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Genome-wide SNP data support species boundaries in sympatric *Polylepis* Ruiz & Pav. (Rosaceae) species from Bolivia and Ecuador

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ABSTRACT

Species delimitation in the South American genus *Polylepis* is notoriously challenging due to high morphological similarity and phenotypic plasticity, likely driven by hybridization and gene flow. Previous phylogenetic studies suggested that genetic structure aligns more strongly with geography than with taxonomy, questioning existing species concepts and hampering conservation efforts. We used double-digest RAD sequencing (ddRADseq) to generate genome-wide SNP data for 11 *Polylepis* species sampled across multiple localities in Bolivia and Ecuador. Population genetic analyses, phylogenetic inference, and network approaches were combined to assess whether genetic structure aligns more closely with taxonomy or geography. Morphologically defined species formed largely cohesive genetic lineages across regions, with species identity explaining substantially more genetic variation than locality. While localized admixture and reticulation were detected among closely related taxa, widespread species showed strong genetic cohesion and clear separation from congeners. Our results indicate that the sampled *Polylepis* species from Bolivia and Ecuador maintain distinct genetic identities despite localized signals consistent with gene flow. This genome-wide support for current taxonomy highlights *Polylepis* as a valuable model for studying speciation under gene flow and indicates that multiple geographic sampling will be essential in reconstructing a robust phylogeny of the genus, with important implications for conservation planning in Andean montane forests.

1. Introduction

Polylepis Ruiz & Pav. (Rosaceae) forms one of the world's highest tree lines, reaching elevations of up to 5000 m asl in the Andes, where the occurrence of trees is unique and comparable only to the high-elevation conifers on the Tibetan plateau (Simpson, 1979; Boza-Espinoza &

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Kessler, 2022). The genus is distributed throughout the tropical Andes, and these forests are ecologically isolated from other vegetation types. Besides their remarkable presence at high elevations, *Polylepis* species are easily recognized by their characteristic multilayered, shredding bark (Fig. 1), from which the genus name is derived. Polylepis is economically and culturally important for Andean societies, providing critical ecosystem services, including erosion control, water regulation, soil protection, nutrient retention, and local microclimate regulation, and serve as important habitats for wildlife (Rowe, 1981; Liberman, 1986; Flores-Ochoa, 1990; Pärssinen, 1993; Fjeldså & Kessler, 1996; Gisbert et al., 1996; Michel, 1996, 2000; Sagárnaga, 1997; Capriles & Flores, 1999). However, *Polylepis* forests are highly vulnerable to human pressures such as burning, livestock grazing, timber extraction, agricultural expansion, and mining activities, which has led to a fragmented distribution restricted to microsites such as ravines, steep slopes, rock faces, or to planted areas near human settlements (Fjeldså, 2002; Kessler, 2002; Renison et al., 2002; Rundel et al., 2003; Boada and Campaña, 2008; Cierjacks et al., 2008; Guerrero, 2009; Navarro et al., 2010; Renison et al., 2011; Urquiaga-Flores et al., 2024). Kessler (1995a) and Fjeldså and Kessler (1996) estimated that more than 90% of *Polylepis* forests in Peru and Bolivia have been lost over time. Recognition of the increasing threats has stimulated extensive scientific research and prompted the establishment of multiple protected areas and conservation initiatives to preserve the remaining *Polylepis* forests (Sevillano-Ríos & Rodewald, 2017; Quispe-Melgar et al., 2019; Pinos, 2020; Ames-Martínez et al., 2021). Gareca et al. (2013) reviewed the effects of fragmentation in *Polylepis* forests and showed that this fragmentation is likely of recent origin (after 1533 CE), highlighting the importance of conservation efforts for maintaining genetic connectivity and promoting forest regeneration. In Ecuador and Peru, *P. racemosa* Ruiz & Pav. has been widely used in reforestation programmes mainly due to its high reproductive vigour and establishment success (Montalvo et al., 2018; Rosero et al., 2018). However, this species has been planted outside of its native range and hybridizes with the native species, so that to avoid negative ecological impacts, it is essential to plant ecologically adapted and compatible species, with phylogenetic relationships informing conservation strategies (Forest et al., 2007; Cadotte et al., 2009; Mouquet et al., 2012; Srivastava et al., 2012; Rosauer and Mooers, 2013). Despite its ecological importance and iconic status in the Andes, a comprehensive and well-resolved phylogenetic reconstruction of the genus *Polylepis* is still lacking.

The most recent monograph of *Polylepis* was published by Boza-Espinoza & Kessler (2022), wherein they recognized 45 species based on morphological, ecological, and biogeographical distinctness. This consolidation provided a clear framework for identifying and describing novel species (e.g., Quispe-Melgar et al., 2024; Gamarra & Valdivia, 2024; Gamarra & Boza-Espinoza, 2025; Quispe-Melgar et al., 2026). Prior to this monograph, taxonomic treatments of the genus were published by Bitter (1911) and later by Simpson (1979), each applying different species concepts and thus including 31 and 15 species, respectively. A consistent conclusion, however, is that species delimitation within *Polylepis* is exceptionally challenging in contrast to the well-defined delimitation of the genus itself. This challenge arises from the high phenotypic variability within species, coupled with a striking morphological similarity among species, which is likely due to a combination of hybridization, gene flow, polyploidization, and possibly apomictic reproduction, although the relative contribution of these processes is still poorly understood (Bitter, 1911; Simpson 1979; Boza-Espinoza & Kessler, 2022). Testing species delimitations is therefore a priority for reconstructing the phylogenetic history of *Polylepis*.

Previous studies investigating the phylogeny of the genus were done by Kerr (2004) using Sanger sequencing data of chloroplast and nuclear markers from over 50 *Polylepis* accessions. She showed that *Polylepis* is nested within *Acaena* Mutis ex L. possibly indicating a hybrid origin of the genus. Similar results were reported in a later study by Jaureguí-Lazo & Potter (2021). It must be noted, however, that their conclusions were limited by sparse sampling and the reliance on the ribosomal ITS marker, whose multiple copies potentially complicate phylogenetic reconstruction by producing topologies differing from plastome phylogenies (Olivar et al., 2025). Another study by Schmidt-Lebuhn et al. (2006) based on Amplified Fragment Length Polymorphisms (AFLP) data from 46 *Polylepis* accessions recorded low phylogenetic resolution but strong geographic clustering that often overrode species boundaries. Combined with morphological data, their analyses nevertheless identified species groups broadly consistent with those proposed by Simpson (1979) based on morphology. Segovia-Salcedo (2014), using Hyb-Seq data from 256 nuclear and chloroplast genes, also recovered a strong geographic signal and detected clades that did not correspond to previously proposed taxonomic groups. However, because widespread species were not sampled with replicates, the study could not test

Fig. 1. Characteristic multilayered, shredding bark of *Polylepis* (left) and populations of *P. subtusalbida* sticking out from the steep slopes of Parque Nacional Tunari, Bolivia at 3930 masl. Photos taken by C Segovia-Salcedo & JE Olivar.

whether geographically disjunct populations of the same species form coherent phylogenetic units. Overall, phylogenetic relationships within *Polylepis* remain poorly understood and previous studies have suggested reticulate evolutionary patterns, although the relative roles of hybridization, introgression, and incomplete lineage sorting remain unresolved.

In recent years, next-generation sequencing (NGS) technologies have opened new opportunities for studying evolutionary histories by enabling the collection of genome-scale data at unprecedented depth, speed, and affordability (Goodwin et al., 2016). Particularly for non-model organisms where the lack of reference genome assemblies constrains the scale and scope of genomic studies, reduced representation genotyping methodologies, such as double digest restriction-site associated DNA sequencing (ddRADseq; Peterson et al., 2012) greatly facilitate genomic analyses and have been widely applied in phylogenetics and phylogeography to unravel complex evolutionary histories, resolve species boundaries, and detect patterns of divergence and admixture in diverse taxa (e.g., Manurung et al., 2023; Vasile et al., 2024; Böhnert et al., 2025; Daubert et al., 2025). At present, complete plastome sequences are available for only three *Polylepis* species (Contreras-Díaz et al., 2025; Olivar et al., 2025) providing the groundwork for broader phylogenomic studies. In this study, we employed ddRADseq to assess the species boundaries proposed by Boza-Espinoza & Kessler (2022), and we specifically tested whether these boundaries are maintained in sympatric populations of different species.

As mentioned above, previous studies (Kerr, 2004; Schmidt-Lebuhn et al. 2006; Segovia-Salcedo, 2014) have shown that *Polylepis* species tend to cluster geographically rather than forming recognizable morphological units. This pattern may arise from two non-mutually exclusive processes: First, reticulate evolutionary processes, including hybridization and introgression, may have contributed to species diversification leading to evolutionary histories that are not readily resolved by classical bifurcating phylogenetic trees. Second, ongoing gene flow may occur among co-occurring species, as proposed by the genic species concept of Wu (2001), which posits that species can maintain their identity despite gene flow, with only a few loci determining species-specific traits while much of the genome remains freely exchangeable (Lexer et al., 2010). In genomic analyses, this process can cause populations to cluster more closely with geographically proximate populations of other species than with their conspecifics.

