



Genetic structure and conservation status of sympatric *Polylepis incana* and *Polylepis racemosa* (Rosaceae) populations in Paluguillo, Pichincha, Ecuador

Estructura genética y estado de conservación de poblaciones simpátricas de *Polylepis incana* y *Polylepis racemosa* (Rosaceae) en Paluguillo, Pichincha, Ecuador

Dominique Vargas-Salinas¹ , Lisbeth Cuenca-Chasi¹ , Gabriela Miño¹ , Carlos Cuenca² , Valeria Ochoa¹ ,
M. Claudia Segovia-Salcedo^{1,3} , Karina Proaño¹ 

Abstract:

Background and Aims: *Polylepis* forests occur at high elevations and provide essential ecosystem services, including water regulation and soil protection. In Ecuador, reforestation programs have introduced the Peruvian species *Polylepis racemosa* without prior evaluation of potential gene flow or hybridisation risks with native congeners, which could compromise the genetic integrity of native *P. incana* populations. This study aims to characterise the genetic structure of sympatric populations of *P. incana* and *P. racemosa* in the Paluguillo area, Pichincha, Ecuador.

Methods: The genetic structure of *P. incana* and *P. racemosa* was analysed using inter-simple sequence repeat (ISSR) markers in 60 adult individuals from six populations (three per species, ten adults per population). Genetic diversity parameters, including the proportion of polymorphic loci and expected heterozygosity, were calculated based on ISSR data. Population structure was evaluated through analysis of molecular variance (AMOVA), UPGMA clustering and Bayesian assignment analysis. Seedlings were not sampled because none were present during the survey period; therefore, all genetic inferences refer to adult cohorts.

Key results: ISSR data revealed low genetic diversity in both species and evidence of gene flow between them. Despite this exchange, the species remained moderately differentiated, with 59% of genetic variation occurring within populations.

Conclusions: *Polylepis incana* and *P. racemosa* populations exhibit low genetic diversity but clear genetic differentiation. Gene flow between species suggests potential long-term risks to the genetic integrity of native *P. incana*. These results provide a molecular baseline for monitoring and managing genetic integrity in Andean reforestation programs, emphasising the priority use of native species.

Key words: gene flow, genetic diversity, ISSR marker, native species.

Resumen:

Antecedentes y Objetivos: Los bosques de *Polylepis* se distribuyen en elevaciones altas y brindan servicios ecosistémicos esenciales, como la regulación hídrica y la protección del suelo. En Ecuador, los programas de reforestación han introducido la especie peruana *Polylepis racemosa* sin evaluar previamente los riesgos de flujo génico o hibridación con especies nativas, lo que podría comprometer la integridad genética de *P. incana*. Este estudio tiene como objetivo analizar la estructura genética de poblaciones simpátricas de *P. incana* y *P. racemosa* en la zona de Paluguillo, Pichincha, Ecuador.

Métodos: La estructura genética de *P. incana* y *P. racemosa* se analizó mediante marcadores inter-simple sequence repeat (ISSR) en 60 individuos adultos pertenecientes a seis poblaciones (tres por especie, diez adultos por población). Los parámetros de diversidad genética, incluyendo la proporción de loci polimórficos y la heterocigosidad esperada, se calcularon a partir de los datos ISSR. La estructura poblacional se evaluó mediante análisis de varianza molecular (AMOVA), agrupamiento UPGMA y análisis de asignación bayesiana. No se muestrearon plántulas, ya que no se encontraron durante el período de muestreo; por lo tanto, todas las inferencias genéticas se refieren a cohortes adultas.

Resultados clave: Los datos ISSR revelaron baja diversidad genética en ambas especies y evidencia de flujo génico entre ellas. A pesar de este intercambio, las especies se mantuvieron moderadamente diferenciadas, con 59% de variación genética dentro de las poblaciones.

Conclusiones: Las poblaciones de *P. incana* y *P. racemosa* presentan baja diversidad genética, pero diferenciación significativa. El flujo génico detectado podría, a largo plazo, comprometer la integridad genética de *P. incana*. Estos resultados proporcionan una base molecular para el monitoreo y manejo de programas de restauración andina, priorizando el uso de especies nativas.

Palabras clave: diversidad genética, especies nativas, flujo génico, marcadores ISSR.

¹Universidad de las Fuerzas Armadas ESPE, Departamento de Ciencias de la Vida y la Agricultura, Laboratorio de Biotecnología Vegetal, 171103 Sangolquí, Ecuador.

²Universidad de las Fuerzas Armadas ESPE, Departamento de Ciencias Exactas, 171103 Sangolquí, Ecuador.

³Author for correspondence: mcsegovia@espe.edu.ec


Received: November 24, 2025.

Reviewed: January 26, 2026.

Accepted by Marie-Stéphanie Samain: March 13, 2026.

Published Online first: April 6, 2026.

Published: Acta Botanica Mexicana 133 (2026).

 This is an open access article under the Creative Commons 4.0 Attribution-Non commercial Licence (CC BY-NC 4.0 International)

To cite as: Vargas-Salinas, D., L. Cuenca-Chasi, G. Miño, Carlos Cuenca, V. Ochoa, M. C. Segovia-Salcedo and K. Proaño. 2026. Genetic structure and conservation status of sympatric *Polylepis incana* and *Polylepis racemosa* (Rosaceae) populations in Paluguillo, Pichincha, Ecuador. Acta Botanica Mexicana 133: e2539. DOI: <https://doi.org/10.21829/abm133.2026.2539>

e-ISSN: 2448-7589

Introduction

Paramos are high-elevation Neotropical ecosystems situated between the upper limit of montane forest and the lower boundary of permanent snow (Hofstede et al., 2014). In Ecuador, they cover approximately 7% of the national territory (Chuncho Morocho and Chuncho, 2019). The arboreal stratum is dominated by the genus *Polylepis* Ruiz & Pav., whose species can grow at elevations above 3500 m, making them the world's highest-growing trees (Kessler, 2006). *Polylepis* woodlands play a crucial role in ecosystem functioning by reducing soil erosion and surface runoff, intercepting cloud moisture, increasing soil depth and water-holding capacity, and enhancing sediment and nutrient retention (Cuyckens and Renison, 2018). They also harbour numerous endemic taxa, establishing them as critical biodiversity hotspots (Ojala-Barbour et al., 2019).

Despite their high ecological value, *Polylepis* forests are increasingly threatened by fragmentation, primarily due to anthropogenic pressures such as overgrazing and land-use change (Teich et al., 2005). Additionally, climate change is shifting elevational ranges and altering population dynamics within the genus, potentially leading to severe population declines or even local extinction (Caballero-Villalobos et al., 2021). It is estimated that 95% of the original *Polylepis* forest cover has already been lost (Kessler, 2006). Consequently, urgent conservation priorities must be considered for the restoration and strict protection of the remaining native stands (Caiza et al., 2021).

According to the most recent taxonomic revision, 45 species of *Polylepis* are currently recognised (Boza Espinoza and Kessler, 2022). However, high morphological variability within populations, low interspecific divergence and complex speciation patterns complicate taxonomic delimitation in the genus (Kessler and Schmidt-Lebuhn, 2006). Evolutionary processes such as hybridisation, allopolyploid and possibly apomixis have also contributed to its diversification (Kerr, 2004). In Ecuador, 12 species of *Polylepis* have been documented, 11 native and one introduced (Boza Espinoza and Kessler, 2022). Among the native species, *Polylepis incana* Kunth stands out for its wide distribution within Ecuador's National System of Protected Areas (SNAP) (Caiza et al., 2021). In contrast, *Polylepis racemosa* Ruiz & Pav.,

introduced from Peru, now occupies large areas that were previously covered by native forest.

