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Authors: Reyes-Ortiz, José Luis, Octavio-Aguilar, Pablo, López-Mata, Lauro, and Sánchez-González, Arturo

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Research Article

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José Luis Reyes-Ortiz[1], Pablo Octavio-Aguilar[1], Lauro López-Mata[2], and Arturo Sánchez-González[1]

Abstract

Background and Research Aims: *Dicksonia navarrensis* is a species of tree fern at risk of extinction, distributed in the montane cloud forest (MCF) of the Americas from the central region of Mexico to Ecuador. In Mexico, populations of this species grow in primary vegetation of the MCF, in a matrix with a high degree of fragmentation and under threat of disappearance.

Methods: In the present study, the diversity and genetic structure of seven populations of *D. navarrensis* that are distributed in the cloud forest of the Sierra Madre Oriental were evaluated, with both standard laboratory and statistical analysis techniques, using 11 microsatellites developed for the genus Dicksonia.

Results: A total of 33 alleles were found. Genetic diversity differed between populations, and some presented low heterozygosity. Using assignment tests, three genetic groups were identified, associated with the geographical distribution of the populations; those from the north maintain connectivity with each other but diverge highly from the populations in the south, probably due to processes of isolation by distance (local environment), genetic drift, and natural selection.

Conclusion: The northernmost population, which is more isolated from the rest, has a broader genetic reservoir, which can be useful for maintaining the genetic diversity of the species. In the other populations, with less genetic diversity, the introduction of individuals and/or the dispersion of spores is important, to maintain and increase the genetic variability that they still possess, but which could disappear in a short time if their habitat continues to deteriorate at a high degree.

Implications for Conservation: The results obtained provide basic information that can be used in management and conservation plans, because the populations with the greatest genetic diversity and the possible processes that are influencing the genetic structure of the species were identified.

Keywords

connectivity, conservation, genetic diversity, heterozygosity, montane cloud forest, tree ferns

Introduction

Dicksonia navarrensis Hook is a tree fern species that was recently separated from *D. sellowiana* (Noben et al., 2017). In Mexico, its distribution is limited to the environmental conditions prevailing in the montane cloud forest (MCF) in the southeast (Chiapas, Oaxaca, and Veracruz), Pacific Coast (Guerrero), Trans-Mexican Volcanic Belt (Puebla), and Sierra Madre Oriental, in Hidalgo, Puebla, and Veracruz (Villaseñor,

[1]Centro de Investigaciones Biológicas, Universidad Autónoma Del Estado de Hidalgo, Mineral de la Reforma, México
[2]Posgrado en Botánica, Colegio de Postgraduados, Texcoco, México

Corresponding Author:
Arturo Sánchez-González, Centro de Investigaciones Biológicas, Universidad Autónoma Del Estado de Hidalgo. Ciudad Universitaria, Carretera Pachuca-Tulancingo Km. 4.5, Colonia Carboneras, Mineral de la Reforma 42184, México.
Email: arturosg@uaeh.edu.mx

2010; Reyes-Ortiz et al., 2019) between elevations of 1000 and 2300 m (Large & Braggins, 2004; Mickel & Smith, 2004). Among these regions, the Sierra Madre Oriental is considered of particular relevance because it concentrates more than 25% of the floristic richness of Mexico, in addition to presenting a great variety of ecosystems, which exhibit a wide range of environmental conditions. Despite being a key area for the conservation of biological diversity at the national level, it is one of the country's regions most susceptible to the effects of climate change, and disturbances caused by human activities (Villaseñor y Ortiz, 2021).

In the current Mexican legislation (Semarnat, 2010), the nomenclature of the species has not been updated, so *Dicksonia navarrensis* is still considered as *D. sellowiana*, in the category of "especial protection"; and its commercial trade is regulated by international agreements (Cites, 2021). One of the criteria included in these agreements is evaluation of the intrinsic vulnerability of a taxon, through the estimation of its genetic variability and, based on this, establishment of protection measures. However, integration of the evaluation of genetic diversity as an effective strategy for the conservation of biodiversity has been little explored in Mexico (Rocha & Gasca, 2007), mainly due to the economic and technical resources involved in the analysis of genetic variability; so, the main focus is towards the evaluation of geographic areas, ecosystems, ecological communities, and species (Coates et al., 2018; Hanson et al., 2020).