Here, we tested these hypotheses by focusing on two morphologically distinct, widely distributed species of *Polylepis*: *P. incana* Kunth in Ecuador and *P. neglecta* M. Kessler in Bolivia, together with their respective sympatric congeners. We selected *P. incana* and *P. neglecta* because they are among the most morphologically distinctive species recognized in the current taxonomic treatment of *Polylepis* (Boza-Espinoza & Kessler, 2022). Their clear morphological distinctiveness from sympatric congeners minimizes the likelihood that any observed genomic patterns simply reflect taxonomic uncertainty arising from overlapping morphological variation. Moreover, their broad distributions enabled assessment of species cohesion across a wider geographic range and under sympatry. By analysing two geographically separated sympatric species assemblages independently, we were able to assess whether patterns of species cohesion were consistently recovered across geographically independent assemblages or whether they instead reflect characteristics of a single local species complex. Our objectives were to (1) determine whether genome-wide genetic structure corresponds more closely to morphology-based species identity or geographic proximity, and (2) evaluate whether sympatric species exhibit genomic patterns consistent with admixture while maintaining distinct genetic identities. Our study provides a genome-wide SNP-based framework for assessing species boundaries in *Polylepis* and for evaluating patterns of genetic structure that may reflect reticulate evolutionary processes. Our study also highlights the importance of replicated geographic sampling for future phylogenomic studies in *Polylepis* and provides a framework for interpreting species boundaries in the context of conservation and restoration of Andean forests. More broadly, our study contributes to the ongoing evaluation of plant species concepts by demonstrating how genome-wide SNP data and replicated geographic sampling can be used to assess species cohesion in recently diverged lineages.

2. Materials and methods

2.1. Sampling

Polylepis incana and congeners were sampled at four localities in Ecuador (Carchi, Imbabura, Napo, and Azuay), whereas *P. neglecta* and congeners were collected at three localities in Bolivia (Acasio, Nuevo Mundo, and Cruz Qasa) (Fig. 2). *Polylepis incana* is distinguished from its co-occurring congeners by the presence of a single pair of lateral leaflets, whose abaxial surfaces are covered with very short white pannose hairs and resinous exudate (Boza-Espinoza & Kessler, 2022). *Polylepis neglecta* is distinguished from its congeners by its finely pinnate, light green leaflets and the long, pendant inflorescences with reddish, strongly winged fruits (Boza-Espinoza & Kessler, 2022). We sampled a total of 165 individuals representing 11 species. These comprised four co-occurring species from Bolivia (*P. neglecta, P. besseri* Hieron., *P. hieronymi* Pilg., *P. subtusalbida* (Bitter) M.Kessler & Schmidt-Leb.) and seven co-occurring species from Ecuador (*P. incana, P. lanuginosa* Kunth, *P. longipilosa* T.Boza, Romol. & M.Kessler, *P. ochreata* (Wedd.) Bitter, *P. pauta* Hieron., *P. reticulata* Hieron., *P. simpsoniae* T.Boza & M.Kessler).

Young leaflets were silica-dried for molecular analyses and voucher specimens were prepared for deposition in BOLV, HAL, QCA, and Z. Fig. 2 shows the collection localities and corresponding species, and the supplementary information in Table S1 provides all voucher information.

2.2. DNA extraction, ddRAD library preparation, and sequencing

Genomic DNA was extracted from 20 mg silica-dried material using NucleoSpin Plant II kit (Macherey-Nagel, Germany) following manufacturer's protocol, with the addition of 30 μL β-mercaptoethanol and 2% polyvinylpyrrolidone (PVP) to the lysis buffer. DNA concentration was quantified using a Qubit Fluorometer, and only samples with concentrations $\geq$ 15 ng/μL were retained. Samples below this threshold were either re-extracted or concentrated by vacuum centrifugation. Preparation of ddRAD libraries was carried out following the protocol of Peterson et al. (2012), using the restriction enzymes *Eco*RI and *Msp*I as detailed in Durka et al. (2025). Sequencing of the ddRAD libraries was performed by Biomarker Technologies (BMK) GmbH (Germany) on an Illumina Novaseq 6000 (PE150) with the addition of 10% phiX.

2.3. SNP genotyping

Sequence reads were de-multiplexed using *process_radtags* from the Stacks 2.53 pipeline (Rochette et al. 2019). The number of allowed mismatches when rescuing barcodes was set to 2 and checking was disabled if the RAD site is intact. Demultiplexed sequence data were deposited in the European Nucleotide Archive (PRJEB104402/ERP185696, sample accession numbers ERS28080667-ERS28080851, https://www.ebi.ac.uk/ena). Reads were assembled and SNPs were called using dDocent 2.9.4 (Puritz et al. 2014) following a *de novo* approach, after trimming the first three and five bases from the forward and reverse read with *cutadapt*. For the assembly, the minimum within-individual coverage required for a read to be retained was set to K1 = 5; the minimum number of individuals in which a read had to be present was set to K2 = 6; the clustering similarity threshold was set to 0.90; all other parameters were kept at their default values. Indels were removed using vcfallelicprimitives from the library vcflib v. 1.0.0 (Garrison et al. 2022) and vcftools v. 0.1.16. Only biallelic SNPs were retained, with a minimum allele count (mac) of 3, a minimum genotype read depth (minDP) of 10, a minimum mean sequence quality (minQ) of 30, and a maximum missingness across individuals (max_missing) of 50%. To

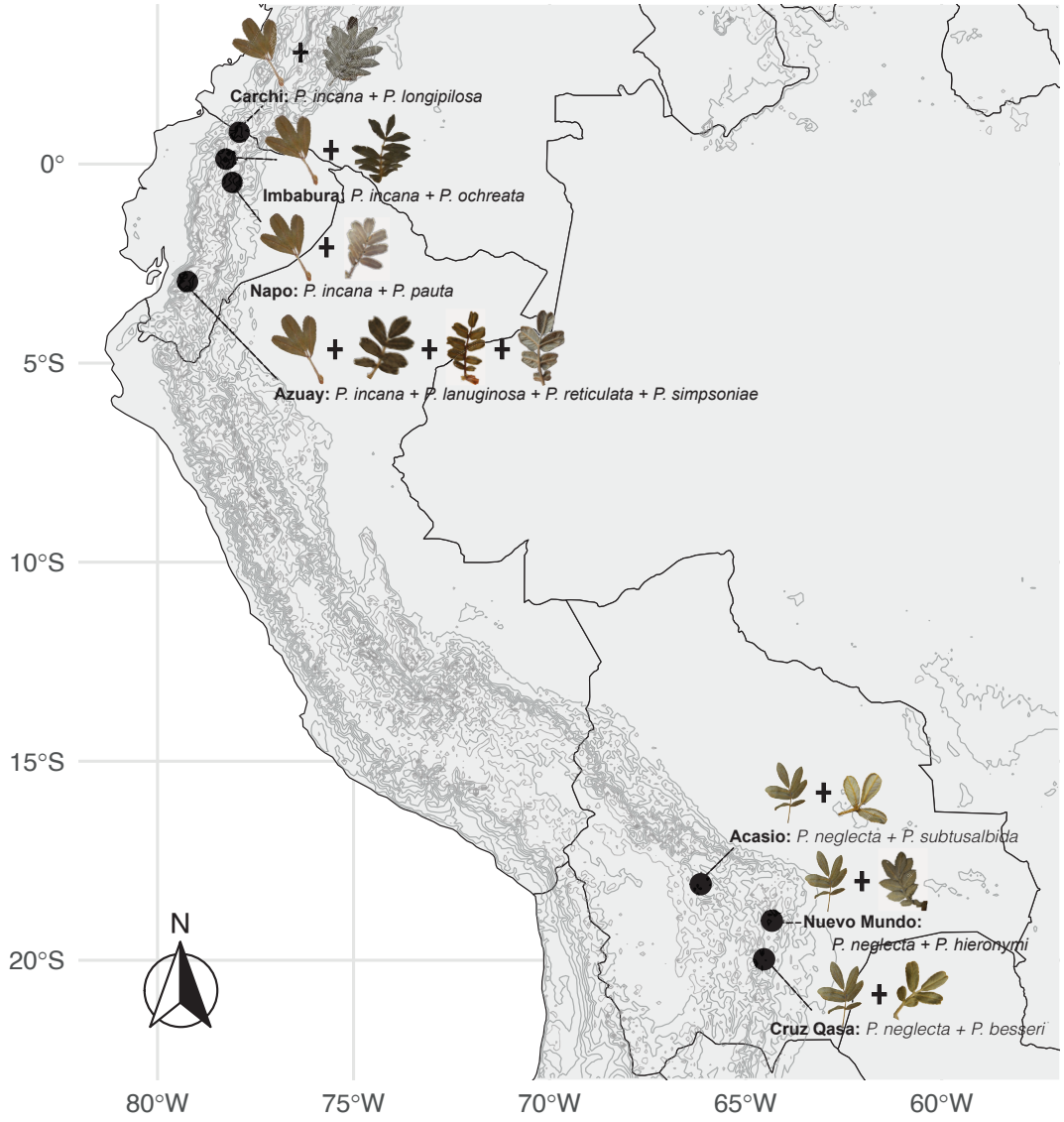

Fig. 2. Sampling design showing the distribution of study sites and species.