Polylepis incana plays a key role in maintaining Andean montane forest ecosystems (Purcell and Brelsford, 2004). However, its low germination rate and slow growth make it particularly vulnerable (Canales and Huarasa, 2020). In efforts to accelerate restoration, *P. racemosa*, a faster-growing congener non-native to the country, has been used in Ecuadorian reforestation projects for over 50 years (Segovia-Salcedo, 2011). In Paluguillo (Pichincha) and other areas nationwide, plantations of *P. racemosa* are found in close proximity to natural stands of *P. incana*.

The taxonomic and spatial proximity of these two species raises the possibility of hybridisation. Hybrid formation is widespread in *Polylepis* and wind-mediated pollination further facilitates gene flow among congeners. Historical evidence of hybridisation within the genus (Romoleroux, 1996; Kessler and Schmidt-Lebuhn, 2006; Villota, 2012; Segovia-Salcedo and Quija-Lamiña, 2014) suggests a high probability of admixture between *P. incana* and *P. racemosa* where they occur sympatrically (Simpson, 1979).

Gene flow and hybridisation can increase genetic diversity, enhancing the adaptive potential of populations. Conversely, they can also lead to the decline or loss of parental lineages through demographic swamping (outbreeding depression) or genetic swamping (parental displacement) (Ellstrand, 2014). These contrasting outcomes are particularly relevant for native taxa of conservation concern, such as *P. incana*. Anthropogenic hybridisation resulting from the introduction of a congeneric species disrupts long-standing reproductive isolation and may necessitate intensive management interventions (Bohling, 2016). Potential gene flow between *P. incana* and the introduced *P. racemosa* thus presents a significant conservation challenge for *Polylepis* woodlands and paramo ecosystems more broadly.

However, the extent of interspecific introgression in Ecuador remains unquantified, hindering a rigorous assessment of reforestation impacts. Because the presence or absence of gene flow can influence the success of conservation actions and inform regulatory decisions (Ellstrand, 2014), robust genetic and morphological data are essential for developing sound management policies. Highly poly-



morphic inter-simple sequence repeat (ISSR) markers, successfully applied to Ecuadorian *Polylepis* species (Ochoa, 2008; Villota, 2012), provide an effective tool for resolving population structure and detecting introgression.

This study aims to characterise genetic diversity, population structure, and interspecific gene flow between *P. incana* and *P. racemosa* in and around the Ponce Paluguillo Water Conservation Area (Pichincha, Ecuador), using inter-simple sequence repeat (ISSR) genotyping. The resulting dataset will inform evidence-based restoration and conservation strategies for these high-Andean forests.

Material and Methods

Study area and plant material collection

The study was conducted in the Ponce Paluguillo Water Conservation Area and its surrounding zones, located west of Quito, in Pifo Parish, Pichincha Province, Ecuador. Sampling sites distributed across the study area are shown in figure 1. The area is characterized by an average annual temperature of 6 °C (range: 0-21 °C) and a mean annual precipitation of approximately 1300 mm (Almeida, 2016).

A total of six populations were sampled for molecular analyses, including three populations of *Polylepis incana* (I1-I3) and three of *P. racemosa* (R1-R3), with ten adult individuals collected per population (n=60; Table 1). Given the extensive and continuous nature of the forest stands, population size could not be accurately quantified. However, random transects conducted across the sampled forests confirmed the presence of numerous reproductively mature individuals within each population. This sampling effort was considered adequate for population-level inference using dominant markers, as a high number of reproducible ISSR loci were scored (296 bands), increasing multilocus resolution and allowing robust detection of genetic patterns despite moderate per-population sample sizes (Nelson and Anderson, 2013).

For the purposes of this study, a population was operationally defined as a spatially discrete and contiguous stand of the focal *Polylepis* taxon, delimited by the absence of intervening adult individuals and by clear discontinuities in crown connectivity. These discontinuities often coincided with anthropogenic or geomorphological barriers (e.g., paved roads or abrupt topographic features) and were verified

through direct field observations and GPS records. Straight-line (Euclidean) geographic distances among populations, calculated from GPS coordinates of population centroids, ranged from 94 to 4370 m (Appendix 1). From each individual, fully expanded, undamaged leaves were collected for DNA extraction. Leaf material was desiccated in silica gel in the field, stored at -20 °C in polypropylene tubes, and subsequently genotyped using inter-simple sequence repeat (ISSR) markers.

Seedlings were not sampled because none were present during the survey period; therefore, all genetic inferences pertain to adult cohorts. Prior to collection, a morphological reconnaissance was conducted to confirm target species and ensure correct field identification. The process was led by an experienced botanist specialized in *Polylepis* taxonomy: author MCSS. Species identification and differentiation were based on diagnostic foliar characters commonly used in *Polylepis* taxonomy, including the number of lateral leaflet pairs, the degree of marginal dentition, and qualitative differences in the indumentum of the abaxial leaflet surface (e.g., hair length and pubescence density), as illustrated in figure 2. Representative voucher specimens of each species are being prepared for deposition in the Herbario Nacional del Ecuador (QCNE), Instituto Nacional de Biodiversidad (INABIO).

Genomic DNA extraction and quantification

Genomic DNA was isolated with the 2X CTAB protocol optimised for *Polylepis* by Andrade et al. (2013), based on the original method of Doyle and Doyle (1987). After extraction, 1.5 µl of RNase A (20 mg/ml) was added to eliminate residual RNA. DNA concentration and purity were assessed using a NanoDrop™ 2000/2000c UV-Vis micro-volume spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and the DNA integrity was verified on 1% agarose gels stained with SYBR™ Safe (0.035 µl/ml gel).

Inter-simple sequence repeat (ISSR) amplification

Eight ISSR primers previously validated for *Polylepis* were used: seven from Ochoa (2008) and one from Julio et al. (2008) (Table 2). PCR reactions were carried out in a total volume of 25 µl following the thermal profile described by



