TITLE: UPSLOPE DIVERSIFICATION AND GENE FLOW AMONG CONGENERIC ANDEAN TREES

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SUMMARY

- The exceptional biodiversity of tropical Andean forests is characterized by rapid turnover of many closely related species across elevation and other environmental gradients. The eco-evolutionary relationships among these congeneric species are often poorly understood—specifically, how they diversify, sort into their elevational niches, and the extent to which species boundaries are maintained through time.
- In this study, we examined the phylogenetic relationships and patterns of ancient and ongoing gene flow among sympatric *Prunus* species occurring along elevation gradients in Peru and Bolivia to explore the evolutionary processes underlying this turnover.
- Phylogenetic relationships reconstructed a pattern of upslope adaptive diversification concurrent with Andean orogeny during the Miocene. Despite deep divergence times, species show evidence of widespread and persistent gene flow, with little constraint imposed by ecological or evolutionary distance.
- Together, these findings show that during adaptive radiations, observable ecological and morphological differences may emerge despite ongoing gene flow and long before reproductive isolation is complete.

Keywords: Andes, biodiversity, diversification, elevation gradient, gene flow, hybridization, tropical trees

INTRODUCTION

Clarifying how species diversify and assemble into communities, and the degree to which species boundaries persist through time is essential for understanding the origin and maintenance of tropical tree biodiversity and its conservation. Coexistence theories of ecology generally assume species are discrete evolutionary units (e.g. Hubbell 2000, Chesson 2000), though interspecific gene flow is known to have been common throughout the evolutionary history of plants (reviewed by Stull et al. 2023). No system lends itself to tests of these theories better than hyper-diverse tropical forests. Until recently, hybridization was considered an extremely rare occurrence in tropical trees, as hybrids were thought to be poor competitors in high-diversity systems and few individuals with intermediate morphologies have been identified (e.g. Ashton 1969; Ehrendorfer 1970; Gentry 1982). Mounting genomic evidence suggests hybridization has been widespread throughout the diversification of the Neotropical flora (reviewed by Schley et al. 2022) and may be an important evolutionary mechanism for maintaining high tree diversity with low population densities (Cannon and Lerdau 2015; Quinlan et al. 2025). These findings collectively point to the importance of reevaluating the impact of hybridization in our understanding of species diversity and coexistence theories in tropical ecosystems.

The tendency for inter-specific gene flow and hybridization to occur amongst closely related, sympatric trees has been widely documented in temperate zones (De La Torre et al. 2014, Tsuda et al. 2017, Cavender-Bares et al. 2018, Chhatre et al. 2018, Menon et al. 2018), yet species cohesion and morphological similarity is often maintained (Grant 1971; Gailing and Curtu 2014; Cannon and Petit 2019). This may be partially due to assortative mating and variable mating behaviors (selfing, conspecific outcrossing, and interspecific gene flow), determined by population densities and pollinator abundance (Hamrick and Murawski 1990; Ward et al. 2005;

Cannon and Lerdau 2015). When present, conspecific pollen can outcompete heterospecific pollen, reinforcing assortative mating (Williams et al. 1999). However, incomplete reproductive isolation and the ability to accept heterospecific pollen may become adaptively beneficial when conspecific pollen is rare, as the benefit of avoiding local extinction overrides potential negative consequences of hybridization such as reduced hybrid fitness (Barton 2001; Wu 2001; Cannon and Lerdau 2015). Hybridization may be particularly beneficial in times of rapid environmental change, as it can produce novel gene combinations within a single generation, increasing adaptive potential and population resilience (Anderson and Stebbins 1954; Baskett and Gomulkiewicz 2011; Suarez-Gonzalez 2018; Brauer et al. 2023; Kulmuni et al. 2024). Such density-dependent mating has been reported in oaks (Lepais et al. 2009) and may be particularly relevant to tropical trees which maintain extremely low local population densities (Pires et al. 1953; Pitman et al. 1999; ter Steege et al. 2013).