exclude paralogous SNPs, putatively fixed heterozygous SNPs were filtered by calculating the p-value for heterozygosity excess with vcftools and removing SNPs with P-HET_EXCESS < 1e-15. Individuals with >75% missing values were excluded. Subsequently, SNPs were filtered with vcffilter from vcflib 1.0.0 according to allele balance (AB > 0.2 & AB < 0.8 | AB < 0.01 | AB > 0.99), strandedness (SAF/SAR > 100 & SRF/SRR > 100 | SAR/SAF > 100 & SRR/SRF > 100), mapping quality ratio of the two alleles (MQM/MQMR > 0.9 & MQM/MQMR < 1.05), and proper pairing of alleles (PAIRED > 0.05 & PAIREDR > 0.05 & PAIREDR/PAIRED < 1.75 & PAIREDR/PAIRED > 0.25 | PAIRED < 0.05 & PAIREDR < 0.05). SNPs were further filtered to a maximum missingness of 34% (max_missing 0.66), a minimum minor allele frequency (maf) of 0.05, a minimum mean read depth (min-meanDP) of 10, and a maximum mean depth (max-meanDP) of 1000. A single SNP per contig was retained to reduce physical linkage among markers prior to downstream population genetic analyses, resulting in a final dataset of 15,154 SNPs. Bioinformatic analyses were run on the High-Performance Computing (HPC) Cluster EVE, a joint effort of the Helmholtz Centre for Environmental Research-UFZ (https://www.ufz.de/) and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig (https://www.idiv-biodiversity.de/).

2.4. Downstream population genetic analyses

Unless otherwise stated, downstream analyses were performed on R 4.5.2 (R Core Team, 2024). Principal Component Analysis (PCA) and Discriminant Analysis of Principal Components (DAPC) were performed using the package *adegenet* (Jombart, 2008; Jombart & Ahmed, 2011). Analysis of Molecular Variance (AMOVA) was performed using the package *pegas* (Paradis, 2010). To evaluate whether patterns of genetic structure align more closely with taxonomy or geography, we performed DAPC and AMOVA using species identity and collection locality as the grouping factor and compared their explanatory power. DAPC was performed separately for the Bolivian and Ecuadorean datasets, respectively, using species identity and collection locality as predefined grouping. Prior to DAPC, SNP matrices were converted to numeric matrices, and missing genotypes were replaced by the mean genotype value for each locus following the default implementation in *adegenet*. To reduce the risk of overfitting, the number of retained principal components (PCs) was selected using cross-validation with the *xvalDapc* function in *adegenet*. For each dataset and grouping factor, cross-validation was performed with a maximum 50 PCs (n.pca.max = 50) and 30 replicate runs (n.rep = 30), with centering enabled and scaling disabled. The number of PCs achieving the highest mean assignment

success was retained for the final DAPC (Bolivia: 5 PCs for species grouping and 10 PCs for locality grouping; Ecuador: 35 PCs for both species and locality grouping), thereby minimizing overfitting while maximizing assignment success. The number of discriminant functions was set to the maximum permitted by the number of predefined groups (n.da = number of groups – 1). To compare relative contributions of taxonomy and geography to genetic structure, AMOVAs were performed separately using species identity and collection locality as grouping factors. Because species composition differed among localities, these analyses were intended to compare the relative partitioning of genetic variance between alternative grouping schemes rather than to completely partition the independent effects of taxonomy and geography. Accordingly, the analyses were intended to compare alternative grouping schemes rather than to statistically partition the independent contributions of taxonomy and geography. Population structure was inferred using the maximum-likelihood clustering algorithm implemented in ADMIXTURE v1.3.0 (Alexander et al., 2009). Input files were generated by converting the filtered VCF file to PLINK binary format using PLINK (Purcell et al. 2007). ADMIXTURE analyses were performed separately for the Bolivian and Ecuadorean datasets for values of K = 1–10. For each value of K, 20 independent replicate runs were performed using different random initializations and 10-fold cross-validation. The optimal number of genetic clusters was determined from the mean cross-validation (CV) error across the 20 replicates for each value of K, with the model exhibiting the lowest mean CV error considered the best-supported solution. Consistency among replicate analyses was assessed using the R package *pophelper* (Francis, 2017), which was used to compare and align individual ancestry coefficient (Q) matrices across replicate runs to account for label switching. Because the SNP dataset had previously been filtered to retain a single SNP per contig, no additional linkage disequilibrium pruning was performed prior to ADMIXTURE analyses. All analyses were conducted using the default convergence criteria implemented in ADMIXTURE.

2.5. Phylogenetic analysis

Phylogenetic relationships were reconstructed using a Maximum Likelihood (ML) approach implemented in IQ-TREE version 2.4.0 (Minh et al., 2020). Analyses were conducted on the generated SNP-only dataset. ModelFinder Plus (Kalyaanamoorthy et al., 2017) was used to identify the best-fitting substitution model, applying ascertainment bias correction (+ASC, Lewis, 2001) to account for the absence of invariant sites. TVMe + ASC + R2 was selected as the best-fitting model under the Bayesian Information Criterion (BIC). Node support was assessed using 1000 ultrafast bootstrap (BS) replicates (UFB, Hoang et al., 2018). The resulting ML tree was visualized using Figtree v1.4.4 (Rambaut, 2018). Separate ML trees were inferred for the Bolivian and Ecuadorean datasets. Each tree was rooted using representatives from the alternate regional dataset to facilitate visualization.

2.6. Network analysis

A GENPOFAD distance matrix, an approach appropriate for quantifying genetic differentiation from SNP-only datasets, was calculated using the *pofadinr* package (Joly et al., 2015). The resulting distance matrix was then used to construct a phylogenetic network in SplitsTree App v6.4.17 (Huson & Bryant, 2024). A NeighborNet network (Bryant & Moulton 2004; Bryant & Huson 2023) was generated using default settings, producing 473 cyclic splits. The ShowSplits method was used to obtain a Split Network visualization. Unlike traditional bifurcating trees, phylogenetic networks can visualize non-tree-like evolutionary patterns that may arise through processes such as hybridization or incomplete lineage sorting and therefore provide a more informative framework for examining relationships among closely related species (Huson & Bryant, 2005).

2.7. Patterson's D-statistics

To evaluate whether excess allele sharing exceeded expectations under incomplete lineage sorting, Patterson's D-statistics (ABBA-BABA tests) were calculated using Dsuite v.0.5 (Malinsky et al., 2021). Analyses were performed separately for the Bolivian and Ecuadorean datasets. Species assignments were specified in a sets file corresponding to the morphology-based species delimitations adopted in this study, with samples from the alternate geographic region designated as the outgroup. A species-level guide tree derived from the maximum-likelihood phylogeny was provided to arrange trios according to the inferred phylogenetic relationships. Dsuite Dtrios was used to calculate Patterson's D-statistics, Z-scores, associated p-values, and f4-ratio estimates for all possible species trios. Because multiple trio comparisons were performed, the resulting p-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) procedure, and statistical significance was assessed using the FDR-adjusted p-values. Branch-specific excess allele sharing was subsequently evaluated using Dsuite Fbranch. Because branch-specific estimates depend on the accuracy of the guided tree, Fbranch results were interpreted only for relationships supported by the maximum-likelihood phylogeny. Exploratory analyses treating selected individuals (H_09 and S_08) as separate groups were additionally performed to assess whether individuals occupying intermediate positions in the previous analyses also exhibited high levels of allele sharing.

3. Results

3.1. SNP matrix

Data from ddRADseq analyses yielded a total of 1.4×10^9 paired-end reads, averaging 3.7×10^6 reads per individual. After filtering for base-call quality, indels, biallelic loci, sequencing depth, Hardy–Weinberg equilibrium, allele balance, and missingness, the final dataset comprised 149 individuals and contained 15,154 unlinked SNPs with an average missingness of 12%. Thirteen individuals of *P. incana* (one from Napo, seven from Imbabura, four from Carchi, and one from Azuay), as well as one individual each of *P. ochreata* and *P. simpsoniae,* were removed because they exceeded the maximum allowed missingness threshold of 50%. Although several *P. incana* individuals from Imbabura were excluded during quality filtering, the remaining samples retained enough genetic signal to assess population structure, particularly in comparison with the sympatric *P. ochreata*.