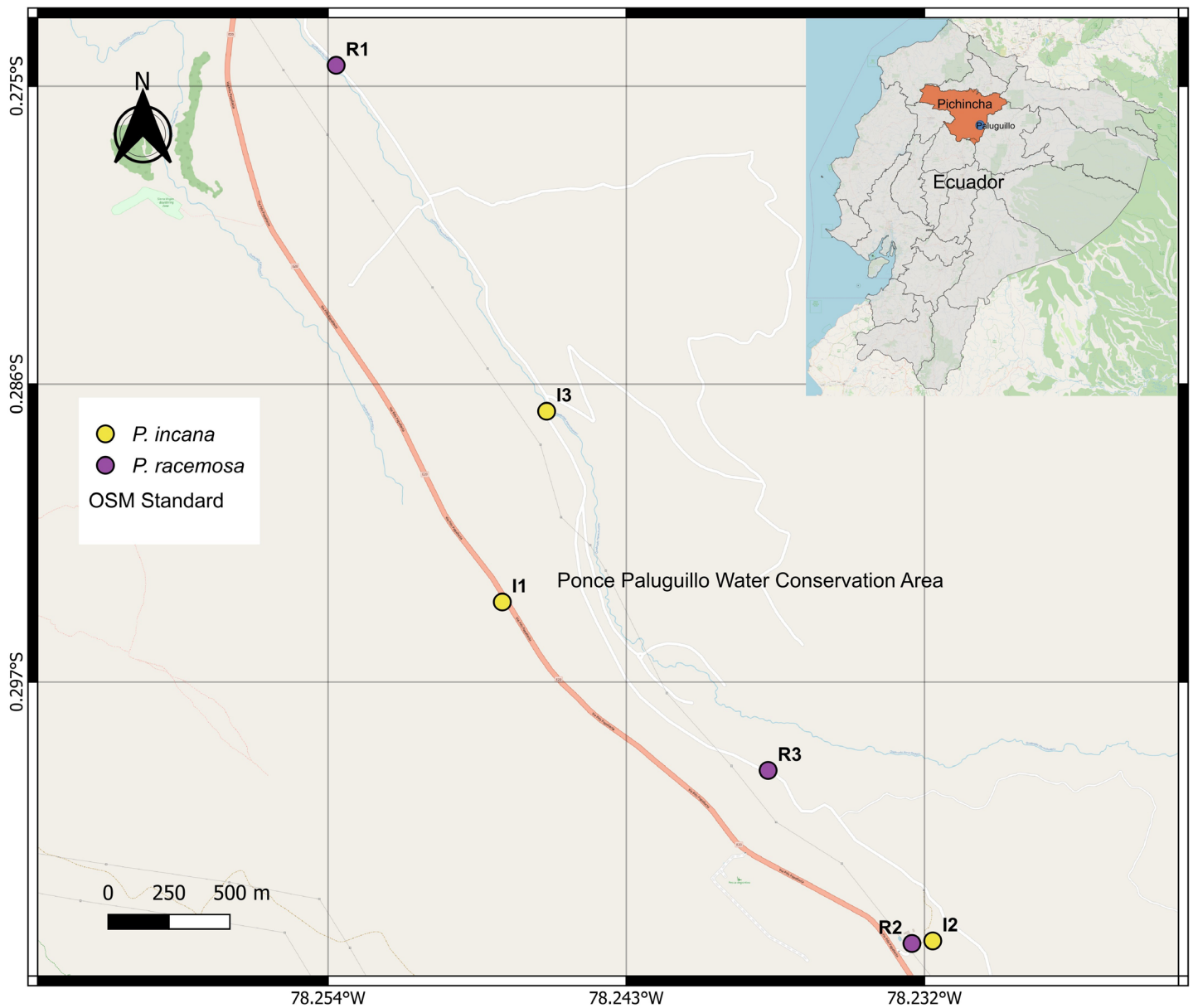


Figure 1: Study area in Paluguillo for the molecular survey using ISSR markers. Circles indicate molecular sampling sites of *Polylepis incana* Kunth (yellow) and *Polylepis racemosa* Ruiz & Pav. (purple). The orange line represents the main road crossing the area. The inset shows the location of the study area within Pichincha province, Ecuador. Map created by Lisbeth Cuenca-Chasi using QGIS v. 3.42 (QGIS Development Team, 2025).

Table 1: Geographic coordinates and elevation of the sampled *Polylepis incana* Kunth (I1-I3) and *Polylepis racemosa* Ruiz & Pav. (R1-R3) populations.

Especie	Code population	Individuals collected	Geographical coordinates	Elevation (m)
<i>Polylepis incana</i> Kunth	I1	10	0°17'38.4"S, 78°14'51.0"W	3399
	I2	10	0°18'23.0"S, 78°13'54.0"W	3735
	I3	10	0°17'13.3"S, 78°14'45.4"W	3829
<i>Polylepis racemosa</i> Ruiz & Pav.	R1	10	0°16'27.2"S, 78°15'13.0"W	3690
	R2	10	0°18'23.9"S, 78°13'56.9"W	3708
	R3	10	0°18'00.9"S, 78°14'16.0"W	3830



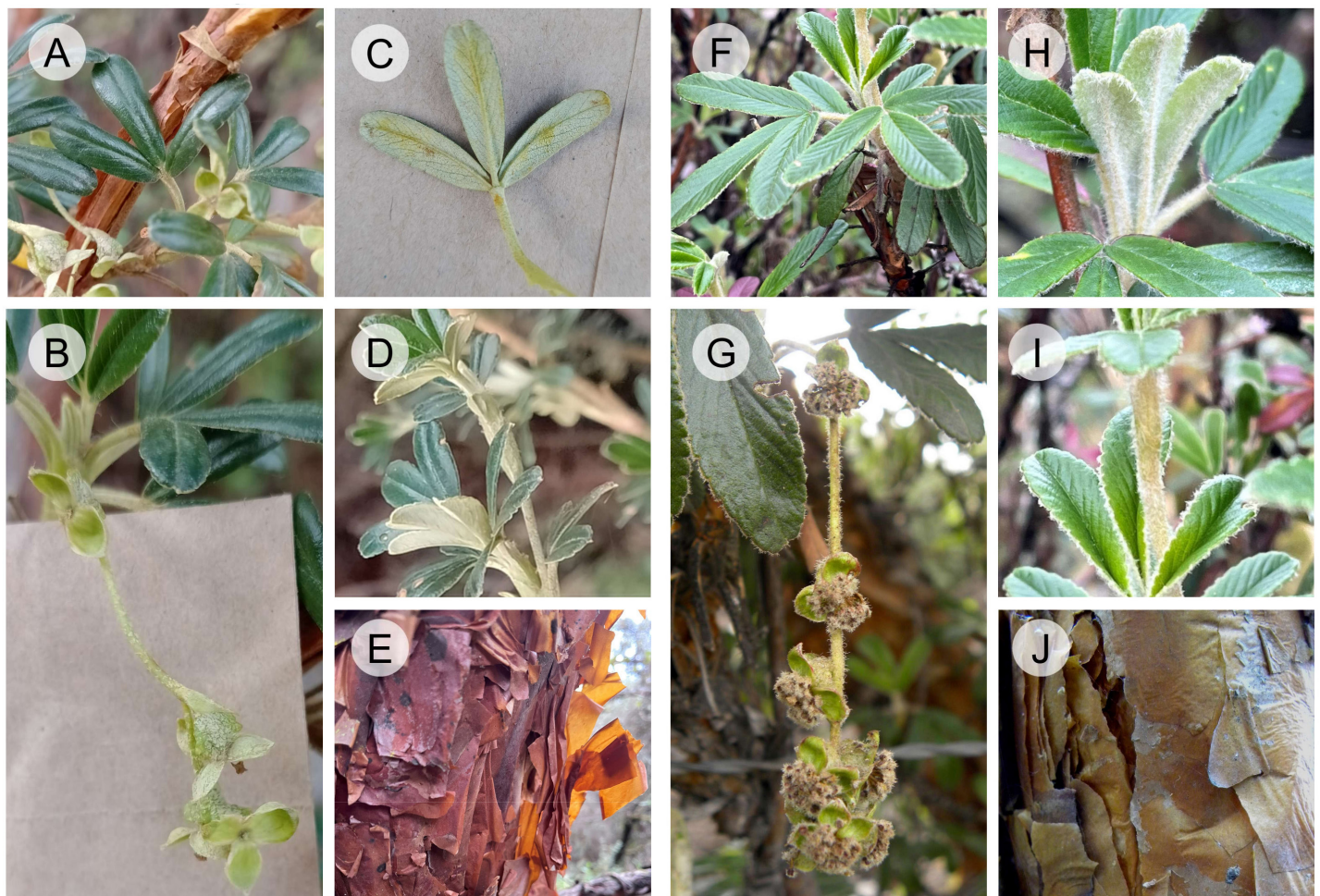


Figure 2: Morphological features of *Polylepis incana* Kunth and *Polylepis racemosa* Ruiz & Pav. from Paluguillo, Pichincha, Ecuador. A-E. *P. incana*: A. adaxial leaf surface; B. flowering branch; C. abaxial leaf surface; D. stipule sheaths; E. bark. F-J. *P. racemosa*: F. adaxial leaf surface; G. flowering branch; H. abaxial leaf surface; I. stipule sheaths; J. bark. Photographs: M. Claudia Segovia-Salcedo and Lisbeth Cuenca-Chasi.

