 µL of primer (5 µM), 2.5 µL of total genomic DNA (1 ng $\mu L^{-1}$) and 4.5 µL of nuclease-free water (Thermo Scientific). The amplification was performed in a Swift MaxPro (ESCO) thermocycler under the following conditions: an initial denaturation at 94 ºC for 3 min, followed by 45 cycles of 5 s at 98 ºC, 5 s at 52 ºC, and 40 s at 72 ºC, with a final extension at 72 ºC for 4 min. PCRs with three RAPDs (OPB-19, OPF-08 and OPAI-05) was carried out in a total volume of 24 µL containing 12 µL of SapphireAmp Fast PCR Master Mix 2X (Takara), 5 µL of primer (5 µM), 5 µL of total genomic DNA (1 ng $\mu L^{-1}$) and 2 µL of nuclease-free water (Thermo Scientific). RAPD amplifications were performed under the following conditions: an initial denaturation at 91 ºC for 1 min, followed by 35 cycles of 1 min at 91 ºC, 1 min at 36 ºC, and 1.5 min at 72 ºC, with a final extension at 72 ºC for 2 min. PCR products were mixed with loading buffer and separated by gel electrophoresis on 1.8 % (w/v) agarose gel with 4.5 µL of ethidium bromide (10 mg $mL^{-1}$), in 0.5X TBE at 100 V for 110 min. Finally, DNA fragments were visualized in UV transilluminator and photographed.

*Data analyses.* Each of the ISSR and RAPD bands were considered an independent character, or locus, and polymorphic bands were scored as either present (1) or absent (0) for each of the 38 individuals. Three independent evaluations were performed on each gel, and only consistently scored bands were considered for analyses. The total number of ISSR bands (total number of band, TNB), percentage of polymorphic bands (P%) at 99 %, number of different genotypes (NG), Resolving Power (Rp) and number of private bands (NPB) obtained with each primer were estimated. The Resolving Power (Rp) of a primer was calculated as $Rp = \Sigma\ Ib$, where Ib (band informativeness) takes the value of: $1 - [2 \times (0.5 - p)]$, p being the frequency of the lines containing the band (Prevost and Wilkinson 1999). The Polymorphic Information Content (PIC) was estimated as PIC = 2p (1-p) (Roldan-Ruiz *et al.* 2000).

The binary matrix was applied to determine corrected allele frequency based on Lynch and Milligan (1994). *A* Pair-wise genetic distance matrix was generated among nine populations (38 individuals) using the approach of Nei in GenAlEx 6.5 software (Peakall and Smouse 2012). To evaluate the genetic variation among and within populations, an analysis of molecular variance (AMOVA) was performed using GenAlEx 6.5 software. The significance correlation between genetic and geographic distance matrices was evaluated using a Mantel test in GenAlEx 6.5 software, and the value was obtained by computing 999 random permutations. Based on the matrix of genetic similarity, cluster analyses were performed using UPGMA with the PAST program (Hammer *et al.* 2001); bootstraps of 1,000 replicates were performed to test the robustness of the dendrograms. The Principal Component Analysis (PCA) was performed in PAST program for the clustering analyses of the *G. decorticans* specimens.

Genetic diversity was calculated for biallelic DNA marker loci using the relative Shannon-Weaver diversity index (H) (Hutchenson 1970): $H = -(pi \ln [pi] + qi \ln [qi])$, where p is the frequency for marker amplification and q the frequency of the null allele (Kolodinska *et al.* 2004). The Shannon-Weaver diversity index was calculated for each primer in nine populations: Azapa, Chaca, Pachica, San Pedro, Calama, Copiapó, Copiapó river-mouth, Alto del Carmen and Vicuña. With data from the Shannon-Weaver diversity index, a one-way ANOVA, followed by a Tukey test, was performed to compare population genetic variation.

## RESULTS

Eight ISSR and three RAPD primers, of a total of nine (UBC 807, UBC 810, UBC 811, UBC815, UBC817, UBC823, UBC826, UBC880 and UBC864) and eight (OPM-12, OPX-04, OPP-18, OPA-19, OPAI-14, OPB-19, OPH-19 and OPJ-18) respectively were selected based on the number of bands and their reproducibility. Of the