3.2. PCA, DAPC, and AMOVA results

We first conducted PCA using the entire dataset, grouping the individuals by region. The analysis revealed clear genetic differentiation between Bolivian and Ecuadorean species (Fig. S1). Consequently, we partitioned the dataset by region and conducted all subsequent analyses independently for each subset. The Bolivian dataset included 64 individuals from four species, whereas the Ecuadorean dataset included 85 individuals from seven other species. PCA of the two datasets (Fig. 3a,b) showed clear genetic clustering among species. In both analyses, the two widely distributed, morphologically distinct species (*P. incana* and *P. neglecta*, respectively) each formed discrete clusters, clearly separated from their respective sympatric taxa. In the Bolivian dataset (Fig. 3a), a single individual of each, *P. hieronymi* (H_09) and *P. subtusalbida* (S_08), plotted in an intermediate position, closer to *P. neglecta* than to their conspecific clusters. In the Ecuadorean dataset (Fig. 3b), the sympatric species *P. lanuginosa, P. reticulata*, and *P. simpsoniae* (collected from Azuay), as well as *P. ochreata* and *P. pauta* formed genetic clusters. Probabilities of DAPC assignments were 1.0 for all analyses except for the Bolivian dataset using collection locality as the grouping factor, which showed a slightly lower assignment probability of 0.98 (see Table S2). The DAPC scatterplots for both datasets, with species as the

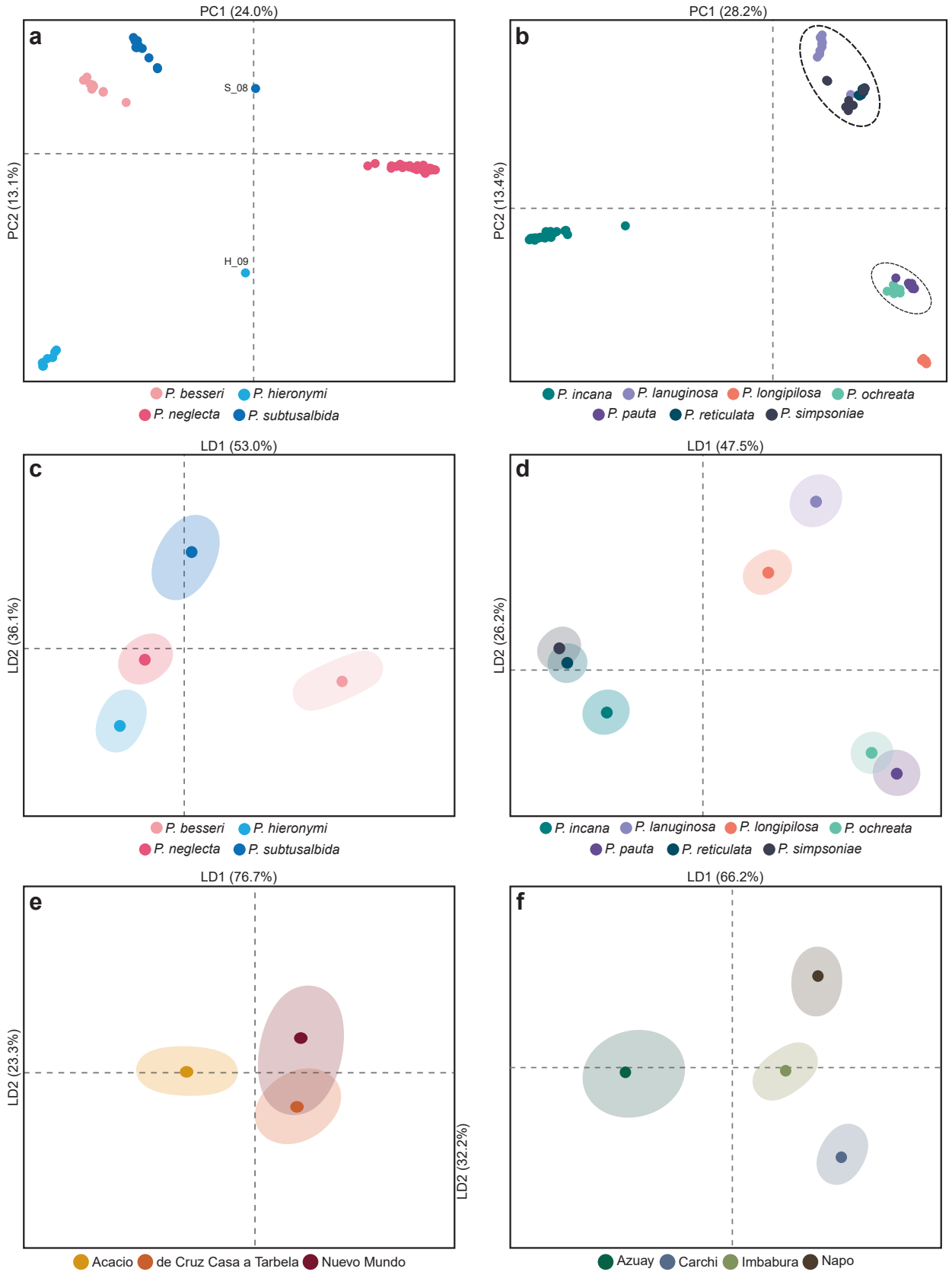

Fig. 3. PCA and DAPC results. Principal Component Analysis of individuals from (a) Bolivia and (b) Ecuador, colored by species. Discriminant Analysis of Principal Components scatterplots showing group centroids and dispersions for (c) Bolivia and (d) Ecuador using species as the grouping factor, and for (e) Bolivia and (f) Ecuador using locality as the grouping factor.

grouping factor (Fig. 3c,d), showed that morphologically defined species formed distinct genetic clusters. Some overlap in dispersion was observed between *P. reticulata* and *P. simpsoniae*, and between *P. ochreata* and *P. pauta* (Fig. 3d), which is expected since these species pairs belong to the same sections *sensu* Boza-Espinoza & Kessler (2022). *Polylepis reticulata* and *P. simpsoniae* are members of sect. *Reticulatae* T. Boza & M. Kessler, whereas *P. ochreata* and *P. pauta* belong to sect. *Sericeae* T. Boza & M. Kessler. The DAPC scatterplot for the Bolivian dataset with collection locality as the grouping factor (Fig. 3e) showed overlap between individuals collected from Cruz Qasa and Nuevo Mundo. This reflects the genetic cohesiveness of populations of the widely distributed, morphologically distinct *P. neglecta* across localities. In contrast, the DAPC scatterplot for the Ecuadorean dataset with collection locality as the grouping factor (Fig. 3f) showed that individuals collected from the same locality are genetically clustered.

Results from AMOVA revealed strong genetic structuring by species in both countries. In the Bolivian dataset, species identity explained a substantially larger proportion of genetic variation ($\Phi = 0.57$) than collection locality ($\Phi = 0.10$; Fig. 4). A similar pattern was observed in the Ecuadorean dataset, where species identity likewise accounted for most of the genetic variance ($\Phi = 0.72$), compared with that of locality ($\Phi = 0.17$; Fig. 4). These results demonstrate that species boundaries explain genetic differentiation across the sampled *Polylepis* populations, whereas geographic separation among localities contributes comparatively little to the overall genetic structure. See Table S3 for complete AMOVA results.

3.3. Phylogenetic and ADMIXTURE Analysis

Phylogenetic analyses of the SNP dataset and ADMIXTURE produced highly concordant patterns of genetic structure in both the Bolivian and Ecuadorean datasets (Figs. 5–6). In Bolivia, *P. neglecta* and *P. hieronymi* each formed strongly supported monophyletic clades (BS > 95%), with ADMIXTURE assigning individuals almost exclusively to their respective species clusters at the best-supported value of K (K = 3; Fig. S2). Within *P. hieronymi*, a single individual (H_09) exhibited partial ancestry shared with *P. neglecta*, corresponding to its intermediate position in the PCA (Fig. 3a). Similarly, one individual of *P. subtusalbida* (S_08) was recovered as sister to the *P. neglecta* clade in the ML tree and showed shared ancestry with *P. neglecta* in the ADMIXTURE analysis, consistent with its intermediate position in the PCA. In contrast, *P. besseri* and *P. subtusalbida* were not recovered as well-supported monophyletic groups. Individuals of *P. besseri* showed mixed ancestry shared with P. *hieronymi* and *P. subtusalbida*.