Ochoa (2008): an initial denaturation at 94 °C for 2 min; 40 cycles of 94 °C for 30 s, primer-specific annealing for 45 s, and 72 °C for 2 min; followed by a final extension at 72 °C for 7 min. Each reaction contained 1X PCR buffer, 0.8 μM of each primer, 1.8-2.0 mM MgSO₄ (depending on the primer), 0.2 mM of each dNTP and 1 U of Taq DNA polymerase.

Gel electrophoresis and image acquisition

PCR products were resolved on 1.5% agarose gels prepared in 1X TBE buffer and stained with SYBRTM Safe (0.035 μl/ml gel). Electrophoresis was carried out in a horizontal electrophoresis chamber (Enduro, Labnet International, Edison, USA) connected to a power supply (4000PECA-115, Thermo Fisher Scientific, Whaltman, USA) at 120 V and 300 mA for 140 min. Gels were imaged using a ChemiDoc™ MP system (Bio-Rad, Hercules, USA).

ISSR data analysis

Band sizes (bp) were estimated using the point-to-point semi-logarithmic method in ImageLab v. 6.1 (Bio-Rad, 2017) and recorded in Microsoft Excel 2010. ISSR loci were scored as dominant binary markers (presence/absence), for which allelic dosage and complete genotypes cannot be inferred. Given the documented occurrence of polyploidy in *Polylepis*, including *P. racemosa*, and the absence of ploidy information for individual samples, all downstream analyses were conducted under an operational diallelic framework. This framework was adopted exclusively to allowed standard estimators for dominant markers in PopGene v. 1.32 (Yeh et al., 2000) and GenAEx v. 6.5 (Peakall and Smouse, 2012), following Lynch and Milligan (1994).

Importantly, this analytical assumption does not imply biological diploidy of the studied taxa. Consequent-

Table 2: Characteristics of the eight ISSR primers used in *Polylepis* Ruiz & Pav. For each primer, the primer sequence, length (bp), $MgSO_4$ concentration, and annealing temperature (Ta) are shown.

Primer	Sequence	length (pb)	[$MgSO_4$] (mM)	Ta (°C)
844A	CTCTCTCTCTCTCTAC	18	1.8	46.4
17898A	CACACACACAAC	14	1.5	41.9
17898B	CACACACACAGT	14	2	45.4
17899A	CACACACACAAG	14	1.8	40.4
HB9	GTGTGTGTGTGTGG	14	1.8	46.4
HB11	GTGTGTGTGTGTCC	14	1.8	43.6
HB12	CACCACCACGC	11	2	50.3
PA1	GAGAGAGAGAGAGAC	17	1.8	52.2

ly, estimates of genetic diversity and population structure are interpreted primarily as band-based patterns of genetic variation, while allele-frequency-based parameters are considered cautiously and only in a comparative, relative context.

Dice dissimilarity coefficients were calculated in DARwin v. 6.0.21 (Perrier and Jacquemoud-Collet, 2019), and the resulting matrix was used to construct dendrograms under the UPGMA algorithm. Analysis of molecular variance (AMOVA) was performed in GenAlEx v. 6.5 with 999 permutations and a significance threshold of 1%. Population structure was inferred with STRUCTURE v. 2.3.4 (Pritchard et al., 2000), using 10,000 burn-in iterations followed by 10,000 Markov chain Monte Carlo (MCMC) iterations. The optimal number of genetic clusters (K) was determined using STRUCTURE Harvester (Earl and vonHoldt, 2012).

Results

ISSR marker data analysis

The eight primers used in the inter-simple sequence repeat (ISSR) analysis generated a total of 296 bands, of which 292 were polymorphic. These results indicate that the primers were highly informative. However, the overall genetic diversity within populations was low (Table 3).

The percentage of polymorphic loci per population ranged from 23.79% in R2 (*P. racemosa*) to 59.12% in I1 (*P. incana*). Expected heterozygosity values varied between 0.089 in I1 and 0.156 in R2, further supporting the observation of limited genetic variability across populations.

The analysis of molecular variance (AMOVA) revealed that 59% of the total genetic variation occurred within populations, while 41% resulted from differences among populations (Table 4). The overall genetic differentiation coefficient (GST) was 0.36487 and the estimated number of effective migrants per generation (Nm) was 0.9338, suggesting restricted gene flow between populations.

A UPGMA dendrogram (Fig. 3) was constructed based on a dissimilarity matrix generated using the Dice coefficient. The clustering pattern revealed two main groups. The first group included *P. racemosa* populations R2 and R3. The second group was subdivided into two subclusters: one consisting of *P. incana* populations I1 and I2, and the other comprising population I3 (*P. incana*) and R1 (*P. racemosa*).

The Bayesian clustering analysis performed with STRUCTURE v. 2.3.4 (Fig. 4) identified K=5 as the optimal number of genetic clusters based on the DeltaK method of Evanno et al. (2005), which showed its highest peak at K=5 (Appendix 2). A secondary peak was observed at K=3, suggesting a higher-level or hierarchical structuring among

Table 3: Genetic diversity parameters for each studied *Polylepis incana* Kunth (I1-I3) and *Polylepis racemosa* Ruiz & Pav. (R1-R3) populations.

Population	Total number of polymorphic loci	Polymorphism rate (%)	Expected heterozygosity
I1	88	23.79	0.089
I2	111	37.5	0.102
I3	140	47.30	0.127
R1	113	38.18	0.118
R2	175	59.12	0.156
R3	147	49.66	0.147

Table 4: Analysis of molecular variance (AMOVA) among the studied populations. df=degrees of freedom, SS=sum of squares, Var. comp=variance component, % var=percentage of variation.

Source	df	SS	Mean square	Var. comp	% var
Within populations	5	918.883	183.777	16.027	41
Among populations	54	1269.500	23.509	23.509	59
Total	59	2188.383		39.536	100