38 specimens examined, the eight ISSR primers were able to generate 142 scorable bands (on average, 18 bands were amplified per ISSR primer), of which 138 (97 %) were polymorphic. For all ISSR primers, the size of amplified products ranged from 250 to 3,000 bp (table 3). The maximum total number of bands (TNB) was 21 (UBC823), and the minimum 14 (UBC807). The UBC811 primer was able to distinguish 37 *G. decorticans* genotypes, and the UBC807 primer 26 (NG). The resolving power (Rp) of the ISSR primers ranged from 4.2105 (UBC807) to 12.2105 (UBC811). For all ISSR primers analyzed, only the UBC826 primer presented exclusive bands (NEB). The PIC was calculated for each primer, with UBC811 showing the highest PIC value (0.41), and UBC807 the lowest (0.22). The average PIC value for the eight ISSR primers was 0.31 (table 3). The three RAPD primers were able to generate 38 scorable bands (13 bands on average were amplified per RAPD primer) of which 32 (81 %) were polymorphic. For all RAPD primers, the size of amplified products ranged from 270 to 3,000 bp (table 3). The maximum total number of band (TNB) was 22 (OPAI-05) and the minimum 7 (OPB-19). The OPAI-05 primer was able to distinguish 35 *G. decorticans* genotypes and the OPB-19 primer 13 (NG). The resolving power (Rp) of the RAPD primers ranged from 2.7368 (OPB-19) to 10.8421 (OPAI-05). Regarding PIC calculations, primer OPAI-05 revealed a maximum value of 0.33, while a minimum value of 0.22 was obtained with OPF-08. The average PIC value obtained for three RAPD was 0.26 (table 3). The amplification products obtained on *G. decorticans* by primers UBC823, UBC807, UBC815, OPAI-05 and OPB-19 are ilustrated in figure 1, which allow efficient discrimination between DNA banding patterns of genotypes from different localities.

Analysis of molecular variance (AMOVA) in population analyses of *G. decorticans* indicated that most of the total variance was attributable to genetic variations within populations (65 %) rather than among them (35 %) (table 4). AMOVA revealed a significantly low level (0.35; $P < 0.001$) of genetic differentiation among populations.

The genetic cluster analysis of the 38 *G. decorticans* individuals was generated using the unweighted pair group method with arithmetic mean (UPGMA) based on Jaccard

**Table 3.** Information regarding ISSR and RAPD primers used for genetic analyses of *Geoffroea decorticans*. Total number of bands (TNB), number of polymorphic bands (NPB) at 99 %, percentage of polymorphic bands (P%) at 99 %, resolving power (Rp), number of different genotypes (NG), number of exclusive bands (NEB) and poyimorphic information content (PIC).

Información de los cebadores ISSR y RAPD utilizados para el análisis genético de *Geoffroea decorticans*. Número total de bandas (TNB), número de bandas polimórficas (NPB) al 99 %, porcentaje de bandas polimórficas (P%) al 99 %, poder de resolución (Rp), número de genotipos diferentes (NG), número de bandas exclusivas ( NEB) y contenido de información poimórfica (PIC).

| Primer | Sequence | Band sizes (bp) | TNB | NPB (99 %) | P% (99 %) | NG | Rp | NEB | PIC |
|---|---|---|---|---|---|---|---|---|---|
| UBC880 | $(GGAGA)_3$ | 350-3,000 | 18 | 17 | 94 | 36 | 7.5789 | 0 | 0.2966 |
| UBC823 | $(TC)_8C$ | 550-3,000 | 21 | 21 | 100 | 31 | 11.0526 | 0 | 0.3558 |
| UBC815 | $(CT)_8G$ | 500-3,000 | 17 | 17 | 100 | 31 | 8.2105 | 0 | 0.3375 |
| UBC811 | $(GA)_8C$ | 300-3,000 | 19 | 19 | 100 | 37 | 12.2105 | 0 | 0.4110 |
| UBC825 | $(AC)_8T$ | 330-2,000 | 15 | 14 | 93 | 33 | 6.8421 | 0 | 0.2814 |
| UBC807 | $(AG)_8T$ | 280-3,000 | 14 | 13 | 93 | 26 | 4.2105 | 0 | 0.2254 |
| UBC826 | $(AC)_8C$ | 250-1,500 | 19 | 18 | 95 | 31 | 8.1053 | 2 | 0.2882 |
| UBC856 | $(AC)_8YA$ | 280-2,000 | 19 | 19 | 100 | 32 | 7.7368 | 0 | 0.2956 |
| TOTAL | | | 142 | 138 | | | | 2 | |
| Average | | | 18 | | 97 | 32 | 8.2434 | 0.25 | 0.3114 |
| OPB-19 | ACCCCCGAAG | 590-3,000 | 7 | 6 | 86 | 13 | 2.7368 | 0 | 0.2473 |
| OPF-08 | GGGATATCGG | 550-3,000 | 9 | 6 | 67 | 17 | 3.1579 | 0 | 0.2262 |
| OPAI-05 | GTCGTAGCGG | 270-3,000 | 22 | 20 | 91 | 35 | 10.8421 | 0 | 0.3306 |
| TOTAL | | | 38 | 32 | | | | 0 | |
| Average | | | 13 | | 81 | 22 | 5.5789 | 0 | 0.2681 |

Y = C, T.