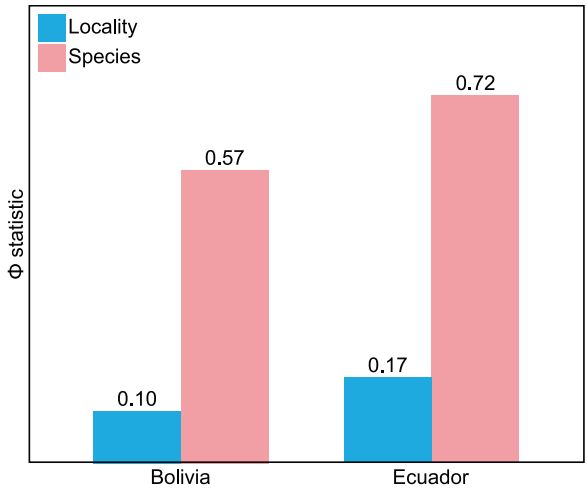

Fig. 4. Analysis of Molecular Variance (AMOVA) results showing phi (Φ) statistic among populations when grouped by locality versus by species of Bolivian and Ecuadorean datasets. All comparisons are statistically significant ($p < 0.001$).

The Ecuadorean dataset likewise exhibited stronger species-level genetic structure (Fig. 6). *Polylepis incana*, *P. lanuginosa*, *P. longipilosa*, and *P. pauta* each formed strongly supported clades (BS > 95%) and showed little or no evidence of admixture at the best-supported value of K (K = 6; Fig. S2). Populations of *P. incana* from Azuay formed a distinct ancestry component at K = 6 and K = 7 but remained genetically cohesive with the remainder of the species. In contrast, *P. ochreata* exhibited ancestry shared with *P. incana*, *P. longipilosa*, and *P. pauta*, with the largest ancestry component shared with *P. pauta*. Although *P. ochreata* formed a strongly supported clade together with *P. longipilosa* and *P. pauta*, its ancestry profile differed from that of the other species in this lineage. Finally, *P. reticulata* and *P. simpsoniae* formed closely related clusters in both the phylogenetic and ADMIXTURE analyses. At K = 7, two individuals of *P. simpsoniae* (Si_03 and Si_04) formed a distinct ancestry component relative to the remaining individuals of the species.

3.4. Network analysis

The NeighborNet network based on GENPOFAD distances recovered species-level clustering broadly consistent with the PCA, phylogenetic, and ADMIXTURE analyses (Fig. 7). The widespread species *P. incana* and *P. neglecta* each formed compact clusters with short internal branches and little reticulation, whereas the remaining species exhibited varying degrees of network complexity.

Within the Bolivian dataset, *P. besseri*, *P. hieronymi*, and *P. subtusalbida* formed recognizable but interconnected clusters. The strongest reticulation occurred around *P. besseri*, which was connected by multiple network edges to both *P. hieronymi* and *P. subtusalbida*. In addition, secondary connections from the *P. neglecta* cluster toward *P. hieronymi* and *P. subtusalbida* corresponded to the intermediate placement of individuals H_09 and S_08 observed in the PCA, phylogenetic, and ADMIXTURE analyses.

In the Ecuadorean dataset, *P. lanuginosa*, *P. longipilosa*, *P. ochreata*, and *P. pauta* formed closely related clusters connected by short internal edges. *Polylepis ochreata* and *P. pauta* showed the greatest degree of reticulation within this lineage, whereas *P. reticulata* and *P. simpsoniae* formed a closely associated cluster. Two individuals of *P. simpsoniae* (Si_03 and Si_04) were positioned outside the main cluster, corresponding to the distinct ancestry component identified in the ADMIXTURE analyses.

Overall, the NeighborNet analyses recovered reticulation patterns that were concordant with the phylogenetic and ADMIXTURE analyses, particularly for *P. besseri* in Bolivia and the *P. ochreata–P. pauta* and *P. reticulata–P. simpsoniae* lineages in Ecuador.

3.5. Patterson's D-statistics

To determine whether excess allele sharing among *Polylepis* species exceeded expectations under incomplete lineage sorting, Patterson's D-statistics were calculated (Figs. 5–6). Among the four possible Bolivian species trios, all remained significant following multiple-testing correction (Table S4). The strongest signal was recovered for the *P. subtusalbida–P. neglecta–P. hieronymi* trio (D = 0.233, Z = 15.91, FDR-adjusted $P < 0.001$), followed by *P. besseri–P. hieronymi–P. neglecta* (D = 0.096, Z = 5.90, FDR-adjusted $P < 0.001$), *P. hieronymi–P. besseri–P. subtusalbida* (D = 0.075, Z = 5.53, FDR-adjusted $P < 0.001$), and *P. subtusalbida–P. neglecta–P. besseri* (D = 0.034, Z = 2.50, FDR-adjusted P = 0.012). Estimated f4-ratios ranged from 0.06 to 0.36 (Table S4), indicating varying magnitudes of excess allele sharing among species.

Branch-based Fbranch analyses were concordant with Patterson's D-statistics, identifying the strongest branch-specific excess allele-sharing signal along the *P. besseri* lineage (fb = 0.267), followed by *P. hieronymi* (fb = 0.178), whereas little or no excess allele sharing was associated with the remaining branches (Table S4). Although the strongest individual Patterson's D-statistic involved the *P. subtusalbida–P. neglecta–P.*

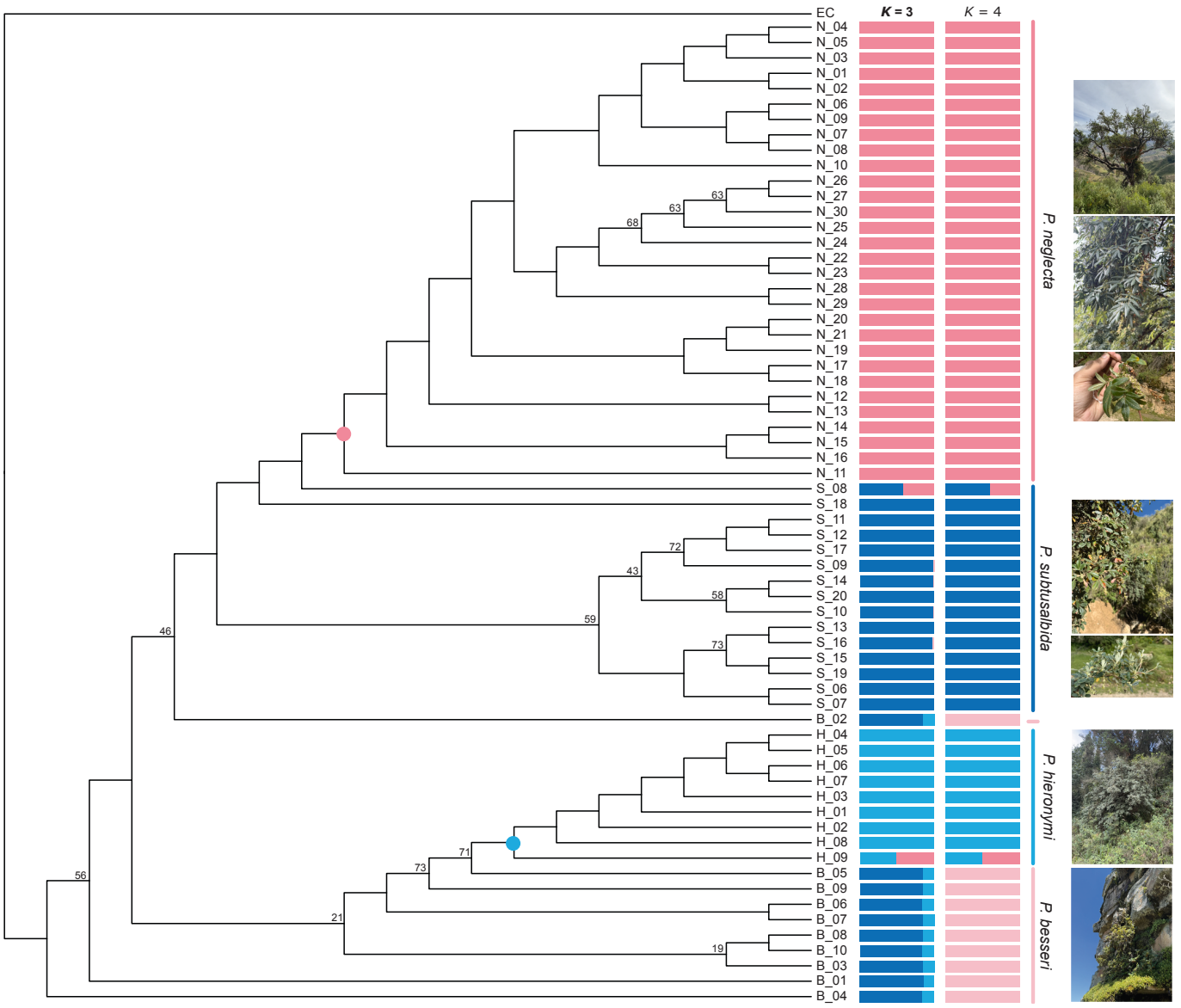

Fig. 5. Maximum-Likelihood (ML) phylogeny inferred from the SNP-only dataset for Bolivian *Polylepis* species. Numbers on nodes indicate bootstrap support (BS) values <75% while coloured nodes indicate strongly supported (BS > 95%) species clades. ML phylogeny is shown alongside ADMIXTURE ancestry proportions for the best-fitting *K* (bold) and *a priori K* values, corresponding to the number of localities and species. Photographs to the right show species in the wild, taken by JE Olivar & M Fernández.

hieronymi trio, the highest Fbranch value was recovered for *P. besseri* because Fbranch summarizes excess allele sharing across all trios associated with a branch rather than ranking individual species trios. This pattern is consistent with the extensive reticulation involving *P. besseri* observed in the NeighborNet network (Fig. 7) and its mixed ancestry recovered by ADMIXTURE (Fig. 5).