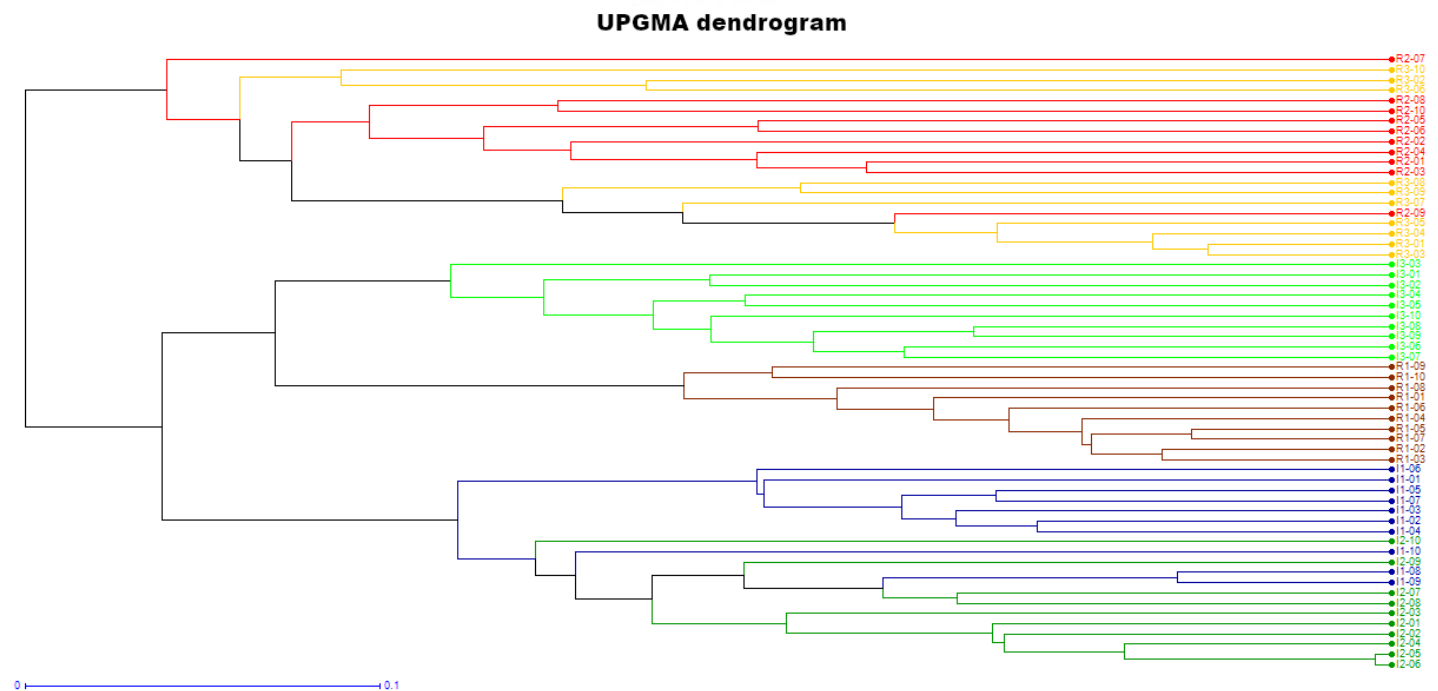


Figure 3: UPGMA dendrogram depicting relationships among *Polylepis* samples from six field clusters. Terminal labels identify population codes (*Polylepis racemosa* Ruiz & Pav. R1-R3; *P. incana* Kunth I1-I3), while branch colours match those codes.

populations. At K=5 (Fig. 4), populations of *Polylepis racemosa* and *P. incana* exhibited a clearer subdivision, while the clustering pattern at K=3 broadly mirrored the UPGMA dendrogram, grouping populations R2 and R3 into one cluster (*P. racemosa*), I1 and I2 into a second cluster (*P. incana*), and I3 together with R1 into a third one. This hierarchical pattern indicates genetic differentiation both between and within species, as well as partial genetic overlap among some populations.

Discussion

ISSR marker data analysis

The 292 polymorphic bands identified represent 98.65% of the total bands generated, confirming that the inter-simple sequence repeat primers used are highly informative. Previous studies using dominant markers on *Polylepis* have also reported high levels of polymorphism, ranging from 92.3% to 95.7% (Ochoa, 2008; Julio et al., 2011; Villota, 2012).

The low percentages of polymorphic loci (23.79% in R2 to 59.12% in I1) and expected heterozygosity (0.156

in R2 to 0.089 in I1) are consistent with prior findings. For instance, *P. incana* populations from Papallacta exhibited polymorphism levels from 39% to 52% (Hensen et al., 2012) and expected heterozygosity values comparable to those reported by Villota (2012), who found a value of $H=0.31$ in *P. incana*. According to Hennink and Zeven (1990), even when individual traits exhibit low variability, populations may still maintain substantial overall genetic variation.

Traits commonly associated with high genetic variability include long generation times and wind pollination (Hamrick et al., 1979). Nevertheless, this study reveals low genetic diversity in both the *Polylepis* species analysed. The low genetic variability in *P. incana* populations is consistent with previous studies in Ecuador (Ochoa, 2008; Hensen et al., 2012; Villota, 2012) and may be explained by the species' mixed reproductive system (sexual and clonal) (Cierjacks et al., 2007), as well as clonal propagation at higher elevations (Hensen et al., 2012). Hamrick et al. (1979) also noted that species with both sexual and asexual reproduction tend to exhibit reduced genetic variation. In the case