Habitat fragmentation decreases the suitability and availability of areas for wildlife (Mullu, 2016). As a result, the distribution area of plant species has been severely reduced (González-Astorga et al., 2006; Shrestha et al., 2002), the degree of isolation of the populations has increased and the population size has decreased (Lienert, 2004). All the factors mentioned above have a negative impact on the genetic diversity, adaptation and probability of populations being conserved. Although the level of impact may vary depending on the organism, habitat type, and the genetic measure used in the analysis (Aguilar et al., 2019; Schlaepfer et al., 2018), some species can survive and adapt to habitat fragmentation (Cheptou et al., 2017). Another factor that has an adverse effect on plant population genetics is the illegal extraction of individuals (Bernabe et al., 1999; Eleutério & Pérez-Salicrup, 2009; Young et al., 1996), which can increase loss of genetic diversity and result in inbreeding, genetic drift, and bottlenecks (Parker & Mac Nally, 2002).

Populations of *D. navarrensis* in the Mexican Sierra Madre Oriental, are rapidly declining, mainly as a consequence of illegal extraction of individuals, fragmentation, and land use changes (Eleutério & Pérez-Salicrup, 2009). Therefore, it is essential to determine the genetic diversity of their populations, as a first step to establish conservation strategies in this region (Reyes-Ortiz et al., 2019). It has been suggested that low levels of diversity are common in species and populations that are at risk of extinction (Wang & Guan, 2011); the disappearance of even one population implies a serious loss of genetic diversity (De Groot et al., 2012). A demographic population study is not a reliable predictor of levels of genetic variation within populations, although it is an excellent indicator of what is likely to happen to genetic variation over time. For this reason, it is essential to complement ecological information with genetic studies (Ramírez-Barahona & Eguiarte, 2014; Varvio et al., 1986).

Therefore, the objectives of this study were (i) to assess the level of genetic diversity and estimate the genetic structure of the populations of this endangered tree fern species, in the MCF of the Sierra Madre Oriental, Mexico; (ii) estimate the variance partition components: within and among population, and (iii) obtain basic information for the development of conservation strategies *D. navarrensis* populations, in the northern area of their distribution in America.

Material and Methods

Study Area

The study area includes only the north region of the current distribution of *Dicksonia navarrensis* in America, the cloud forest of the Sierra Madre Oriental (SMOR), Mexico. This type of vegetation grows in conditions of high atmospheric humidity, frequent fog, and low light. Most plant species retain their foliage throughout the year, with some exceptions during the dry season (Gual-Díaz & Rendón-Correa, 2014; Rzedowski, 2006). The elevation distribution of *D. navarrensis* ranges between 900 and 2200 m (Figure 1), in sites with slopes of 15°–80°. In the sampling sites Agua Blanca, Medio Monte, Tizapan, Tlanchinol (Hidalgo), and Zilacatipan (Veracruz), the Cwa climate predominates (temperate, with dry winter and hot summer); in Naupan (Puebla) the Cwb climate (temperate, with dry winter and hot summer) and in Xocoyolo (Puebla) the Cfb climate (temperate, without a dry season and with a hot summer; Peel et al., 2007). The average annual temperature ranges between 12 and 23°C, and the total annual rainfall between 1000 and 3000 mm. During the year there is a season of low rainfall that lasts from 0 to 4 months.

Sampling

Seven wild populations of *D. navarrensis* distributed in the Mexican Sierra Madre Oriental were sampled. These populations were relatively close to human settlements, in an elevation interval of 1300 to 2200 m, on steep slopes of up to 70° with northeastern and northwestern orientation, and with canopy covers of 50–78% (Reyes-Ortiz et al., 2019). The distance between populations ranged from 12 to 163 km (Figure 1). Fresh foliar tissue was collected from 30 individuals randomly selected from each population (a total of 210 samples). The samples were labeled, cleaned with 70% ethanol, stored individually in plastic bags filled with silica gel, and preserved at −20°C until they were used for