Most tropical forest trees are highly outcrossed (Bawa et al. 1985a, b; Hamrick and Murawski 1990; Ward et al. 2005), though individuals retain variable capacities for different mating behaviors, even within populations and across flowering events (Murawski and Hamrick 1991; Nason and Hamrick 1997). Self-fertilization has been shown to be inversely correlated with population density (Hamrick and Murawski 1990; Ward et al. 2005), but few studies have assessed the extent of inter-specific gene flow. Those that have (e.g. Schley et al. 2020 – *Brownea*, Larson et al. 2021 – *Eschweilera,* Schley et al. 2025 – *Inga*), detected widespread admixture. Evolutionary distance may be one important constraint on interspecific gene flow among congeners in sympatry, as hybridization is thought to decrease with increasing divergence due to the accumulation of pre- and postzygotic barriers through time (Mendelson et al. 2004; Levin 2012; Comeault and Matute 2018). Linan et al. (2021) found evidence of this in the

Mascarene Island *Diospyros* clade, suggesting the processes by which species diversify and assemble into communities may have lasting effects on their eco-evolutionary dynamics. For example, adaptive radiations across gradients typically result in stepwise diversification patterns with sister taxa occurring closest in niche proximity (Simpson 1953; Schluter 2000; Stroud and Losos 2016), whereas communities assembled predominantly via the immigration and sorting of pre-adapted clades demonstrate greater phylogenetic distance among co-occurring taxa (Donoghue 2008; Linan et al. 2021).

In tropical montane forests, genera commonly comprise many rare species that turn over rapidly with elevation (Terborgh 1971; Gentry 1982). Turnover in the abiotic environment and biotic interactions leads to narrow, shoestring-like distributions and the packing of many closely related species across elevation gradients (e.g. Janzen 1967, Jankowski et el. 2009, Hillyer and Silman 2010, Freeman et al. 2022). In the tropical Andes, this is consistent across taxa and is especially true for forest trees (e.g. Terborgh 1977, Meier et al. 2010, Jankowski et al. 2012). While many tree species have elevation ranges of < 500 m, the distributions of entire genera are often much broader, with congeneric species occurring in partial sympatry, especially at range margins (Feeley et al. 2011; Griffiths et al. 2020). At the genus level, immigration and sorting of pre-adapted clades has been shown to be the primary mechanism structuring tree diversity across elevation gradients in the Andes (Griffiths et al. 2020; Segovia et al. 2020; Linan et al. 2021). However, many examples of adaptive diversification have been found within individual plant clades (e.g. Nŭrk et al. 2013, Lagomarsino et al. 2016, Nevado et al. 2016, among many others). Still, few studies have explored how congeners within individual tree clades have diversified and assembled into their elevation niches, and characterizing these evolutionary relationships is essential for understanding potential gene flow and its ecological implications.

Prunus L. (Rosaceae) is one such genus that exhibits high congeneric turnover with elevation in the Andes (Figure 1). Globally, it has diversified into a range of environments, and though typically considered a temperate group (Wen et al. 2008; Chin et al. 2014), a recent revision found its highest diversity occurs in the tropics and primarily the Neotropics (Pérez-Zabala 2022). *Prunus* is one of the only tree genera with species spanning the entire Andes-Amazon gradient, from treeline (~3800 m) into the lowlands (<500 m), though individual species have more narrow ranges, typically 500–1000 m or less (e.g. Achá 2013). It is a keystone clade, whose fruits are important in the diets of frugivorous birds, primates, Andean bears, and humans (Achá 2013). The genus also contains many globally important crop species (peach, plum, almond, cherries) and others used as ornamentals, timber, and medicine (Andro and Riffaud 1995; Bortiri et al. 2001; Lee and Wen 2001; Wen et al. 2008).

The genus *Prunus* is divided into three major clades that correspond to inflorescence type: solitary, corymbose, and racemose (Rehder 1940, Su et al. 2023). All species endemic to the Americas and tropical regions are racemose (Perez-Zabala 2022). Though historically understudied, the exclusively South American species form a strongly supported clade nested within the tropical Racemose group (Hodel et al. 2021, 2023). Studies have placed the origin of the genus in either North America (Chin et al. 2014) or East Asia (Hodel et al. 2023) during the Paleocene (ca. 68–55 Ma), with the three major clades diverging by the early-mid Eocene as part of the Boreotropical flora (Chin et al. 2014; Su et al. 2023; Hodel et al. 2023). Divergence in the tropical Racemose group occurred in several regions including Asia, Africa, North America, and Europe ca. 55–40 Ma (Hodel et al. 2023). Chin et al. (2014) hypothesized migration into South America occurred via the dispersal of a North American relative ca. 34 Ma through Central

America and the Caribbean. Hodel et al. (2023) found evidence of a long period of stasis in the Neotropical lineages followed by rapid diversification during the Miocene.