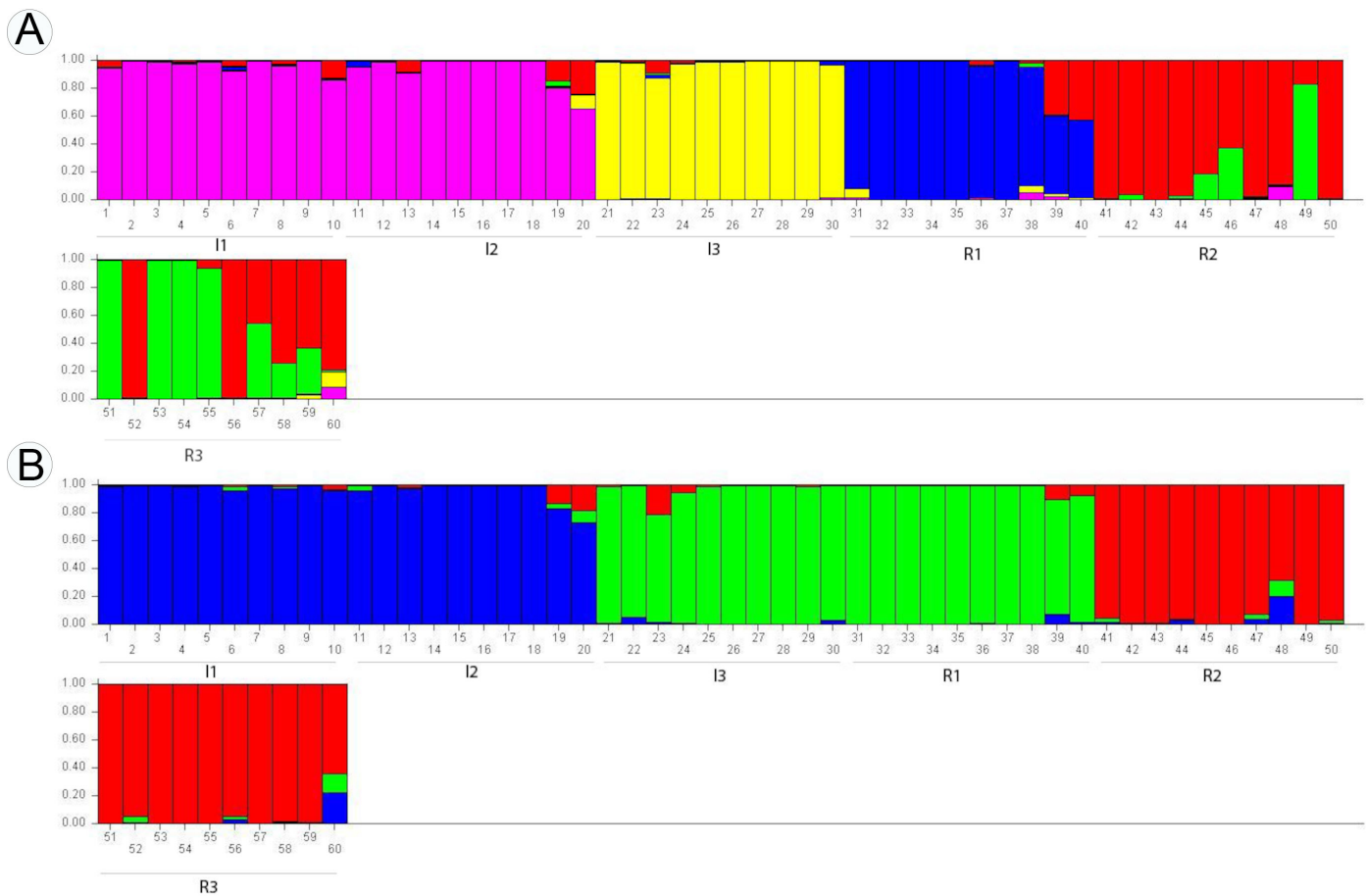


Figure 4: Bayesian structure of the *Polylepis incana* Kunth (I1-I3) and *Polylepis racemosa* Ruiz & Pav. (R1-R3) populations, generated using STRUCTURE v. 2.3.4 (Pritchard et al., 2000). A. clustering pattern for K=5; B. clustering pattern for K=3. Each vertical bar represents an individual; colours indicate genome proportions assigned to each cluster. Individuals are grouped by population along the x-axis, and membership probability (Q) is shown on the y-axis (0-1 scale).

of *P. racemosa*, the low genetic diversity is likely due to the fact that all sampled populations were cultivated or reforested and propagated via cuttings.

The AMOVA results indicated that 59% of genetic variation occurred within populations, while 41% was distributed among populations. The genetic differentiation index ($G_{ST}=0.36487$) suggests that approximately 36% of the total genetic variation corresponds to interpopulation divergence. These patterns agree with findings by Kessler and Schmidt-Lebuhr (2006), who also reported low but significant differentiation among *Polylepis* populations. Similarly, Aragundi et al. (2011) proposed that such low differentiation reflects a shared evolutionary history influenced by recent habitat fragmentation rather than long-term isolation.

The estimated number of effective migrants ($N_m=0.9338$) suggests roughly one migrant between *P. incana* and *P. racemosa* populations per generation. While this indicates gene flow, it is relatively low compared to other *Polylepis* species. This is notably lower than values reported for other species pairs, such as *P. incana* and *P. pauta* Hieron. ($N_m=3.651$; Villota, 2012) or *P. australis* Bitter ($N_m=10.1$; Julio et al., 2011).

Clustering patterns and gene flow

Dendrogram analysis identified three major clusters (Fig. 3): R2 and R3 (*P. racemosa*; average similarity=0.613), I2 and I1 (*P. incana*; 0.423), and a mixed cluster of I3 (*P. incana*) and R1 (*P. racemosa*; 0.591). While most populations of the same species clustered together, suggesting a shared

origin, certain interspecific groupings (e.g., I3 and R1) imply the occurrence of gene flow rather than hybridisation.

Bayesian clustering analysis identified two likely population structures: K=5 and K=3 (Appendix 2). Meirmans (2015) noted that estimates of K should be interpreted cautiously as population structure is often complex. Rather than seeking a single “true” K, it is more informative to evaluate biologically meaningful patterns across multiple values. In this case, K=3 is congruent with both the UPGMA dendrogram and known demographic patterns: it groups R2 and R3 together, I1 and I2 together, and I3 with R1. This suggests some degree of genetic overlap between *P. incana* and *P. racemosa*, particularly in sympatric zones.

This limited yet detectable gene flow raises questions about its ecological and evolutionary implications, particularly in regions where both species co-occur. As shown in Figure 4, gene exchange appears restricted and asymmetric, a pattern typical of early-stage introductions or incipient introgression events.

Implications for the conservation and management of *Polylepis* forests

The genetic structures observed in adult individuals reflect historical gene flow, shaped by evolutionary processes acting on previous generations (Ellstrand, 2014). This means that the current rate of gene flow may have changed over time and could be increasing. One possible explanation lies in the long-distance wind pollination characteristic of the genus *Polylepis*. For example, *P. australis* pollen has been reported to disperse over distances of up to 80 km (Kessler and Schmidt-Lebuhn, 2006). Nevertheless, determining pollen dispersal distances specific to the studied species remains a research priority.

The ecological consequences of localized gene flow are difficult to predict. Hybrid individuals may vary in phenotype and fitness, potentially producing a range of evolutionary outcomes. In some cases, hybridisation can lead to species extinction (Ellstrand and Rieseberg, 2016).

For example, genetic swamping may occur when hybrids gradually replace one or both parental species, ultimately leading to the merging or extinction of the original taxa, a process known as speciation reversal. In contrast, demographic swamping arises when hybrids exhibit

reduced reproductive success (outbreeding depression), which can either result in premature reproductive isolation or increase extinction risk due to inefficient reproduction (Ellstrand, 2014; Runemark et al., 2019). Conversely, hybridisation may also lead to the emergence of novel hybrid species through the evolution of reproductive barriers against both parental lineages (Runemark et al., 2019).

Given these potential outcomes, the gene flow detected between *P. incana* and *P. racemosa* represents a potential threat to the conservation of *P. incana*. Additionally, the low genetic diversity observed in *P. incana* is a disadvantage, as it may increase the species' vulnerability to selective pressures and reduce its long-term viability (Esfandani Bozchaloyi et al., 2017). Small, threatened populations are particularly sensitive to increased gene flow, which can further elevate the risk of extinction (Ellstrand, 2014).

This study has several limitations that should be considered when interpreting the results. First, only adult individuals were sampled because seedlings were not available in the field, which means our findings primarily reflect the genetic structure of established cohorts and do not capture recent recruitment. Second, the use of dominant ISSR markers, while valuable for detecting overall genetic patterns, does not allow the distinction between heterozygotes and homozygotes, thereby limiting the resolution of genetic diversity estimates. As a result, our data cannot directly confirm whether hybridisation events are currently occurring between *P. incana* and *P. racemosa*, although patterns of gene flow between populations were identified.

Future studies should incorporate codominant markers such as microsatellites or SNPs, together with a more inclusive sampling of seedlings and juveniles, to better assess contemporary hybridisation dynamics and guide the conservation and management of *Polylepis incana* in sympatric zones.

Conclusions

The sampled populations of *P. incana* and *P. racemosa* from the Ponce Palugillo Water Conservation Area and its surrounding regions exhibit low genetic diversity, as evidenced by the low percentage of polymorphic loci and expected heterozygosity. Furthermore, greater genetic variation was detected within populations than among them. Genetically



similar population pairs included I1 and I2 (*P. incana*), R2 and R3 (*P. racemosa*), and I3 and R1 (*P. incana* and *P. racemosa*, respectively). These groupings were consistently identified through distance-based clustering (UPGMA) and Bayesian inference, which revealed a population structure best described by $K=3$.

Although our ISSR data do not allow for the reliable identification of hybrid individuals, we detected patterns consistent with gene flow between *P. incana* and *P. racemosa*, particularly between populations I3 and R1. The estimated migration rate of 0.9 individuals per generation indicates low but biologically meaningful gene flow. These results have important implications for the conservation and management of native *P. incana* populations in the region.

To better understand the dynamics of gene flow and population structure, future research should include multi-generational analyses and employ more informative molecular markers than ISSRs. Additionally, empirical studies are needed to quantify pollen and seed dispersal distances in both species. This information is critical for determining the spatial scale at which gene flow occurs and for designing effective conservation strategies.