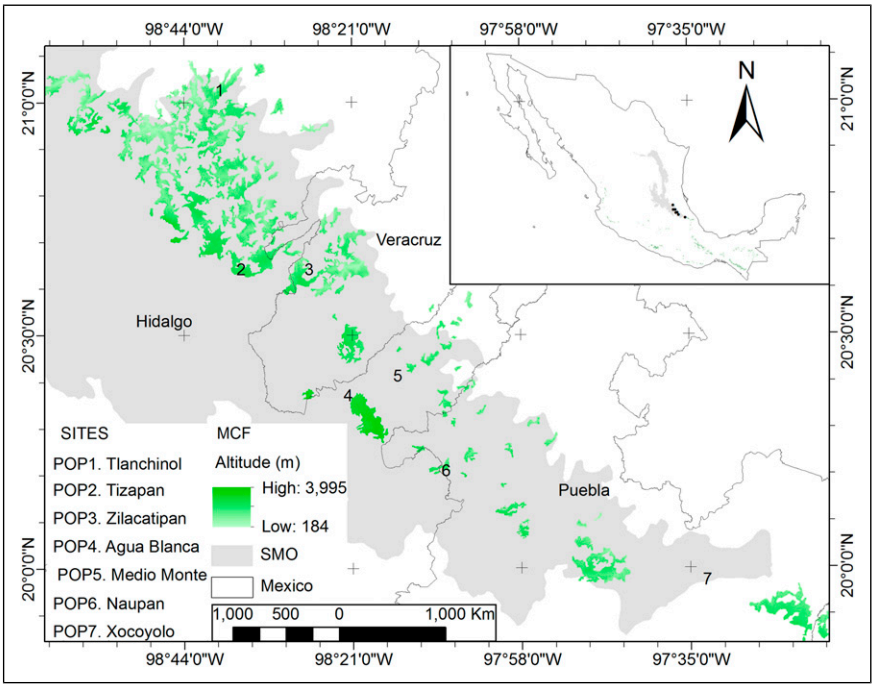

Figure 1. Geographic location of the analyzed wild populations of *Dicksonia navarrensis* in the Sierra Madre Oriental, Mexico

subsequent DNA extraction at the Biological Research Center of the Autonomous University of Hidalgo State, Mexico.

DNA Extraction

A modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987) was used to isolate genomic DNA from leaf tissue. DNA concentration was measured in an Eppendorf™ Bio Spectrometer basic. The quality and integrity of the molecule were assessed with 0.8% agarose gel electrophoresis.

Amplification

Eleven specific SSR markers (Dic01–Dic04, Dic06, Dic08, Dic10, Dic11–Dic14), developed for *D. sellowiana*, were used for the polymerase chain reaction (PCR); of which, eight were polymorphic for the populations of the south and southeast of Brazil (Supplementary Material 1; Nazareno et al., 2013). PCRs were carried out in a Thermo Scientific™ Arktik™ Thermal Cycler with a final volume of 8 µL containing 50 ng of genomic DNA, buffer Taq 1X, $MgCl_2$ (2.5 mM), dNTPs Mix (0.6 mM), forward primer (25 p.m.), reverse primer (25p.m.), Taq DNA (Promega™) polymerase (0.8 U) and sterile H_2O. PCR conditions were as follows: 94°C for 3 min of initial denaturation, 38 cycles of 94°C for 30 s, 45 s of annealing at 49–63°C for 45 s (Supplement Material 1), 1 min of elongation at 72°C, and a final extension step of 72°C for 10 min. Amplified fragments were separated into 15% polyacrylamide gels (140 V, 90 min), stained with ethidium bromide, and visualized under UV light on a transilluminator (2 UVTM-UVP™) with a molecular ladder of 100 bp (Promega™).

Data Analysis

Genetic Diversity. A matrix was created based on the size of the amplified fragments, which was corrected by the Chybicki and Burczyk (2009) method. GenAlEx ver. 6.5 (Peakall & Smouse, 2006) was used to estimate the percent of polymorphism (*% P*), the average alleles per *locus* (N_a), effective allele number per *locus* (A_e), Shannon's index (I), and the observed and the expected heterozygosity (H_O, H_E, respectively). To test whether the *loci* and populations were in Hardy-Weinberg equilibrium (HWE), the χ^2 test was used.

Genetic Structure. An analysis of molecular variance (AMOVA) was run to obtain F differentiation indicators (Excoffier et al., 1992). The genetic flow between populations was estimated from the genetic differentiation index (F_{ST}) using the formula $Nm = 1 - F_{ST}/4F_{ST}$ (Slatkin and Barton, 1989), where Nm is the number of migrants per generation. The resulting matrix of distances was used to construct a neighbor-joining cluster tree by bootstrap (1000 iterations) using PAST v.2.17 (Hammer et al., 2001). The isolation by distance model was analyzed by the Mantel test using geographic distances (km) and Nei's genetic distances matrix among all populations, using the TFPGA tools for genetic analysis (Miller, 1997). The genetic relationship between