While most species in the solitary-flower and corymbose groups are diploids, the racemose lineage is known for higher ploidy levels across temperate and tropical species and the lineage is thought to be the result of a single or multiple ancient hybridization events during the Eocene (Chin et al. 2104, Zhao et al. 2016, Su et al. 2023, Hodel et al. 2023). Domesticated *Prunus* (particularly plums) readily hybridize in agricultural settings (Cici and Acker 2010), resulting in the creation of some crops (e.g. *P. armeniaca* L. × *P. cerasifera* Ehrh. – 'black apricots,' *P. salicina* × *P. armeniaca* Lindl. – plumcots, pluots, and apriums; Guerrero et al. 2022), as well as among cultivated varieties and wild conspecifics (*P. yedoensis* - Baek et al. 2018). Species in this genus are highly outcrossing and typically self-incompatible with generalist insect pollinators (Cici and Acker 2010), though we are unaware of any study that has specifically looked at either breeding systems or pollination in Neotropical *Prunus*.

In this study, we investigate the patterns, timing, and extent of divergence among partially sympatric tree species which turn over across elevation gradients in the tropical Andes, and the degree to which species boundaries are maintained through time. Specifically, we ask: (1) how and when co-occurring species diversified into their elevational niches, (2) whether species are genetically isolated, and, if not, (3) is introgressive gene flow related to evolutionary or ecological distance. We reconstructed the phylogenetic relationships among sympatric *Prunus* species, estimated the timing of divergence, and tested for historical introgression and recent admixture. The results of these analyses expand current understanding of the origin and maintenance of tropical Andean tree diversity and provide important context for predicting how these species may respond to rapid environmental change.

MATERIALS AND METHODS

Study site and sample collection

Sampling for this study primarily focused on species which co-occur across the Andes Biodiversity and Ecosystem Research Group (ABERG; andesbiodiversity.org) plot network in Manu National Park, Peru (Figure 1). This site is located on the eastern slope of the tropical Andes in southeastern Peru and is a 3.5 km elevational gradient from the Andean tree line to the Amazonian lowlands. With 23-1 ha and 33-0.1 ha forest plots along a series of descending ridges, this is the most continuous transect in the tropical Andes. To date, ~19,000 individual trees have been documented in these plots from 100 families, 292 genera, and 1255 species (Farfan-Rios et al. 2025).

Intensive field sampling was conducted for *Prunus* species along this transect, with botanists searching in lateral transects centered on the 1-ha plots (approximately every 250 m) for up to 6 hrs. Leaf tissue was field collected and stored in silica following the Smithsonian Institution guidelines for collecting vouchers and tissues intended for genomic work (Funk et al. 2017). Samples were also included for species known to co-occur across an additional plot network spanning 4 km of elevation in Madidi National Park, Bolivia, and from at least one external population when possible. These samples were collected from herbarium specimens previously not treated with alcohol or fresh tissue stored in silica collected from individuals in the Cotapata region of Bolivia (Figure 1, Table S1). In total, we sampled 158 individuals from 13 species including two outgroups (*P. myrtifolia* and *P. caroliniana*) and a reference (Table S1).

ddRAD-seq data

High molecular weight DNA was extracted from ~50 mg of dried leaf tissue using Aboul-Maaty and Oraby's (2019) modified CTAB protocol for non-model plants with a few

173 modifications: samples were ground with a bead-beater, incubated at -20 ℃ overnight (and up to
174 24 hrs), and the DNA pellet was washed twice with 500 µl 70% ethanol. DNA was checked for
175 quality and quantity on a 1.5% agarose gel and Qubit before being sent to Floragenex
176 (Beaverton, Oregon, USA) for ddRAD library prep and sequencing. Samples were ligated with 6
177 bp barcodes and digested with the PstI/MseI+0 enzyme pair. Genomic data were demultiplexed
178 and assembled to sample using ipyrad v. 0.9.95 (Eaton and Overcast 2020) with an average of
179 2,215,444 ± 1,419,356 million reads (mean ± s.d.) recovered per sample.