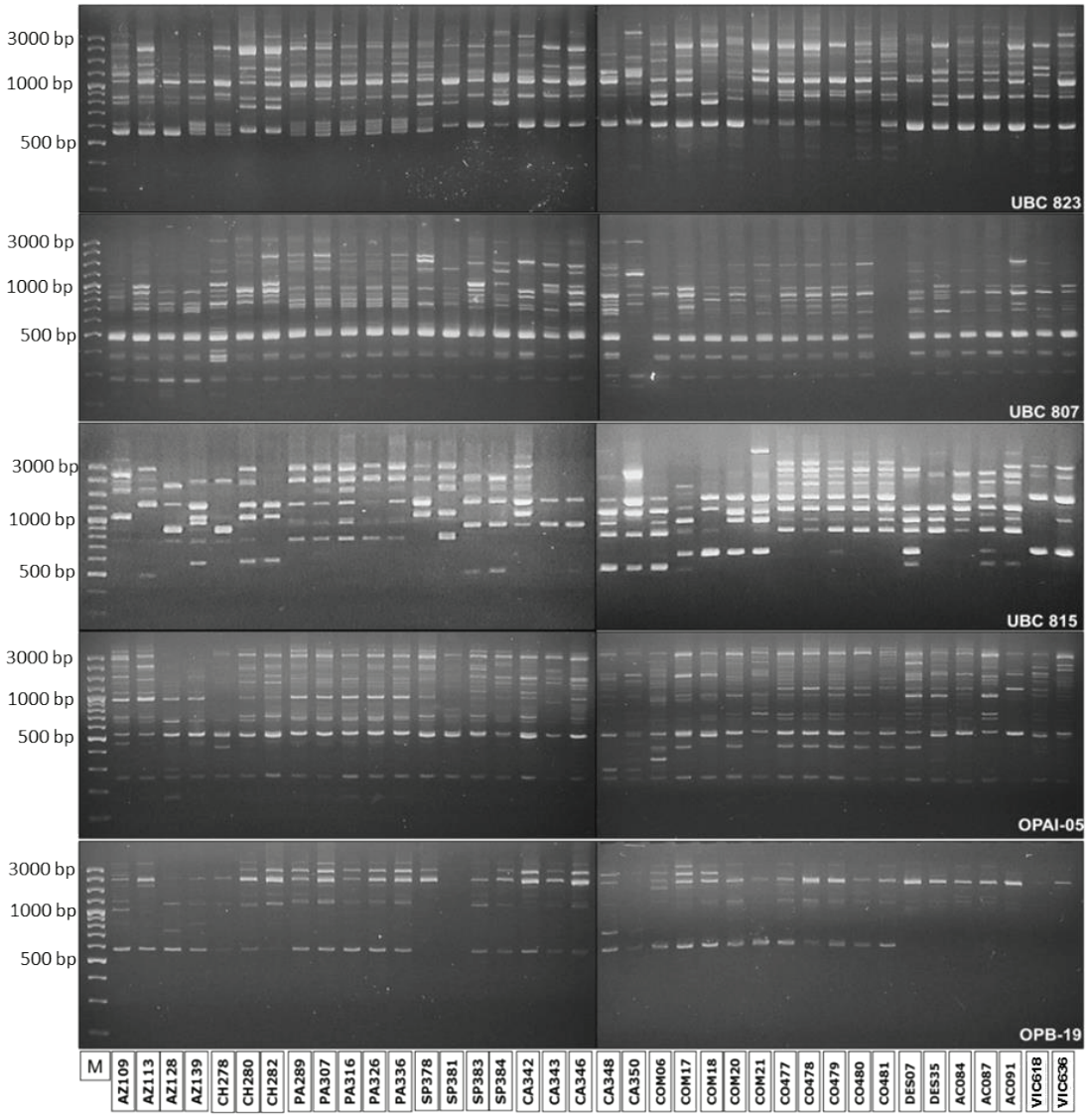


**Figure 1.** Electrophoresis pattern obtained on agarose gel using ISSR (UBC823, UBC807 and UBC815) and RAPD (OPAI-05 and OPB-19) primers, and thirty eight *Geoffroea decorticans* specimens (AZ,CH, PA, SP, CA, CO, DES, AC and VIC populations). Lane M is a DNA ladder ranged 100-3,000 bp. For a complete visualization of PCR profiles, two agarose gel of 20 wells were linked.

Patrón de electroforesis obtenido en gel de agarosa, utilizando los cebadores ISSR (UBC823, UBC807 y UBC815) y RAPD (OPAI-05 y OPB-19) y ocho geoffroea decorticans (AZ, CH, PA, SP, CA, CO, DES, AC y VIC poblaciones). El carril M es un marcador de ADN con un rango de 100-3.000 pb. Para una visualización completa de los perfiles de PCR, se unieron dos gel de agarosa de 20 pocillos.