populations was analyzed with the STRUCTURE 2.3.4 program (Falush et al., 2007; Pritchard et al., 2000) with an admixture model. Twenty replicates each of 1 million Markov chain Monte Carlo (MCMC) iterations (burn-in of 100,000) were run for each number of populations (K) tested. The optimal value of K was determined according to Janes et al. (2017), Kalinowski (2011), Pina-Martins et al. (2017), Puechmaille (2016), and Wang (2017), who have shown via simulation and empirical data that uneven sampling, the choice of model and parameter priors can cause inaccuracy in the ΔK and lnP (K) methods of Evanno et al. (2005) and Pritchard et al. (2000), respectively. The STRUCTURE output results were evaluated and visualized using the STRUCTURE SELECTOR web server (Li & Liu, 2018), and K = 1 to 14 were analyzed. Estimates of ΔK (Evanno et al., 2005), lnP (K) (Pritchard et al., 2000), and the four independent estimators of Puechmaille (2016), which we refer to collectively as MM K, were generated. The membership (Q value) threshold for any individual to be assigned to a particular cluster was set at 0.5. CLUMPAK (Kopelman et al., 2015) implemented in STRUCTURE SELECTOR (Li & Liu, 2018) was used to generate a consensus Q-matrix from the replicates at optimal K and to visualize the clusters in a histogram labeled by population.

To compare the genetic groups and to infer the clusters under a null model of gene flow (without a priori assignment based on orthogonal metric distances), the discriminant analysis of principal components (DAPC) was used, which is based on a covariance matrix (Jombart et al., 2010). It was complemented with a canonical analysis using STATISTICA v.10 (StatSoft, 2011).

Results

Genetic Diversity

The results revealed the presence of two monomorphic loci (Dic04 and Dic06), which were excluded from the subsequent genetic analyses (mean of polymorphic loci 74.03 ± 3.4%, across the 11 screened markers). A total of 33 alleles were identified at the remaining nine loci, ranging from 2 (Dic13) to 6 (Dic01 and Dic08), with an average of 2.55 alleles per *locus* (Supplementary Material 1). The mean heterozygosity observed (H_O) and expected (H_E) per population were 0.133 and 0.367, respectively. Thus, *D. navarrensis* populations showed an inbreeding tendency, probably due to heterozygosity loss ($H_O = 0.133 \pm 0.023$, $f = 0.65 \pm 0.045$). Low values of effective alleles by *locus* (mean $A_e = 1.766 \pm 0.079$) were estimated. Genetic diversity differed between the analyzed populations (Table 1). Three exclusive alleles were found in POP1 (Dic03: 195 bp, Dic04: 185 and 219 bp), three in POP4 (Dic02, Dic03, and Dic08: 253, 249, and 283 bp, respectively), and only one in POP6 (Dic01: 295 bp).

Genetic Structure

The Nm and F_{ST} values were heterogeneous between the populations analyzed. The values of the index of differentiation ranged between 0.045 and 0.328 (mean $F_{ST(6,\ 419)} = 0.174$, $p < 0.001$). The highest differentiation values were those of population 1, with respect to populations 4–7. The AMOVA indicated greater variation among individuals (58%) than within individuals (24%) or populations (18%). Inter-population flow levels ranged from 0.511 to 5.285, and the average number of migrant individuals per generation (Nm) between populations was 1.19 (Table 2).

The structure of the population inferred with the assignation methods (Evanno et al., 2005; Puechmaille, 2016) revealed that $K = 3$ and $K = 4$ were the numbers of most likely clusters for an admixture model within the predefined populations. The highest homogeneity among individuals was recognized in POP1 and POP6, and major heterogeneity was found in POP2 and POP3. POP1, located in the north, was a divergent population in both K models (Figure 2). The first assignment group (K = 3) includes the three northern populations, the second cluster comprises POP4 and POP7, and POP5 and POP6 form the remaining group. In the K = 4 model, POP1 is isolated from the two remaining north cluster populations. Both assignments are similar to the genetic associations identified by neighbor-joining clustering (Figure 3).

The DACP results indicate that there are significant differences between all populations, consistent with the AMOVA results, but only 4 *loci* were needed to explain 65.3% of the variance ($F_{(17,\ 187)} = 8.59 \pm 4.23$; $p < 0.0002$) correlated with two factors. In the ordination diagram, it is observed that the individuals of POP1 and POP4 are more differentiated, compared to the rest of the populations (Figure 4). The correlation between the geographic distances (km) and the Nei genetic distances of the *D. navarrensis* populations was statistically significant (r = 0.58, p = 0.03) (Supplementary Material 2). Geographic distance between the populations ranged from 12.7 to 163.5 km.