Author contributions

Conceptualization: MCSS, KP; Methodology: MCSS, DVS; Formal analysis: DVS, CC; Investigation: DVS, LCC; Funding acquisition: MCSS, KP; Resources: GM; Writing - original draft: LCC, KP, VO, CC; Writing - review & editing: LCC, MCSS, KP; Project administration: MCSS, KP.

Funding

This research was funded by Universidad de las Fuerzas Armadas ESPE through the HANS-BANK project (code 9189000000.388138).

Acknowledgements

The authors acknowledge the Universidad de las Fuerzas Armadas ESPE. We are also grateful to the Fideicomiso Mercantil Fondo para la Protección del Agua (FONAG) and the Empresa Pública Metropolitana de Agua Potable y Saneamiento (EPMAPS) for providing logistical support and facilitating access to the sampling sites. Field collections used in this study were carried out during 2020-2021 under the

Framework Contract for Access to Genetic Resources MAE-DNB-CM-2020-012. The study is currently framed under the institutional Framework Contract for Access to Genetic Resources MAATE-CMARG-2024-0001, granted by the Ministerio del Ambiente, Agua y Transición Ecológica del Ecuador (MAATE). We further acknowledge the assistance of all institutions and individuals who contributed to the successful execution of this research.

Data availability statement

The dataset that supports the findings of this study has been deposited in SciELO Data and is available at: <https://doi.org/10.48331/SCIELODATA.UKYESY>

Literature cited

- Almeida, P. 2016. Comparación de la composición florística en páramo de pajonal a diferentes rangos altitudinales en el Área de Conservación Hídrica Paluguillo, Ecuador. Master thesis in Ecology. Colegio de Posgrados, Universidad San Francisco de Quito USFQ. Quito, Ecuador. 39 pp. <https://repositorio.usfq.edu.ec/bitstream/23000/6029/1/129244.pdf> (consulted April, 2025).
- Andrade, R., M. Jadán and C. Segovia-Salcedo. 2013. Estudio de genética poblacional de *Polylepis pauta* y *Polylepis sericea* en Pichincha mediante la utilización de marcadores moleculares SSRs. Revista Ecuatoriana de Medicina y Ciencias Biológicas 34(1-2): 27-45. DOI: <https://doi.org/10.26807/remcb.v34i1-2.232>
- Aragundi, S., J. L. Hamrick and K. C. Parker. 2011. Genetic insights into the historical distribution of *Polylepis pauta* (Rosaceae) in the northeastern Cordillera Oriental of Ecuador. Conservation Genetics 12: 607-618. DOI: <https://doi.org/10.1007/s10592-010-0165-x>
- Bio-Rad. 2017. ImageLab Software version 6.1. <https://www.bio-rad.com/es-ec/product/image-lab-software?ID=KRE6P5E8Z#fragment-6> (consulted April, 2025).
- Bohling, J. H. 2016. Strategies to address the conservation threats posed by hybridization and genetic introgression. Biological Conservation 203: 321-327. DOI: <https://doi.org/10.1016/j.biocon.2016.10.011>
- Boza Espinoza, T. and M. Kessler. 2022. A monograph of the genus *Polylepis* (Rosaceae). PhytoKeys 203: 1-274. DOI: <https://doi.org/10.3897/phytokeys.203.83529>



- Caballero-Villalobos, L., F. Fajardo-Gutiérrez, M. Calbi and G. A. Silva-Arias. 2021. Climate Change Can Drive a Significant Loss of Suitable Habitat for *Polylepis quadrijuga*, a Treeline Species in the Sky Islands of the Northern Andes. *Frontiers in Ecology and Evolution* 9: 661550. DOI: <https://doi.org/10.3389/fevo.2021.661550>
- Caiza, J., D. Corredor, C. Galárraga, J. P. Herdoiza, M. Santillán and M. C. Segovia-Salcedo. 2021. Geometry morphometrics of plant structures as a phenotypic tool to differentiate *Polylepis incana* Kunth and *Polylepis racemosa* Ruiz & Pav. reforested jointly in Ecuador. *Neotropical Biodiversity* 7(1): 121-134. DOI: <https://doi.org/10.1080/23766808.2021.1906138>
- Canales, A. and Y. Huarasa. 2020. Poder germinativo de *Polylepis incana* con aplicación de diferentes tratamientos de agua. *Revista Cubana de Ciencias Forestales* 8(3): 495-506. http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S2310-34692020000300495 (consulted April, 2025).
- Chuncho Morocho, C. and G. Chuncho. 2019. Páramos del Ecuador, importancia y afectaciones: Una revisión. *Bosques Latitud Cero* 9(2): 71-83. https://drive.google.com/file/d/1_m4ZobqzjfgTfv2S3CvB4AljSh5lIPnS/view (consulted April, 2025).
- Cierjacks, A., K. Wesche and I. Hensen. 2007. Potential lateral expansion of *Polylepis* forest fragments in central Ecuador. *Forest Ecology and Management* 242(2-3): 477-486. DOI: <https://doi.org/10.1016/j.foreco.2007.01.082>
- Cuyckens, G. A. and D. Renison. 2018. Ecología y conservación de los bosques montanos de *Polylepis*: Una introducción al número especial. *Ecología Austral* 28(1-bis): 157-162. DOI: <https://doi.org/10.25260/EA.18.28.1.1.766>
- Doyle, J.J. and J.L. Doyle. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* 19: 11-15.
- Earl, D. A. and B. M. vonHoldt. 2012. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4(2): 359-361. DOI: <https://doi.org/10.1007/s12686-011-9548-7>
- Ellstrand, N. C. 2014. Is gene flow the most important evolutionary force in plants? *American Journal of Botany* 101(5): 737-753. DOI: <https://doi.org/10.3732/ajb.1400024>
- Ellstrand, N. C. and L. H. Rieseberg. 2016. When gene flow really matters: gene flow in applied evolutionary biology. *Evolutionary Applications* 9(7): 833-836. DOI: <https://doi.org/10.1111/eva.12402>
- Esfandani Bozchaloyi, S., M. Sheidai, M. Keshavarzi, Z. Noormohammadi, M. Hassanzadeh, S. Ghasemzadeh-Baraki and F. Koohdar. 2017. Analysis of genetic diversity in *Geranium robertianum* by ISSR markers. *Phytologia Balcanica* 23(2): 157-166. https://www.researchgate.net/publication/321276224_Analysis_of_genetic_diversity_in_Geranium_robertianum_by_ISSR_markers/references (consulted April, 2025).
- Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14(8): 2611-2620. DOI: <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Hamrick, J. L., Y. B. Linhart and J. B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology, Evolution, and Systematics* 10: 173-200. DOI: <https://doi.org/10.1146/annurev.es.10.110179.001133>
- Hennink, S. and A. C. Zeven. 1990. The interpretation of Nei and Shannon-Weaver within population variation indices. *Euphytica* 51(3): 235-240. DOI: <https://doi.org/10.1007/BF00039724>
- Hensen, I., A. Cierjacks, H. Hirsch, M. Kessler, K. Romoleroux, D. Renison and K. Wesche. 2012. Historic and recent fragmentation coupled with altitude affect the genetic population structure of one of the world's highest tropical tree line species. *Global Ecology and Biogeography* 21(4): 455-464. DOI: <https://doi.org/10.1111/j.1466-8238.2011.00691.x>
- Hofstede, R., J. Calles, V. López, R. Polanco, F. Torres, J. Ulloa, A. Vásquez and M. Cerra. 2014. Los Páramos Andinos ¿Qué sabemos? Estado de conocimiento sobre el impacto del cambio climático en el ecosistema páramo. *Unión Internacional para la Conservación de la Naturaleza y de los Recursos Naturales*. Quito, Ecuador. Pp. 1-79.
- Julio, N., A. Sobral, J. Rondan Dueñas, J. Di Rienzo, D. Reninson and I. Hensen. 2008. RAPD and ISSR markers indicate diminished gene flow due to recent fragmentation of *Polylepis australis* woodlands in central Argentina.