**Table 4.** Analysis of molecular variance (AMOVA) for nine populations of *Geoffroea decorticans* ($P < 0.001$).

Análisis de varianza molecular (AMOVA) para nueve poblaciones de *Geoffroea decorticans* ($P < 0{,}001$).

| Source of variation | df | Sum of Squares | Mean Square | Variance component | % total variance |
|---|---|---|---|---|---|
| Among Pops | 8 | 447.650 | 55.956 | 9.451 | 35 |
| Within Pops | 29 | 508.350 | 17.529 | 17.529 | 65 |
| Total | 37 | 956.000 | - | 26.981 | 100 |

similarity. The dendogram showed an identical pattern of clustering using the Dice similarity matrix. Figure 2 shows a dendogram of the three main clusters, with the two largest divided on the basis of northerly and southerly distributions. Cluster I included 11 individuals (CoM6, CoM17, CoM18, CoM20, Des07, Des35, VIC618, VIC636, AC84, AC87 and AC91) comprising the southern area, cluster II consisted of 25 individuals (CH280, CH282, SP378, SP383, CA342, SP381, SP384, CA343, CA346, PA289, PA326, PA316, PA336, PA307, AZ113, AZ109, CO477, CO478, CO479, CO480, CO481, COM21, CH278, AZ128 and AZ139), most of them in the northern area, with the exception of Copiapó specimens. Cluster III included two individuals from Calama (CA348 and CA350). In addition, cluster I was split into three subclusters (DES,VIC and AC), cluster II into five (AZ, CHA, CA, PA and CO), and cluster III into only one subcluster (CA) (figure 2). The principal component analysis (PCA) revealed that the first three components (Component 1, 2 and 3) explained 14.9 %, 10.9 % and 6.6 % of total variation, respectively. The two-dimensional ordination confirmed cluster analysis results, showing two main clusters from the north and south areas, however, some individuals from Copiapó (CO477, CO478, CO479, CO480 and CO481) and Pachica populations (all PA individuals) were clearly separated from the rest (figure 3). For the nine *G. decorticans* populations, the average Nei's genetic distance was 0.244, ranging from 0.140 (between San Pedro and Calama) to 0.442 (between Pachica and Vicuña) (table 5).

The Mantel test showed no significant correlation between geographic and genetic distances for nine populations ($r = 0.18$, $P = 0.01$) (figure 4A), although a significantly positive correlation ($r = 0.58$, $P = 0.02$) was found for seven populations (figure 4B). The Average Shannon Weaver diversity indices of Pachica, Copiapó river-mouth, Alto del Carmen and Vicuña populations were 0.089, 0.127, 0.154