Discussion

Genetic Diversity

This study provides the first results on the structure and genetic diversity of *Dicksonia navarrensis* (sensu Noben et al., 2017) populations in Mexico; 100% of the specific *loci* included in the work were successfully amplified, and 81.81% were polymorphic, although it is possible that other alleles are masked (Izuno et al., 2012). Here, the polymorphism percentage obtained through the use of microsatellites, is similar to that estimated in *D. sellowiana* in Brazil, the values range between 72.73% (Nazareno et al., 2013) and 100% (Fagundes et al., 2020), with a total number of alleles similar to that of Mexican populations of *D. navarrensis*, 38

Table 1. Genetic Diversity Among Populations of *Dicksonia navarrensis* in Mexico.

Locality	N	Na	Ae	I	H_O	H_E	f	HWE (ChiSq)
POP 1. Tlanchinol	29.67 ± 0.33	2.67 ± 0.29	1.66 ± 0.13	0.6 ± 0.08	0.19 ± 0.08	0.36 ± 0.05	0.49 ± 0.16	23.33*
POP 2. Tizapan	28.11 ± 1.11	2.78 ± 0.4	2.02 ± 0.25	0.73 ± 0.13	0.16 ± 0.07	0.44 ± 0.07	0.65 ± 0.11	33.80*
POP 3. Zilacatipan	28.67 ± 0.73	2.78 ± 0.4	1.94 ± 0.23	0.71 ± 0.12	0.19 ± 0.1	0.43 ± 0.07	0.63 ± 0.15	31.30*
POP 4. Agua Blanca	27.44 ± 0.97	2.67 ± 0.29	1.92 ± 0.24	0.69 ±0.13	0.09 ± 0.04	0.41 ± 0.07	0.81 ± 0.06	32.82*
POP 5. Medio Monte	28.56 ± 1.32	2.22 ± 0.22	1.46 ± 0.13	0.43 ±0.1	0.06 ± 0.03	0.27 ± 0.07	0.75 ± 0.12	23.64*
POP 6. Naupan	28.22 ± 0.60	2.56 ± 0.48	1.73 ± 0.27	0.56 ±0.15	0.12 ± 0.04	0.32 ± 0.08	0.69 ± 0.1	30.32*
POP 7. Xocoyolo	28.33 ± 0.76	2.22 ± 0.22	1.64 ± 0.16	0.55 ± 0.1	0.13 ± 0.05	0.34 ± 0.06	0.58 ± 0.12	24.06*

POP: population, N: sample size, *Na*: Mean number of alleles per *locus*, *Ae*: effective number of alleles by *locus*, *I*: Shannon's index, H_O: Observed heterozygosity, H_E: Expected heterozygosity, *f*: Endogamy coefficient, HWE: Hardy–Weinberg Equilibrium; ChiSq: Chi-square, *: $p < .001$.

Table 2. Genetic Structure Calculated by AMOVA. The F_{ST} and N_m Values Appear Below and Above the Dashes, Respectively.

POP	1	2	3	4	5	6	7
1	—	1.095	1.325	0.651	0.588	0.511	0.529
2	0.186	—	5.285	1.646	1.067	1.593	1.614
3	0.159	0.045	—	1.568	0.966	1.668	1.254
4	0.277	0.132	0.138	—	1.580	2.186	3.032
5	0.298	0.190	0.206	0.137	—	1.623	1.186
6	0.328	0.136	0.130	0.103	0.133	—	2.096
7	0.321	0.134	0.166	0.076	0.174	0.107	—

and 32, respectively (versus 33 in the present work). In *Alsophila spinulosa* populations of China, polymorphism estimated with SSR markers was 21.43% (Zhou et al., 2008), and in *Sphaeropteris brunoniana* populations of China, polymorphism was 80.6%, estimated using AFLP (Wang & Guan, 2011).

Dicksonia navarrensis presented low genetic diversity (mean H_E = 0.36) compared to other species of the same genera, such as *D. sellowiana* (mean H_E = 0.50, Fagundes et al., 2020), or with respect to *Alsophila spinulosa* (mean H_E = 0.45, Zhou et al., 2008), probably as a result of the high degree of fragmentation and loss of its habitat, the montane cloud forest of Mexico (INEGI, 2017), which affects the connectivity between its populations (Graignic et al., 2018). However, it must be considered that habitat loss and fragmentation in other fern species (*Pleopeltis crassinervata* and *Polypodium rhodopleuron*) did not have a strong influence on genetic diversity because it remained high; mean H_E = 0.257 and 0.223, respectively (Winkler et al., 2011). In addition to habitat alteration, the density of *D. navarrensis* populations in the SMO is rapidly declining and geographic isolation is increasing due to illegal harvesting of individuals (Reyes-Ortiz et al., 2019). However, it is necessary to analyze other populations of *D. navarrensis* to establish the relationship between population size and forest fragment size in the different mountainous regions where it is distributed in Mexico, to complement the results obtained here, since the consequences of habitat reduction on the genetic diversity of species cannot be generalized (Carvalho et al., 2019; Yu et al., 2020).