Because individuals H_09 and S_08 consistently occupied intermediate positions in the PCA and NeighborNet analyses and exhibited the strongest admixture signals in ADMIXTURE, these individuals were further evaluated using exploratory Patterson's D-statistics by treating each as an independent group. H_09 exhibited consistently elevated D-statistics across multiple comparisons, with the strongest signal recovered for the *P. hieronymi*–H_09–*P. neglecta* comparison (D = 0.673, Z = 43.29, f4-ratio = 0.525), whereas S_08 showed more moderate but consistently significant excess allele-sharing signals, with a maximum D-statistic of 0.270 (Z = 19.32, f4-ratio = 0.366) (Table S4). These exploratory analyses were concordant with the independent evidence from the PCA, ADMIXTURE, phylogenetic, and NeighborNet analyses, all of which identified H_09 and S_08 as the most genetically intermediate individuals within the Bolivian dataset.

The Ecuadorean dataset comprised 35 possible species trios, of which 30 remained significant (Table S5). The strongest signal was recovered for the *P. pauta–P. ochreata–P. incana* trio (D = 0.172, Z = 12.02, FDR-adjusted P < 0.001), followed by *P. longipilosa–P. ochreata–P. incana* (D = 0.160, Z = 8.26, FDR-adjusted P < 0.001). Significant excess allele sharing was detected across numerous species combinations, particularly involving *P. ochreata, P. incana,* and the *P. reticulata–P. simpsoniae* lineage, whereas only five comparisons were not significant. Estimated f4-ratios were generally low to moderate (Table S5), although one comparison (*P. pauta–P. longipilosa–P. ochreata*) reached the upper boundary of the estimator (f4-ratio = 1.0) and was interpreted cautiously.

Branch-based Fbranch analyses were consistent with the species-level Patterson's D-statistics and identified branch-specific excess allele sharing concentrated along the *P. ochreata* branch, the *P. reticulata* branch, and the ancestral branch uniting *P. longipilosa* and *P. pauta*

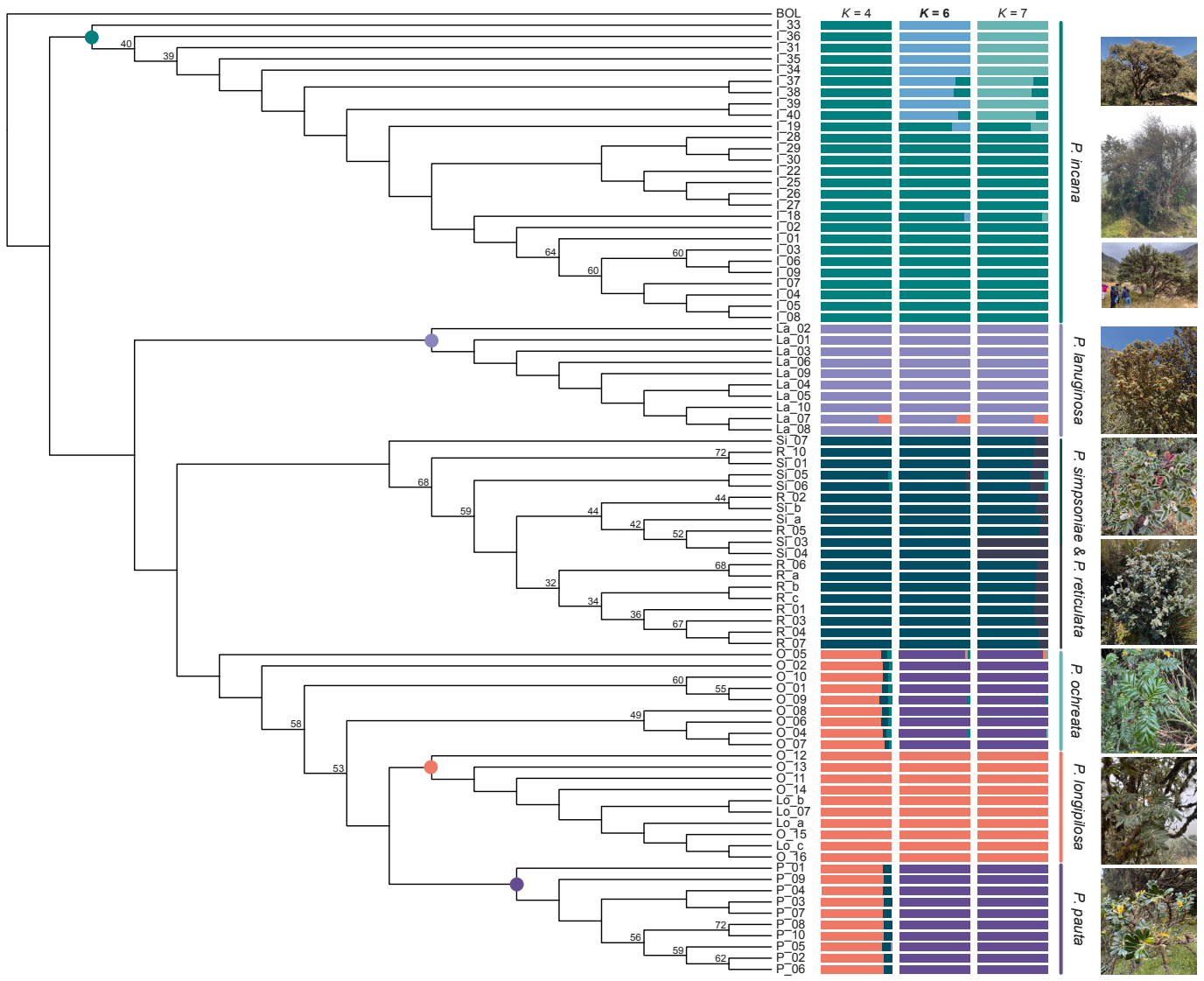

Fig. 6. Maximum-likelihood (ML) phylogeny inferred from the SNP-only dataset for Ecuadorean *Polylepis* species. Numbers on nodes indicate bootstrap support (BS) values <75% while coloured nodes indicate strongly supported (BS > 95%) species clades. ML phylogeny is shown alongside ADMIXTURE ancestry proportions for the best-fitting *K* (bold) and *a priori K* values, corresponding to the number of localities and species. Photographs to the right show species in the wild, taken by C Segovia-Salcedo, JE Olivar, & S Acosta-Villamarín.

(Table S5). Together, the Patterson's D-statistics and Fbranch analyses indicate that excess allele sharing is widespread across the Ecuadorean assemblage rather than being restricted to a small number of species pairs, consistent with the reticulation patterns recovered in the NeighborNet network (Fig. 7) and the shared ancestry identified by ADMIXTURE (Fig. 6).

4. Discussion

This study provides a genome-wide SNP-based test of species boundaries in two geographically independent *Polylepis* assemblages from Bolivia and Ecuador. Across the 11 sampled species, genetic variation was structured primarily by morphology-based species identity rather than by collection locality, and the widely distributed focal species *P. neglecta* and *P. incana* each remained genetically cohesive across multiple localities and in sympatry with congeners. PCA, DAPC, ADMIXTURE, individual-level SNP phylogenies, NeighborNet, and Patterson's D-statistics converged on a common pattern: species boundaries were largely maintained despite significant excess allele sharing among several sympatric taxa. This differs from earlier studies based on Sanger markers, AFLPs, and Hyb-Seq data, which recovered strong geographic structure and limited correspondence with morphology-based species delimitations (Kerr, 2004; Schmidt-Lebuhn et al., 2006; Segovia-Salcedo, 2014). The greater correspondence recovered here likely reflects the combination of genome-wide SNP sampling and replicated sampling of widespread species across multiple localities. Our results therefore support the species circumscriptions of Boza-Espinoza and Kessler (2022) for the sampled Bolivian and Ecuadorean taxa, while also showing that species cohesion can persist in the presence of localized introgression.