- Biochemical Systematics and Ecology 36(5-6): 329-335. DOI: <https://doi.org/10.1016/j.bse.2007.10.007>
- Julio, N. B., J. C. Rondan Dueñas, D. Renison and I. Hensen. 2011. Genetic structure and diversity of *Polylepis australis* (Rosaceae) tree populations from central Argentina: Implications for forest conservation. *Silvae Genetica* 60(1-6): 55-61. DOI: <https://doi.org/10.1515/sg-2011-0007>
- Kerr, S. 2004. A Phylogenetic and Biogeographic analysis of Sanguisorbeae (Rosaceae) with emphasis on the Pleistocene radiation of the high Andean genus *Polylepis*. Doctoral dissertation in Biology. University of Maryland. College Park, Maryland, USA. 202 pp. <https://api.drum.lib.umd.edu/server/api/core/bitstreams/f102a7a6-0be6-480e-be40-8fb897bab624/content> (consulted April, 2025).
- Kessler, M. 2006. Bosques de *Polylepis*. In: Moraes R., M., B. Øllgaard, L. P. Kvist, F. Borchsenius and H. Balslev (eds.). Botánica Económica de los Andes Centrales. Universidad Mayor de San Andrés. La Paz, Bolivia. Pp. 110-120. https://www.researchgate.net/publication/228644927_Bosques_de_Polylepis (consulted April, 2025).
- Kessler, M. and A. N. Schmidt-Lebuhn. 2006. Taxonomical and distributional notes on *Polylepis* (Rosaceae). *Organisms Diversity and Evolution* 6(1): 67-69. DOI: <https://doi.org/10.1016/j.ode.2005.04.001>
- Lynch, M. and B. G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3(2): 91-99. DOI: <https://doi.org/10.1111/j.1365-294X.1994.tb00109.x>
- Meirmans, P. G. 2015. Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology* 24(13): 3223-3231. DOI: <https://doi.org/10.1111/mec.13243>
- Nelson, M. F. and N. O. Anderson. 2013. How many marker loci are necessary? Analysis of dominant marker data sets using two popular population genetic algorithms. *Ecology and Evolution* 3(10): 3455-3470. DOI: <https://doi.org/10.1002/ece3.725>
- Ochoa, V. 2008. Genética poblacional de *Polylepis incana* y *Polylepis pauta* en los páramos de Papallacta y los Ilinizas mediante ISSRs. Biotechnology Engineering thesis. Universidad de las Fuerzas Armadas ESPE. Sangolquí, Ecuador. 104 pp.
- Ojala-Barbour, R., J. Brito and W. R. Teska. 2019. A comparison of small mammal communities in two High-Andean *Polylepis* woodlands in Ecuador. *ACI Avances en Ciencias e Ingenierías* 11(2): 208-221. DOI: <https://doi.org/10.18272/aci.v11i2.516>
- Peakall, R. and P. E. Smouse. 2012. GenALEx 6.5: genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28(19): 2537-2539. DOI: <https://doi.org/10.1093/bioinformatics/bts460>
- Perrier, X. and J. P. Jacquemoud-Collet. 2006. Darwin software: Dissimilarity analysis and representation for Windows. Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD). Montpellier, France.
- Pritchard, J. K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155(2): 945-959. DOI: <https://doi.org/10.1093/genetics/155.2.945>
- Purcell, J. and A. Brelsford. 2004. Reassessing the causes of decline of *Polylepis*, a tropical subalpine forest. *Ecotropica* 10: 155-158. <https://purcelllab.ucr.edu/pdfs/Purcell,Brelsford%202004.pdf> (consulted April, 2025).
- QGIS Development Team. 2025. QGIS Geographic Information System. QGIS Software. <https://qgis.org/download/> (consulted April, 2025).
- Romoleroux, K. 1996. Rosaceae. In: Harling, G. y L. Andersson (eds.). Flora of Ecuador. Vol. 56. University of Gothenburg, Göteborg, Sweden; Riksmuseum, Stockholm, Sweden; Pontificia Universidad Católica del Ecuador (PUCE). Quito, Ecuador. Pp. 1-151.
- Runemark, A., M. Vallejo-Marin and J. I. Meier. 2019. Eukaryote hybrid genomes. *PloS Genetics* 15(11): e1008404. DOI: <https://doi.org/10.1371/journal.pgen.1008404>
- Segovia-Salcedo, M. 2011. Los riesgos de la reforestación de los páramos con especies exóticas: el caso de *Polylepis racemosa*. *Propuestas Andinas* 4(1): 1-4. https://www.researchgate.net/publication/264383781_Los_riesgos_de_la_reforestacion_de_los_paramos_con_especies_exoticasel_caso_de_Polylepis_racemosa (consulted April, 2025).
- Segovia-Salcedo, M. C. and P. Quija-Lamiña. 2014. Citogeografía de cuatro especies de *Polylepis* (Rosaceae) en el Ecuador: Información relevante para el manejo y conservación de los bosques andinos. In: Cuesta, F., J. Sevink, L. Llambí, B. De Bièvre and J. Posner (eds.). Avances en la investigación para

- la conservación de los páramos andinos. Consorcio para el Desarrollo Sostenible de la Ecorregión Andina (CONDESAN). Lima, Perú. Pp. 468-481.
- Simpson, B. B. 1979. A revision of the genus *Polylepis* (Rosaceae: Sanguisorbeae). *Smithsonian Contributions to Botany* 43: 1-62. DOI: <https://doi.org/10.5479/si.0081024X.43.1>
- Teich, I., A. M. Cingolani, D. Renison, I. Hensen and M. A. Giorgis. 2005. Do domestic herbivores retard *Polylepis australis* Bitt. woodland recovery in the mountains of Córdoba, Argentina? *Forest Ecology and Management* 219(2-3): 229-241. DOI: <https://doi.org/10.1016/j.foreco.2005.08.048>
- Villota, S. 2012. Evaluación de la dinámica poblacional en especies simpátricas de *Polylepis* en el Páramo de la Virgen, provincia de Napo y Pichincha mediante marcadores moleculares. Bachelor thesis in Biological Sciences. Pontificia Universidad Católica del Ecuador. Quito, Ecuador. 119 pp.
- Yeh, F., R. C. Yang and T. Boyle. 2000. POPGEN (version 1.32), Microsoft windows-based freeware for population genetic analysis. https://sites.ualberta.ca/~fyeh/popgene_download.html (consulted April, 2025).



Appendix 1: Pairwise straight-line (Euclidean) geographic distances (m) among sampled populations of *Polylepis incana* Kunth (I1-I3) and *Polylepis racemosa* Ruiz & Pav. (R1-R3), calculated in QGIS from representative GPS coordinates.

	I1	I2	I3	R1	R2
I1	0				
I2	800 m	0			
I3	785 m	2680 m	0		
R1	2300 m	4330 m	1670 m	0	
R2	2250 m	94 m	2590 m	4370 m	0
R3	1310 m	970 m	1730 m	3400 m	931 m

Appendix 2: DeltaK profile from STRUCTURE analyses showing the optimum number of genetic clusters (K) inferred using the Evanno method.