In this study, the low values of heterozygosity suggest inbreeding (Frankham et al., 2002), which can result from genetic drift events, founder effect, bottlenecks (Luikart, Allendorf, & Cornuet, 1998), and a reduction in population size (Lönn & Prentice, 2003; Pullin, 2002). It has been suggested that the plant mating system increases inbreeding (Wang et al., 2014). In ferns, in general, the probability of inbreeding is high, because the sporophytes are produced from gametophytes that can self-fertilize (Sessa et al., 2016). However, crossing over can be favored, both by the asynchronous production of ovules and sperm in the gametophyte phase, and by the high vagility of the spores, which allows them to colonize new habitats (Haufler et al., 2016). This happens according to the type of mating system they use: outcrossing versus selfing (Haufler et al., 2016; Nazareno et al., 2013). *Alsophila firma*, for example, has developed a post-zygotic mechanism that enables it to promote outcrossing in its populations (Ramírez-Barahona & Eguiarte, 2015); therefore, it is necessary to analyze the mating system of *D. navarrensis*, to find whether the specific cause of the deficiency in heterozygotes is attributable to natural selection or self-crossing (Wang et al., 2014).

Fagundes et al. (2020) note that the spores of *D. sellowiana* are very small, so they are easily dispersed by wind and rain, which facilitates the movement of individuals and interbreeding. This is plausible for this species, but the environmental conditions in which *D. navarrensis* grows are somewhat different. In Mexico, the primary MCF, which is the ecosystem that meets the ideal conditions for *D. navarrensis*, occupies between 0.4 and 0.9% of Mexican territory and is considered an ecosystem at risk of extinction (Gual-Díaz & Rendón-Correa, 2014; INEGI, 2017). Even though the dispersal capacity of the spores of this species is very high, the probability that they will find suitable conditions for germination, gametophyte production and sporophyte formation is low, but studies are required to confirm that argument. In contrast, populations of *D. sellowiana* are distributed over a larger geographic area in the Atlantic Forest of South America (Mallman et al., 2019), have a high density

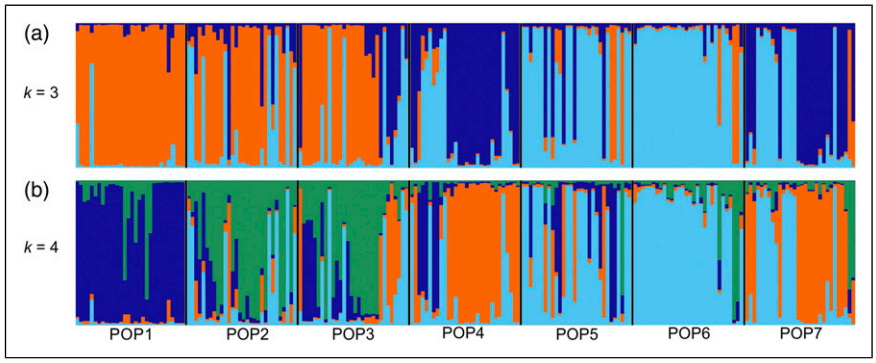

Figure 2. Assignation analysis based on Bayesian model of STRUCTURE software: (a) K = 3, (b) K = 4. Colors represent the groups identified; each population is represented by one chart, showing the proportion of membership of the different clusters.

of individuals, and have a continuous influx of gametophytes into populations (Schmitz et al., 2006; Fagundes et al., 2020); these three characteristics could be related to the corresponding values of H_E, higher than those estimated for *D. navarrensis* in the present study.

Genetic Structure

The F_{ST} values suggest low differentiation among populations. At the population level, POP2 and POP3 had the lowest differentiation rate and a high Nm per generation (5285). The two populations are relatively close (16.1 km apart), they have similar latitude and elevation (1878 and 1732 m, respectively), and their orientation is similar as well. This suggests that both populations were at some time in the past a single population that now is in a process of fragmentation; recent studies indicate that gene flow is one of the most important factors affecting the population genetic structure, in some cases increasing genetic diversity by sexual reproduction among populations (Ranker, 1994; Yu et al., 2018). In this sense, Pelosi and Sessa (2021) point out that in ferns the value of *Nm* can be up to 24 migrants per generation; however, values above four indicate random mating between populations.