4.1. *Multiple geographic sampling reveals robust species boundaries despite localized introgression*

Accurate inference of species boundaries requires sampling that captures genetic variation across the geographic distribution of species. Restricted geographic sampling can confound population structure with species boundaries, particularly in recently diverged lineages where incomplete lineage sorting and gene flow may produce conflicting phylogenetic signals (Degnan & Rosenberg, 2006, 2009). Consequently,

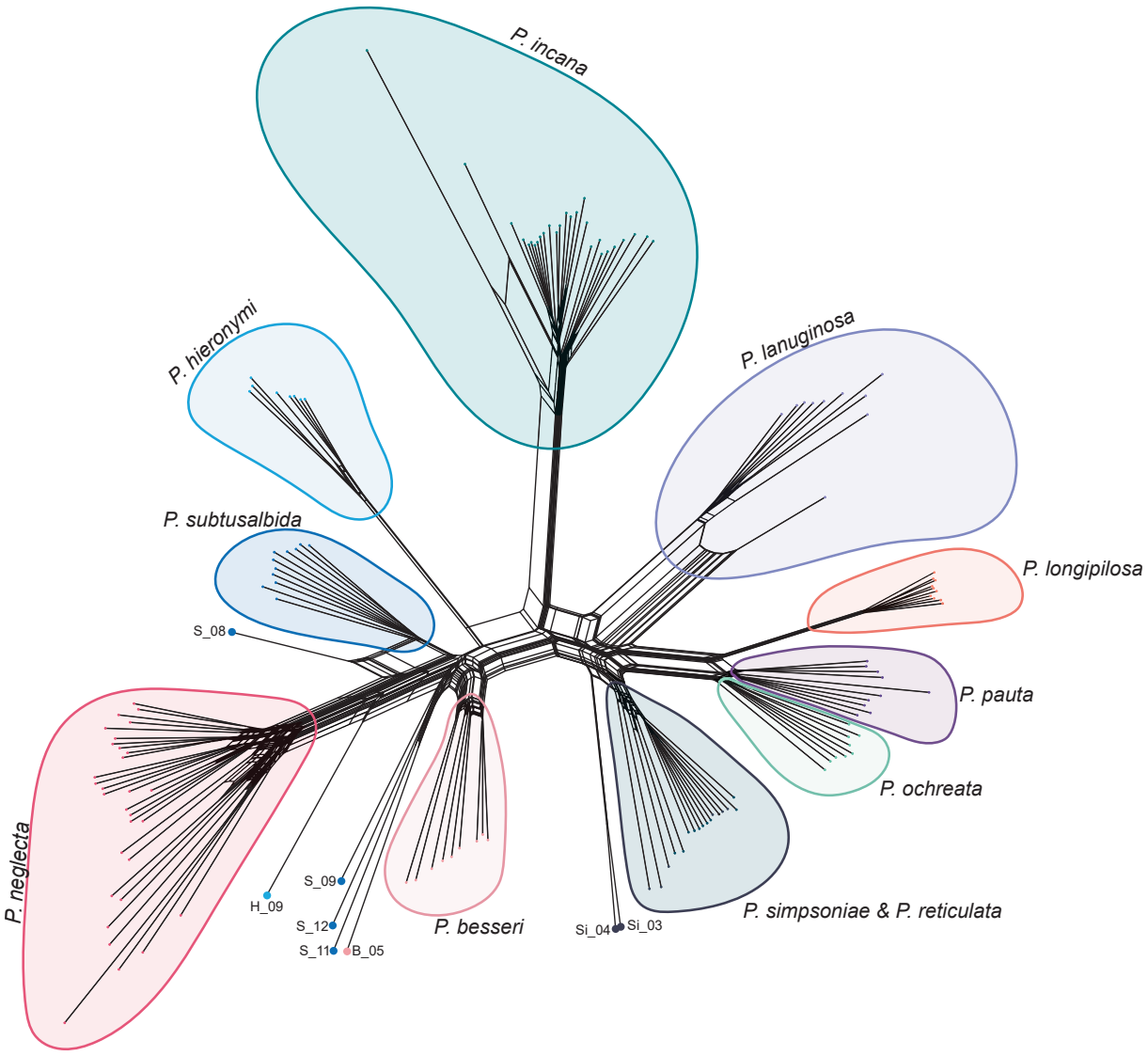

Fig. 7. NeighborNet network constructed with SplitsTree using GENPOFAD distances calculated from the SNP-only dataset. Tip colours indicate species assignment and recognizable species clusters are grouped accordingly.

phylogenetic analyses based on limited sampling or a small number of loci may underestimate species cohesion or overemphasize geographic differentiation. Integrating genome-wide SNP datasets with geographically replicated sampling provides a more comprehensive framework for distinguishing species-level divergence from localized population-level processes.

Our sampling strategy enabled us to evaluate species cohesion across broad portions of the geographic distributions of the two widespread species, *P. neglecta* in Bolivia and *P. incana* in Ecuador, while simultaneously examining closely related sympatric congeners. In both regional assemblages, the widespread species formed genetically cohesive groups across multiple localities. In contrast, excess allele sharing was concentrated among closely related sympatric taxa, including the *P. besseri–P. hieronymi–P. subtusalbida* complex in Bolivia and the *P. ochreata–P. pauta* and *P. reticulata–P. simpsoniae* lineages in Ecuador. Patterson's D-statistics demonstrated that these signals significantly differ from expectations under incomplete lineage sorting for many species combinations, confirming that localized excess allele sharing occurs within both regional assemblages.

At first glance, the strong species differentiation recovered by the AMOVA appears inconsistent with the widespread excess allele sharing detected by Patterson's D-statistics. However, these analyses quantify different aspects of genomic variation. AMOVA measures the overall partitioning of genomic diversity among predefined groups and showed that species identity explained substantially more genetic variation than geography in both Bolivia and Ecuador. In contrast, Patterson's D-statistics detect localized deviations from a strictly bifurcating evolutionary history, identifying species pairs that have exchanged alleles despite remaining genetically distinct across most of their genomes. The concordance between these analyses demonstrates that localized introgression and strong genome-wide species cohesion are not mutually exclusive.

These findings differ from earlier molecular studies based on Sanger sequencing (Kerr, 2004), AFLPs (Schmidt-Lebuhn et al., 2006), and HybSeq data (Segovia-Salcedo, 2014), which concluded that geographic structure equalled or exceeded species-level differentiation. We suggest that this difference primarily reflects the combination of genome-wide SNP sampling and geographically replicated sampling employed here, which allowed widespread species to be evaluated across multiple localities while simultaneously testing relationships among sympatric congeners. For the eleven species examined, our results support the species circumscriptions proposed by Boza-Espinoza and Kessler (2022) and demonstrate that species boundaries can remain robust despite localized introgression among closely related taxa.

4.2. Reticulate evolutionary histories and the maintenance of species boundaries

Although the sampled *Polylepis* species generally formed genetically cohesive groups, several closely related lineages exhibited localized excess allele sharing. In Bolivia, this pattern was concentrated within the *P. besseri–P. hieronymi–P. subtusalbida* complex, whereas in Ecuador it primarily involved the *P. ochreata–P. pauta* and *P. reticulata–P. simpsoniae* lineages. These signals were recovered across several analyses: ADMIXTURE revealed shared ancestry, NeighborNet showed non-tree-like relationships, and Patterson's D-statistics identified significant excess allele sharing inconsistent with a simple model of incomplete lineage sorting. Despite these reticulate patterns, the sampled species retained clear genomic differentiation, indicating that allele exchange has not erased species boundaries within either regional assemblage.

The Bolivian assemblage provides a particularly clear example. *Polylepis neglecta* remained genetically cohesive across the sampled localities, yet significant Patterson's D-statistics revealed excess allele sharing with sympatric congeners. The strongest species-level D-statistic involved the *P. subtusalbida–P. neglecta–P. hieronymi* trio, whereas the highest branch-specific Fbranch estimate was associated with *P. besseri*. These results are not contradictory because Patterson's D-statistics quantify excess allele sharing within individual species trios, while Fbranch summarizes branch-specific signals across all trios involving a lineage. The elevated Fbranch value for *P. besseri* therefore reflects its repeated involvement in allele-sharing patterns, consistent with its mixed ancestry in ADMIXTURE and its highly reticulated position in the NeighborNet network.

Additional evidence for localized introgression comes from the concordant placement of H_09 and S_08 across analyses. Both individuals occupied intermediate positions in the PCA, exhibited shared ancestry with *P. neglecta* in ADMIXTURE, showed atypical positions in the individual-level SNP phylogeny and NeighborNet network, and produced strongly elevated individual-level Patterson's D-statistics. Because these targeted D-statistic analyses treated single individuals as separate groups, they should be interpreted cautiously and not as formal estimates of hybrid class. Nevertheless, the agreement among methods makes recent or inherited introgressed ancestry involving *P. neglecta* a plausible explanation for these individuals.