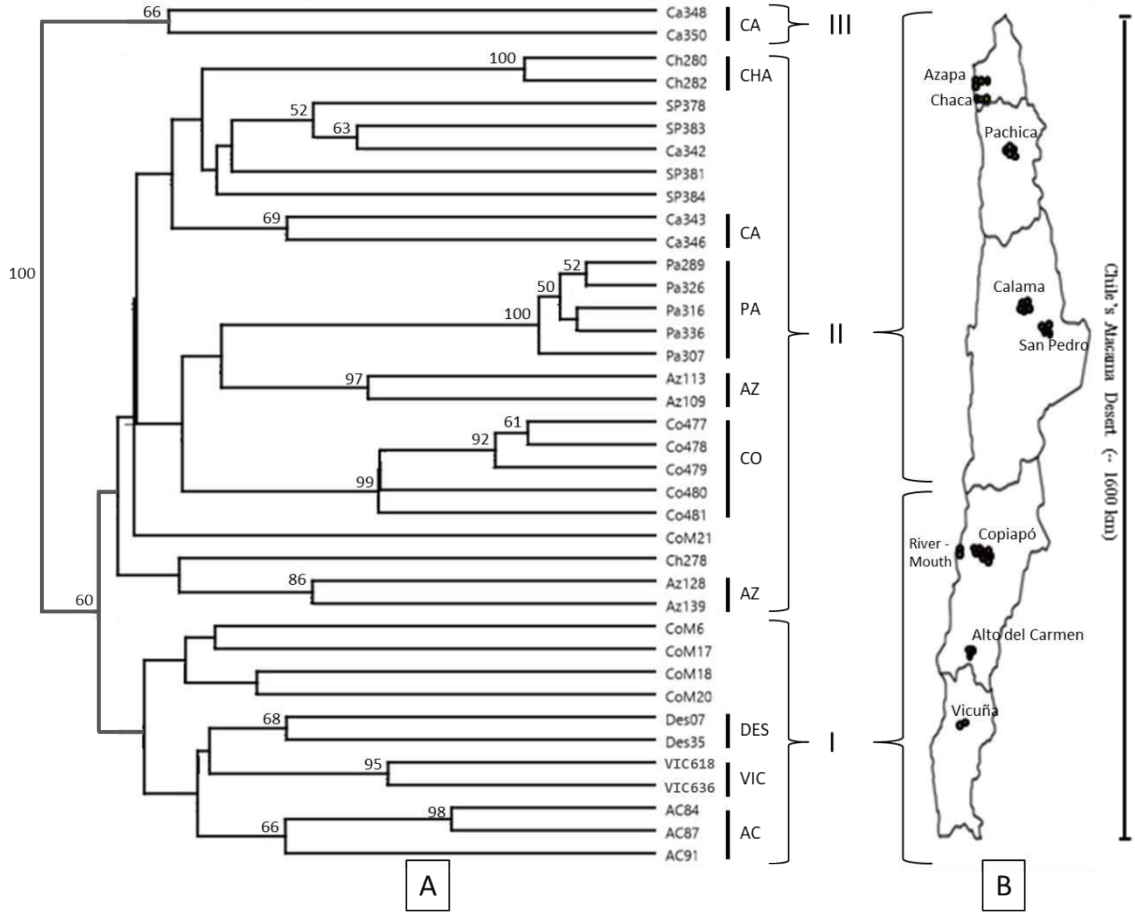


**Figure 2.** A) UPGMA cluster analysis based on Jaccard similarity, calculated based on 180 markers obtained from ISSR and RAPD. Bootstrap value percentages above 50 are indicated. B) Map of northern Chile showing locations of *Geoffroea decorticans* populations (AZ, CHA, PA, CA, SP, DES, CO, and VIC).

A) Análisis de cluster de UPGMA basado en la similitud de Jaccard, calculado sobre la base de 180 marcadores obtenidos de ISSR y RAPD. Se indican los porcentajes de valores de Bootstrap por encima de 50. B) Mapa del norte de Chile muestra la ubicación de las poblaciones de *Geoffroea decorticans* (AZ, CHA, PA, CA, SP, DES, CO, AC,VIC).

and 0.102 respectively, significantly ($P \leq 0.05$) lower than those of Azapa, San Pedro, Calama and Copiapó populations, which exhibited high values of 0.269, 0.297, 0.355 and 0.362 respectively (figure 5); Chaca (0.214), on the other hand, showed intermediate average Shannon Weaver diversity indices not significantly ($P \leq 0.05$) different to those of the remaining populations (figure 5).

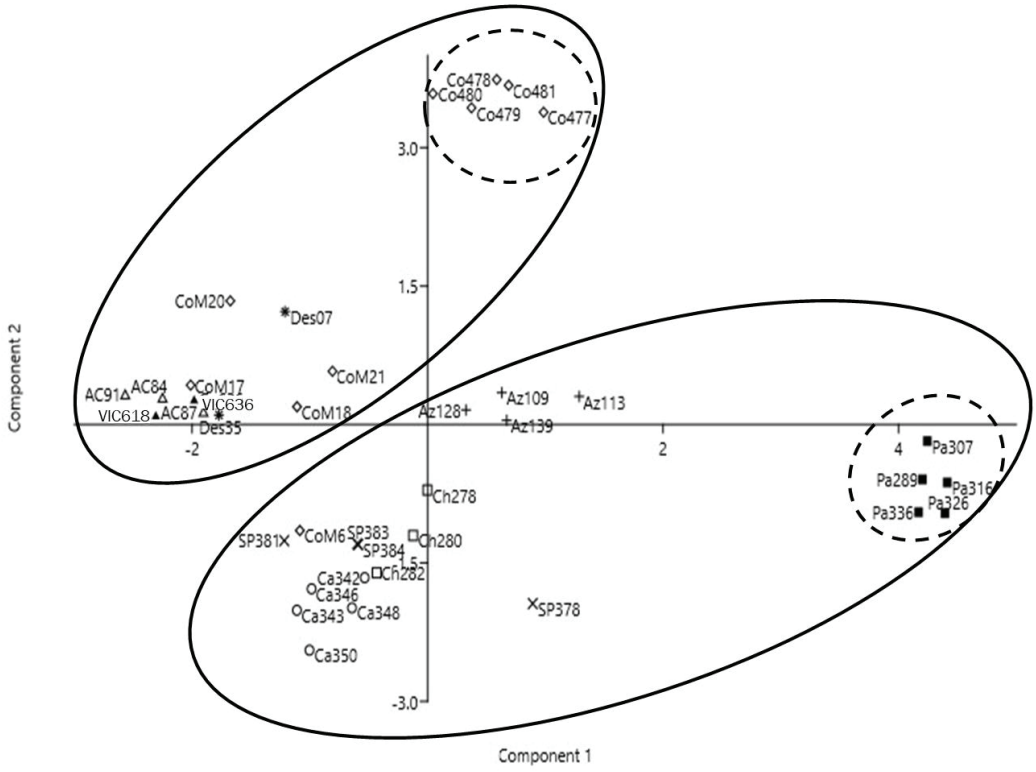


**Figure 3.** Principal Component Analysis based on the ISSR and RAPD markers and the Jaccard similarity index among 38 *Geoffroea decorticans* individuals. The first and second principal components account for 14.9 % and 10.9 % of genetic variation., respectively.