180 Reads were assembled into orthologous loci using reference-based assembly in ipyrad.
181 Default settings were used to trim and filter reads and the clustering threshold was set to 0.85 as
182 recommended by the ipyrad manual and McCartney-Melstad et al. (2019) for phylogenetic
183 analyses. For the reference-based assembly, reads were mapped to the *Prunus persica* (peach)
184 genome (GCF_000346465.2; The International Peach Genome Initiative et al. 2013, Verde et al.
185 2017). The proportion of mapped reads varied across samples (mean = 67%, s.d. = 13%), but
186 variation in the quality of sample preservation and DNA extractions likely explain much of the
187 variation. When calling consensus alleles, we allowed for up to 4 alleles per sample following
188 Donoghue et al. (2022), as all *Prunus* species in the tropical Racemose group studied to date are
189 tetraploids (2n=32; Hodel et al. 2023), but ploidy is not known for most of the species in our
190 study. The final reference-mapped dataset comprised 35,099 loci, representing non-overlapping
191 genomic regions where data from at least 4 samples passed filtering. Further downstream
192 analyses were performed using vcftools and the ipyrad-analysis toolkit, which allows for filtering
193 and subsampling loci, genomic windows, and/or SNPs for phylogenetic inference and D-
194 statistics.

195 *Individual-level phylogeny*

A maximum likelihood (ML) phylogeny was reconstructed from a subset of representative samples with the most data present per species (up to 10 individuals per species, at least 1000 loci per sample; n=47), including conspecifics from multiple localities when possible. The ipa.window_extracter module in the ipyrad-analysis toolkit was used to extract data from the 8 largest scaffolds (the chromosomes) and filter sites to only maintain those shared by at least 25% of individuals. We selected the 25% threshold after testing various other filtering thresholds (10%, 30%, 40%, 50%), as 25% provided for the best phylogenetic resolution while also including as much information as possible. The filtered and concatenated sequence matrix is 2.8 Mb in length, containing 107,708 SNPs and 65.8% missing data. This alignment was used in RAxML (Stamatakis 2014) under the GTR+GAMMA evolutionary model to infer a best phylogeny under the rapid hill-climbing algorithm, with support values from 100 bootstrap replicates. The phylogeny was rooted to the reference genome *P. persica* (a native of China and member of the Solitary-flower group and Amygdalus subgenus) and included two additional Racemose outgroup species – *P. caroliniana* (native of the eastern United States and Carribbean) and *P. myrtifolia* (native to the Caribbean, Central America, and lowland South America).

Species-level phylogeny

To construct the species-level phylogeny, we selected one representative specimen for each species in the individual-level phylogeny (n=13). The samples selected grouped with other members of their species on the individual-level phylogeny and had the most data present. Again, we used the ipa.window_extracter module in the ipyrad-analysis toolkit to extract data from the chromosomes and filter sites, selecting those shared by at least 25% of all species (after testing multiple filtering thresholds). The resulting sequence matrix is 4.2 Mb with 62,262 SNPs and 63.6% missing data. To reconstruct the phylogenetic relationships, we used both maximum

219 likelihood (RAxML) and Bayesian (ExaBayes) analyses. We ran RAxML using GTR+GAMMA
220 to infer a best phylogeny under the rapid hill-climbing algorithm with support values from 100
221 bootstrap replicates. For ExaBayes, we ran two Metropolis-coupling replicates with four
222 coupled-chains (each with three heated chains) for 1×10^6 MCMC generations, sampled every
223 500 generations. Estimated sample size (ESS) for all parameters and branch lengths were
224 summarized with the ProcParam tool and confirmed as sufficiently sampled. The majority-rule
225 consensus phylogeny was generated with the ExaBayes 'consense' tool with a 25% burn-in.

226 To assess the robustness of the resolved relationships and distinguish between conflicted
227 and poorly informed branches, we used Quartet Sampling (Pease et al. 2018;
228 https://www.github.com/ fephyfofum/quartetsampling). We performed Quartet Sampling on the
229 resulting RAxML tree with the full concatenated alignment, allowing 100 replicates per branch
230 (Figure S2).