The Ecuadorean assemblage showed a similar overall pattern. *Polylepis incana* remained genetically cohesive across the sampled localities despite significant excess allele sharing among several sympatric congeners. The strongest Patterson's D-statistics involved *P. ochreata*, which also showed shared ancestry with *P. incana, P. longipilosa,* and *P. pauta* in ADMIXTURE. Fbranch analyses further localized excess allele sharing to the *P. ochreata* and *P. reticulata* branches and to the ancestral branch uniting *P. longipilosa* and *P. pauta*. The close relationships between *P. reticulata* and *P. simpsoniae*, and between *P. lanuginosa* and *P. longipilosa*, were likewise recovered across the SNP phylogeny, ADMIXTURE, NeighborNet, and D-statistic analyses. Together, the Bolivian and Ecuadorean datasets indicate that excess allele sharing is concentrated among sympatric lineages rather than occurring uniformly across all sampled species.

The repeated signals of reticulation observed in *P. besseri* and *P. ochreata* make these taxa promising candidates for investigating the evolutionary consequences of hybridization. These hypotheses could be explicitly tested in known contact zones where hybrid swarms have previously been reported, particularly in Mojón, Cochabamba, Bolivia, and at Lake Mojanda, Ecuador (Kessler, 1995b; Romoleroux, 1996). Applying the analytical framework developed here, together with reference-based genomic analyses, local ancestry inference, demographic modelling, and ploidy analyses, would allow the parental contributions to putative hybrids to be quantified and provide a rigorous test of whether these taxa represent cases of hybridogenic speciation or repeated introgression.

The close relationship between *P. lanuginosa* and *P. longipilosa* is also biologically noteworthy. These species occupy contrasting ecological settings, with *P. lanuginosa* occurring in relatively warm habitats of central and southern Ecuador, whereas *P. longipilosa* is restricted to humid páramos of northwestern Ecuador (Boza-Espinoza & Kessler, 2022). Their consistent genetic affinity across the phylogenetic, ADMIXTURE, NeighborNet, and Patterson's D-statistic analyses, together with their placement in section Sericeae, is consistent with a shared evolutionary history despite subsequent ecological differentiation. Simpson (1986) and Boza-Espinoza and Kessler (2022) further suggested that such localized species, together with *P. multijuga* Bitter from northern Peru, retain some of the most ancestral morphological characteristics within the genus. Although our data cannot directly test this hypothesis, the genomic similarity observed here is compatible with a close evolutionary relationship among these taxa.

A similar pattern was observed for *P. reticulata* and *P. simpsoniae*, both members of section Reticulatae. Despite their close genetic affinity, these species remain morphologically and ecologically distinguishable (Boza-Espinoza & Kessler, 2022). Both occur on relatively dry Andean slopes, but where they are sympatric, *P. simpsoniae* typically occupies lower elevations (approximately 3300–3700 m a.s.l.), whereas *P. reticulata* is generally found at higher elevations (approximately 3800–4000 m a.s.l.; Montalvo et al., 2018). The genomic cohesion observed within each species, despite evidence of excess allele sharing, suggests that ecological differentiation may contribute to maintaining species boundaries even among closely related sympatric lineages. Additional sampling across their contact zones would help determine whether the distinct ancestry observed in individuals Si_03 and Si_04 reflects localized introgression or previously unrecognized population structure.

The persistence of discrete genomic clusters despite significant excess allele sharing is consistent with models of semi-permeable species boundaries, in which some genomic regions remain permeable to gene flow while loci contributing to reproductive isolation resist introgression (Wu, 2001; Harrison & Larson, 2014; Chen et al., 2025). Our ddRADseq data cannot identify which genomic regions are introgressed or determine whether the observed signals reflect recent hybridization, repeated historical introgression, or ancestral population structure. Whole-genome data and explicit demographic analyses will therefore be necessary to distinguish among these alternatives. Nonetheless, the replicated patterns in Bolivia and Ecuador show that detectable allele exchange can coexist with strong genome-wide species cohesion in the sampled Polylepis assemblages.

4.3. Future directions for phylogenomics and diversification in Polylepis

Our results establish that *Polylepis* is a valuable system for investigating how species boundaries are maintained despite localized introgression in the high Andes. Although genome-wide ddRADseq data resolved relationships among the sampled species and identified lineage-specific patterns of excess allele sharing, they capture only a fraction of the genome and cannot determine the genomic distribution, timing, or direction of introgression. Future studies integrating whole-genome resequencing, plastome data, and explicit demographic modelling will allow introgressed genomic regions to be identified, distinguish recent introgression from ancestral population structure, and quantify the extent to which genomic barriers to gene flow differ among lineages.

Extending this framework across the genus would provide an important opportunity to investigate how hybridization, geography, and ecological differentiation have interacted throughout the evolutionary history of *Polylepis*. A densely sampled phylogenomic dataset could test whether the lineage-specific patterns observed here are representative of the genus or are restricted to the Bolivian and Ecuadorean assemblages examined in this study. Such analyses would also provide a robust framework for evaluating hypotheses of hybridogenic speciation in taxa such as *P. besseri* and *P. ochreata*, while identifying genomic regions

associated with reproductive isolation and ecological adaptation.

Because *Polylepis* is restricted to the high Andes, it provides an exceptional system for investigating the origin and diversification of Andean montane floras. Three non-exclusive hypotheses have been proposed to explain diversification in Andean plant lineages: (1) north-to-south colonization from Central or North America (Simpson, 1975; von Hagen & Kadereit, 2001; Hughes & Eastwood, 2006; Muellner et al., 2010; Sklenář et al., 2011; Koecke et al., 2013; Bacon et al., 2018; Muellner-Riehl & Rojas-Andrés, 2022); (2) south-to-north expansion following the development of high-elevation habitats during Andean uplift (Ezcurra, 2002; Jabaily & Sytsma, 2013; Lagomarsino et al., 2016; Sanín et al., 2016; Bacon et al., 2018; Tribble et al., 2024); and (3) in situ diversification associated with increasing topographic complexity and the formation of new dispersal barriers during Andean uplift (Ribas et al., 2007; Ceccarelli et al., 2016; Vasconcelos et al., 2020). A robust dated phylogeny incorporating genome-scale data and comprehensive geographic sampling would provide a powerful framework for evaluating these alternative scenarios while simultaneously placing patterns of introgression and species cohesion into a temporal context.

A well-resolved phylogenomic framework would also facilitate investigations of adaptive evolution in one of the world's highest woody plant genera. *Polylepis* species persist under extreme environmental conditions, including frequent sub-zero temperatures, large diurnal temperature fluctuations, intense ultraviolet radiation, and seasonal drought. Understanding how these environmental pressures have shaped genomic divergence and adaptive evolution would provide new insights into the mechanisms underlying diversification in high-elevation ecosystems. Such knowledge will also have practical implications for conservation by identifying evolutionarily significant lineages, informing species-specific restoration and seed sourcing, and improving management of naturally occurring hybrid zones that may contribute to the long-term adaptive potential of Andean *Polylepis* forests under ongoing environmental change.

CRediT authorship contribution statement

Jay Edneil C. Olivar: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Marcus Lehnert:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Walter Durka:** Writing – review & editing, Validation, Software, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Stefan Michalski:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Christiane M. Ritz:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Jana Ebersbach:** Writing – review & editing, Funding acquisition. **Alexandra N. Muellner-Riehl:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Claudia Segovia-Salcedo:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Sofía Acosta-Villamarín:** Writing – review & editing, Methodology, Investigation. **Katya Romoleroux:** Writing – review & editing, Methodology, Investigation. **Erika Priscilla Muriel Mera:** Writing – review & editing, Resources, Methodology, Investigation. **Emilio J. Trujillo:** Writing – review & editing, Methodology, Investigation, Data curation. **Edgar E. Gareca:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Milton Fernández:** Writing – review & editing, Methodology, Investigation. **Magaly Mercado:** Writing – review & editing, Methodology, Investigation, Data curation. **Adriana Jimenez Rojas:** Writing – review & editing, Methodology, Investigation, Data curation. **Susana Arrazola:** Writing – review & editing, Methodology, Investigation, Data curation. **Isabell Hensen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Michael Kessler:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2026.108722.

Data availability

Demultiplexed individual raw sequence data are available under sample accession numbers ERS28080667–ERS28080851 and run accession numbers ERR15964296-ERR15964480 under European Nucleotide Archive Project ID PRJEB104402. Input files and scripts for all analyses can be found on Zenodo (https://doi.org/10.5281/zenodo.17945289).

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