Análisis de componentes principales basado en los marcadores ISSR y RAPD y el índice de similitud de Jaccard entre 38 individuos de *Geoffroea decorticans*. El primer y segundo componente principal representan el 14,9 % y el 10,9 % de la variación genética, respectivamente.

**Table 5.** Nei's genetic distance between nine populations of *Geoffroea decorticans*.

Distancia genética de Nei entre nueve poblaciones de *Geoffroea decorticans*.

| | Azapa | Chaca | Pachica | San Pedro | Calama | Copiapó | Mouth-River | Alto del Carmen | Vicuña |
|---|---|---|---|---|---|---|---|---|---|
| Azapa | 0.000 | | | | | | | | |
| Chaca | 0.183 | 0.000 | | | | | | | |
| Pachica | 0.254 | 0.304 | 0.000 | | | | | | |
| San Pedro | 0.212 | 0.165 | 0.293 | 0.000 | | | | | |
| Calama | 0.243 | 0.204 | 0.328 | 0.140 | 0.000 | | | | |
| Copiapó | 0.164 | 0.168 | 0.298 | 0.154 | 0.147 | 0.000 | | | |
| Mouth-River | 0.291 | 0.244 | 0.386 | 0.247 | 0.271 | 0.205 | 0.000 | | |
| Alto Carmen | 0.261 | 0.240 | 0.427 | 0.192 | 0.203 | 0.172 | 0.192 | 0.000 | |
| Vicuña | 0.303 | 0.287 | 0.442 | 0.261 | 0.267 | 0.214 | 0.231 | 0.210 | 0.000 |

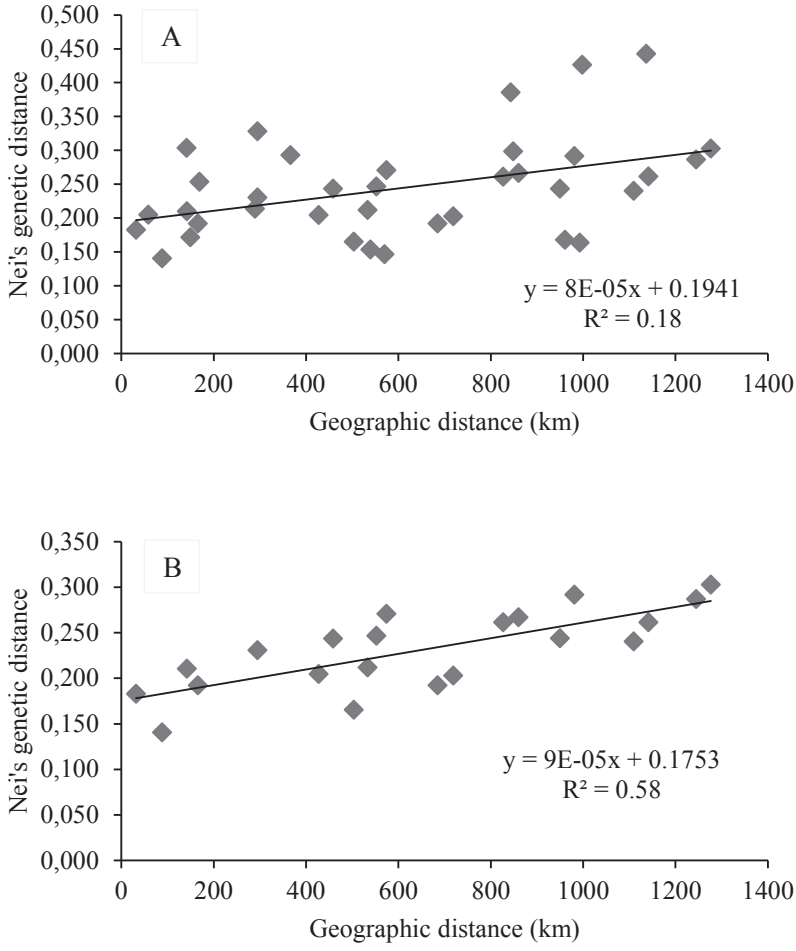


**Figure 4.** Relationship between genetic vs geographic distances among populations of *Geoffroea decorticans*. A) nine *G. decorticans* populations: AZ, CHA, PA, SP, CA, CO, DES, ADC, VIC. B) seven *G. decorticans* populations: AZ, CHA, SP, CA, DES, ADC, VIC.

Relación entre la distancia genética y la distancia geográfica entre las poblaciones de *Geoffroea decorticans*. A) nueve poblaciones de *G. decorticans*: AZ, CHA, PA, SP, CA, CO, DES, ADC, VIC. B) siete poblaciones de *G. decorticans*: AZ, CHA, SP, CA, DES, ADC, VIC.