In ferns species, it has been determined that its genetic structure is affected by the mating system (Pelosi & Sessa, 2021), their evolutionary history (Soltis & Soltis, 1990), and the physical characteristics of their habitat: elevation, precipitation, and latitude, among others (Fagundes et al., 2020). The results indicate that the populations of *D. navarrensis* that are distributed in the northern part of the study area (POP1–POP3) maintain gene flow among them, which could explain their low level of differentiation. The probable lack of genetic exchange between these three populations and the rest could be an indication of isolation by distance, favored by the high degree of habitat fragmentation (Yu et al., 2020). This suggests a synergy effect between natural selection on disturbed populations and isolation by distance in the north that increases the similarity

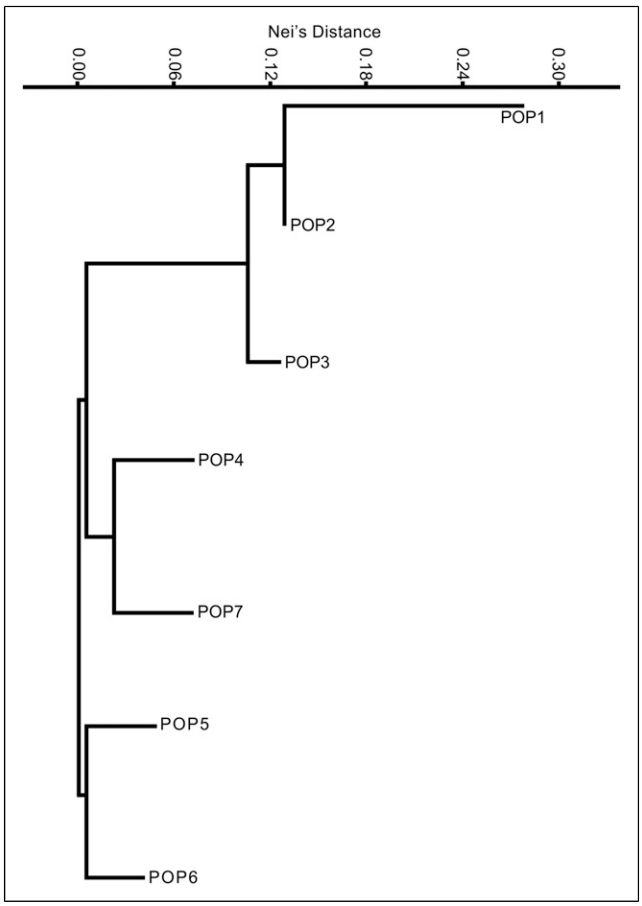

Figure 3. Neighbor-joining clustering in wild populations of *Dicksonia navarrensis* in Mexico.

within populations but increases divergence between latitudinal groups. However, tree ferns produce billions of spores (Large & Braggins, 2004); *D. sellowiana*, for example, produces approximately 4 billion spores per kilogram (Gomes et al., 2006), which facilitates the formation of spore banks (Fiilippini et al., 1999). Moreover, it has been suggested that in general they are able to disperse even over