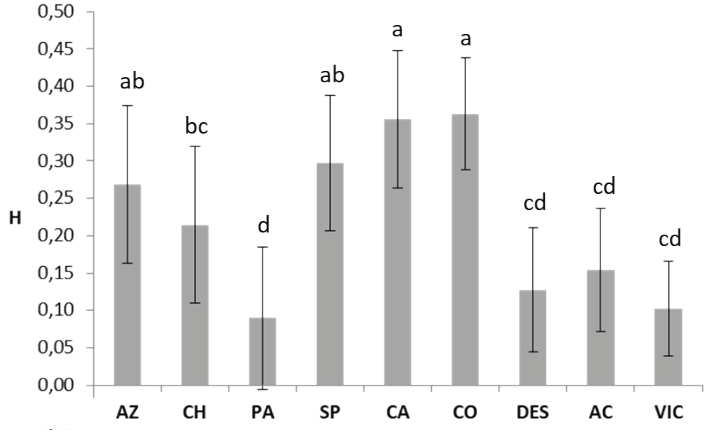


**Figure 5.** Average Shannon-Weaver diversity indices (H) of nine populations of *Geoffroea decorticans*. Bars sharing the same letter are not significantly different according to Tukey's test ($P \leq 0.05$). Bars show standard deviation (SD).

Promedio de índice de diversidad de Shannon-Weaver (H) de nueve poblaciones de *Geoffroea decorticans*. Las barras que comparten la misma letra no son significativamente diferentes según la prueba de Tukey ($P \leq 0,05$). Barras muestran desviación estandar.

## DISCUSSION

In cases where genomic information and DNA sequences of a plant species are not available, ISSR and RAPD molecular markers offer a useful alternative for genetic diversity studies. Our work is the first molecular study assessing the genetic diversity of *G. decorticans* from Atacama. In this study, eight ISSR and three RAPD primers produced 180 reproducible bands, of which 97 % and 81 %, respectively, were polymorphic. Other Fabaceae closely related to the genus *Geoffroea* genera (*Dalbergia* and *Pterocarpus* clades, Lavin *et al*. 2001), such as *D. oliveri* Gamble ex Prain. (Phong *et al.* 2011) and *D. sissoo* Roxb. (Arif *et al*. 2009), have fewer polymorphic bands (38.6 % and 56.7 %, respectively) and little polymorphic information content (PIC) using both ISSR and RAPD primers. *P. angolensis* DC. (Chisha-Kasumu *et al*. 2009), on the other hand, demonstrated higher values, with 88 % of bands polymorphic using RAPDs. Our results also reveal that polymorphism in *G. decorticans* is higher than in other desert tree species, for example *Haloxylon ammodendron* (C. A. Mey.) Bunge ex Fenzl, which shows polymorphism in 84 % of bands (Sheng *et al.* 2005), and *Acacia senegal* (L.) Willd., with fewer than 55.6 % polymorphic bands (Josiah *et al*. 2008). In addition, ISSR and RAPD markers could be used as an alternative method to identify and distinguish *G. decorticans* genotypes of interest. For example, Jiménez-Aspee *et al.* (2017) conclude the need to perform a genetic characterization of *G. decorticans* genotypes, because they revealed a different chemistry in populations of *G. decorticans*. We further demonstrate that, in *G. decorticans*, ISSR primers have higher incidences of polymorphism than those presented by RAPD primers. In summary, the polymorphic information found in *G. decorticans* using ISSR markers is high compared to other species from the same family and desert tree species in general.

AMOVA results for nine populations shows that a high genetic variation level within populations (65 %) and a moderate level among population diversity (35 %) are preserved. *Geoffroea decorticans,* with the highest intrapopulation genetic variation, reveals limited population differentiation. This is potentially a result of the extremely arid conditions in Atacama, which presents a significant barrier to gene dispersal. Chilean Atacama is the driest desert in the world, and certainly the oldest continuously arid region on earth (Clarke 2006).

Pachica specimens demonstrate a clear separation from the rest of *G. decorticans* individuals, suggesting the possibility of notorious fragmentation and may have evidenced a strong endemism. *Geoffroea decorticans* in Pachica occupies a relatively small total area, with more hostile environmental conditions relative to the rest of the sampling region. Few established *G. decorticans* are found here, due to very low water availability, high salinity and a lack of surrounding plant cover. These conditions result in restricted gene flow to outside populations. On the other hand, the five *G. decorticans* from Copiapó (CO477, CO478, CO479, CO480 and CO481) are grouped in one cluster, which may represent an old forest refuge. This because they have large trunk diameter ~ 80 cm (measured one meter from the base) contrasted with other sampled specimens (< 40 cm).

The principal component analysis (PCA) and the cluster analysis (UPGMA) performed on Jaccard similarity and Nei Genetic distance, respectively, reveal two main consistent *G. decorticans* groups associated with the northern (Clade II) and southern (Clade I) areas of Atacama Desert. Similar groupings are noted in other plant species, for example *Patosia clandestine* (Phil.) Buchenau, a Juncaceae species from the high Andean areas of Chile Norte Chico, which show a clear separation among populations from northern and southern areas, demonstrated by means of *rbcL* gene sequencing and AFLP markers (Troncoso *et al.* 2017). Other species from Atacama, such as *Copiapoa* spp. (Cactaceae) and *Nolana* spp. (Solanaceae), also reveal marked differences in clades originating from north and south of Copiapó Valley, suggesting that this formation presents a significant barrier to gene flow (Larridon *et al.* 2015). Moreover, the dendrogram shows that most individuals are grouped in respective locations, which could be a result of the conservation status of each locality. In Atacama Desert, water availability could determine a major vegetation cover and facilities to movement of pollinator insects. Indeed, the precipitation gradient in Atacama ranges from arid to hyper-arid from south to north, which could explain the division between the two main *G. decorticans* clusters. *Geoffroea decorticans* individuals from Cluster I in the southern reaches of the study area inhabit relatively more humid conditions with more important vegetation cover than those of Cluster II to the north, a hyper-arid zone with little vegetation cover, suggesting that climatic steadiness between these zones might limit colonization by new genotypes.

The small Nei genetic distance between Calama and San Pedro populations is anticipated given their close geographic proximity (~100 km). Conversely, the large genetic distance between Pachica and Vicuña populations is consistent with the large geographic distance (~1,384 km) and extreme aridity, which acts as a barrier to gene flow.

In the initial analysis of nine populations, the Mantel test revealed a negative correlation between geographic and genetic distances (r = 0.18). However, by excluding Pachica and Copiapó populations on the basis of the large differences revealed by the PCA, the correlation becomes significantly positive (r = 0.58), indicating significant isolation by distance. Comparable isolation-by-distance effects were observed in naturally fragmented populations of the tree *Potentilla ikonnikovii* Juz. in Gobi Desert (Wesche *et al*. 2006), and *Plumbago zeylanica* L. in India (Panda *et al*. 2015). In addition, *G. decorticans* populations in this study demonstrate a negative correlation between alti-

tude (m a.s.l.) and genetic distance for both nine and seven populations (data not shown).

The Shannon-Weaver diversity index shows significantly low levels of genetic diversity with regards to Pachica, Copiapó river-mouth, Alto del Carmen and Vicuña populations. This may be due to the barrier to pollen dispersal presented by Atacama Desert, resulting in isolation of these populations. Restricted gene flow in these conditions is widely recognized to be a factor affecting genetic diversity (Wesche *et al.* 2006). By contrast, Calama-San Pedro and Copiapó populations demonstrate significantly high genetic diversity relative to the populations mentioned above. We believe that this may be due to the fact that *G. decorticans* inhabits, as forests, at uniquely high densities in these regions. In Atacama Region these native forests comprise a combined area of 900 hectares, dominated by *G. decorticans* as well as *Acacia caven* (Mol.) Mol. and *Prosopis chilensis* (Mol.) Stuntz (Squeo *et al.* 2008). It is possible that these areas historically contained extensive *G. decorticans* forests. Thus, Calama-San Pedro and Copiapó sites, which have sufficient forest cover, may acquire higher levels of genetic diversity, possibly as a result of increased gene flow. Pither *et al.* (2003) recorded a similar result in *Terminalia amazonia* (Gmel.) Exell., they attributed to sites with sufficient forest cover acquire higher level of genetic diversity, while populations in sites with forest cover below it expected low level of genetic diversity.

For future genetic diversity studies focusing on *G. decoticans*, we recommend optimize the DNA extract method to increment the samples number and thus to improve the reliability of the molecular analysis. In our study, several DNA extractions had to be discarded due to poor quality and high levels of contamination, which considerably reduced the number of samples available for analyses. Moreover, to perform the genetic analysis by ISSR and RAPD markers, an optimal DNA quality is required.

In conclusion, we found a high degree of polymorphism in 38 *G. decorticans* individuals using ISSR and RAPD molecular markers. Both, the UPGMA cluster analysis and PCA, supported the grouping of all individuals into two main groups, and a clear geographic trend was evident. A larger proportion of genetic variation was found within populations rather than among them, which may also be related to the good correlation between genetic and geographic distances. Likewise, with the exception of San Pedro and Copiapó populations, little variation in the level of diversity in *G. decorticans* populations was found to occur. Our results evidenced that there is marked fragmentation among *G. decorticans* populations, as well as differences in levels of genetic diversity, possibly a result of isolation due to the extreme aridity of Atacama Desert. Future conservation efforts targeting this species should therefore focus on fostering regeneration within respective geographic zones in the region. This work is the first attempt to evaluate the genetic diversity of this species in northern Chile.

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