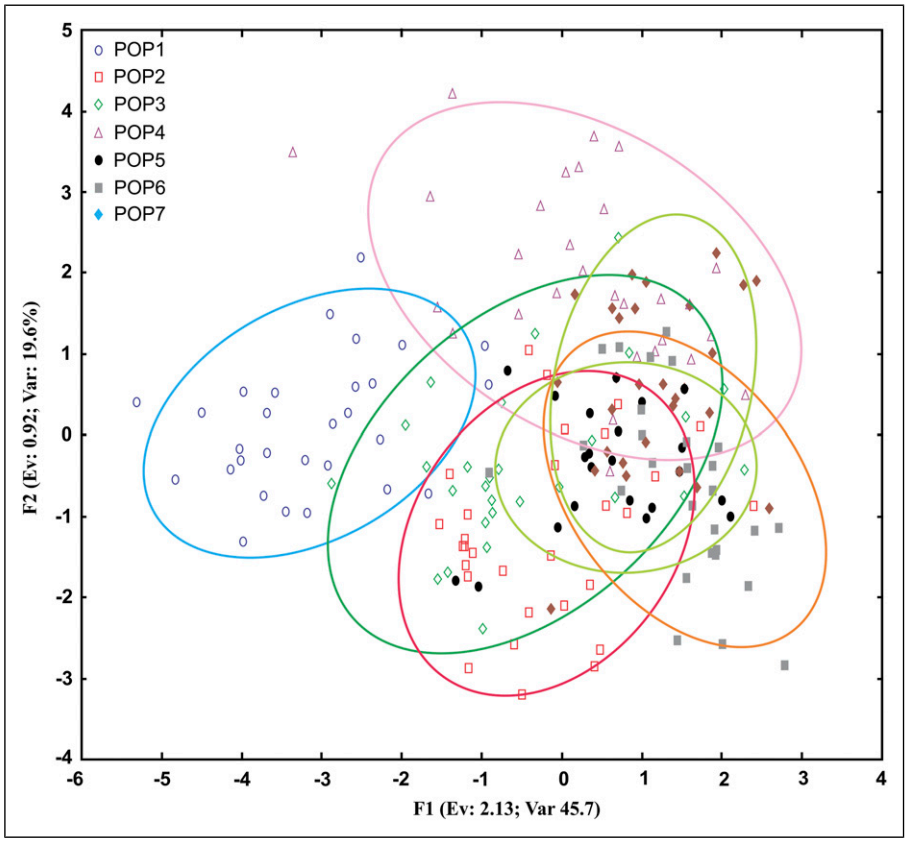

Figure 4. DAPC grouping of wild populations of *Dicksonia navarrensis* in Mexico based on linear distances orthogonalized by correlation matrix.

distances greater than 3000 km (Tryon, 1970; Tryon & Tryon, 1982). In the case of *D. navarrensis*, a probable answer to found divergence is that, within the SMO, there is a wide range of variation in environmental factors such as elevation, climate, topography, edaphology, and degree of anthropic disturbance (Salinas-Rodríguez, 2018), which alone or in synergy, constitute barriers to spores survival or dispersal along different distances, thus inducing genetic isolation (Tryon, 1970; Tryon & Tryon, 1982). This environmental heterogeneity facilitates the existence of populations with a few isolated individuals, which can lead to the loss of genetic variation (Soltis & Soltis, 1990). Despite the large number of spores produced and their wide dispersal distance, well documented in ferns, the effects of multiple colonization contribute to loss of diversity and low genetic structuring in their populations (Vogel et al., 1999), which could explain the divergence between the three geographic groups in *D. navarrensis*.

Implications for Population Conservation in the Sierra Madre Oriental

Currently, the populations of *D. navarrensis* are being decimated through the illegal extraction of individuals, which are sold in local markets as ornamental plants (Reyes-Ortiz et al., 2019). In addition, changes in land use in their distribution area have made sites that meet the ideal high humidity characteristics necessary for the establishment of individuals increasingly scarce, which contributes to reducing their survival rate. The genetic structure and divergence found in this species could be related to the high environmental heterogeneity caused by drastic modifications in its habitat (Carvalho et al., 2019; Yu et al., 2020).

Genetic information on *D. navarrensis* and a recent ecological analysis of its population structure (Reyes-Ortiz et al., 2019) can be very useful in the design of a management and conservation plan for this species. The limited distribution and small population size of species in the SMO should be considered. On the other hand, the heterogeneity of the SMO (Salinas-Rodríguez, 2018) hardly represents an obstacle for illegal extraction of species. Therefore, we should look for alternatives to avoid the loss of *D. navarrensis* genetic resources, principally in the populations at the edge of local extinction, each population must be considered individually due their genetic differences (Shrestha et al., 2002), the close relationship between population size and genetic variation (Izuno et al., 2012), and also because information about the proportion of genetic variation that confers potential adaptability to this species is

unknown (Kim et al., 2005). However, it is evident that we are still far from knowing the total genetic variation of the species (Shah et al., 2008).

The values of genetic diversity estimated for this species probably are the product of evolutionary processes, habitat fragmentation, and excessive extraction of individuals, whether for purposes of exploitation or not (Supplement Material 3; Shah et al., 2008). The degree of habitat disturbance differs between populations, and is most severe in POP6 and POP1; the latter is an important genetic reservoir, containing particular adaptations. *In situ* and *ex situ* conservation are methods that complement each other (Vovides et al., 2013). The priority should be to categorize all populations *in situ*, and subsequently carry out *ex situ* work through the collection of spores or youngest individuals, initially in the populations of highest genetic diversity (Shah et al., 2008) such as POP1 in this study. The ecological characteristics of *D. navarrensis* populations (Reyes-Ortiz et al., 2019), and genetic information provided here, suggest develop strategies to conservation and management of MCF. If its habitat is conserved, *D. navarrensis* will be protected properly.

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ORCID iDs

José Luis Reyes-Ortiz https://orcid.org/0000-0002-3520-6792
Pablo Octavio-Aguilar https://orcid.org/0000-0002-4636-9773
Lauro López-Mata https://orcid.org/0000-0002-1690-1335
Arturo Sánchez-González https://orcid.org/0000-0002-3190-8789

Supplemental Material

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