231 *Divergence time dating*

232 To assess the timing of divergence among species, we generated a time-calibrated
233 phylogeny using BEAST v. 2.7.7 (Bouckaert et al., 2019) with the same alignment used for the
234 species-level phylogenies. We used an optimized relaxed molecular clock and Yule speciation
235 process with the GTR+GAMMA substitution model. A nodal calibration was set for the crown
236 age of *Prunus* using a normal prior with an offset of 61.5 Mya and a standard deviation of 3.0
237 Mya (Chin et al. 2014; Su et al. 2023). The crown age of the racemose group was calibrated from
238 a staminate flower of *Prunus hirsutipetala* D.D. Sokoloff, Remizowa et Nuraliev preserved in
239 the late Eocene (Priabonian) Rovno amber from northwestern Ukraine (Sokoloff et al. 2018),
240 using a normal prior with an offset of 35.0 Mya and standard deviation of 2.0 Mya (following Su
241 et al. 2023). To ensure a more rapid convergence of MCMC runs, we used our RAxML topology

242 as our starting tree. The Markov chain Monte Carlo (MCMC) run was conducted for 20,000,000
243 generations and trees sampled every 2000 generations producing a total of 10,000 trees. The
244 resulting log files were checked in Tracer v.1.5 (Rambaut et al. 2018) and we used TreeAnnotator
245 v.1.10.4 to generate the maximum clade credibility tree discarding 25% of trees as burn-in before
246 visualizing the results in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

247 *Tests of historical introgression*

248 To test for historical introgression, we used the four-taxon Patterson's *D*-statistic (ABBA-
249 BABA), which quantifies allele frequency patterns that are incongruent with the hypothesized
250 species tree topology (Green et al. 2010). We ran the Patterson's *D*tests using the ipa.baba tool as
251 implemented in ipyrad, which is specifically designed for analysis of RAD-seq datasets. We used
252 the species-level RAxML tree as our starting tree hypothesis and constrained the tests setting the
253 outgroup to *P. myrtifolia*, so all tests would focus on ingroup species. To assess significance, we
254 performed 1000 bootstrap replicates for each test with loci resampled with replacement. Z-scores
255 were then calculated as the number of bootstrap standard deviations from the null D=0 (Table 1).
256 Z-scores > 2.5 were considered significant.

257 *Tests of recent admixture*

258 To assess genetic structure and test for recent admixture, we used the Bayesian clustering
259 algorithm STRUCTURE v. 2.3.4 (Pritchard et al. 2000) as implemented in ipyrad with the set of
260 samples included in the individual-level phylogeny (minus the outgroups). SNPs were filtered
261 conservatively to only include those with at least 50% coverage across individuals and then
262 randomly sampled to one SNP per locus to avoid linkage. This resulted in a total of 1493 SNPs
263 included in the analysis. The model was run with a burn-in of 125,000 and run length of 500,000

generations, testing K=1–10 with 10 replicates per K. The assignment of samples into K distinct genetic groups was assessed using DeltaK, which has been shown to be more robust to accurately estimating the correct number of clusters in STRUCTURE, particularly with high amounts of missing data as is common in RADseq (Evanno et al. 2005; Figure S3).

Estimates of genomic diversity

Genetic diversity was estimated within individuals and species to compare to the admixture results. In VCFtools v.1.16 (Danecek et al. 2011), sequences were filtered using two different missing data thresholds, retaining loci present within at least 50% or 70% of the samples for each species. Sites were also filtered if they had a minor allele frequency less than 0.05. We calculated nucleotide diversity (π) for each species – the average pairwise nucleotide differences between sequences in a sample at a site as well as observed (H_o) and expected heterozygosity (H_e) – the proportion of loci where an individual is heterozygous vs. the proportion expected by Hardy-Weinberg equilibrium – within samples and averaged across the species. For these calculations, *P. debilis* and *P. detrita* samples were grouped together as well as *P. huantensis* and *P. brittoniana,* given that STRUCTURE (Figure 4) did not detect any distinct genetic clusters among these species' pairs. Species with < 3 samples were excluded from these analyses given the lack of statistical power from such a small sample size.

RESULTS

Phylogenomic relationships and elevational niche diversification

Both the species-level maximum likelihood (RAxML) and Bayesian (ExaBayes) phylogenetic analyses supported the same evolutionary relationships among the focal species and their outgroups (Figure 2). Subtropical *P. caroliniana* diverged before the tropical species and *P.*

286 *myrtifolia* was sister to the Andean species. Among these partially sympatric Andean species,
287 diversification generally occurred from the lowlands to the highlands, with the lineage including
288 *P. debilis* and *P. detrita* diverging first, followed by the mid-elevation cloud-forest species (*P.*
289 *herthae, P. pleiantha, P. stipulata, P. integrifolia*) and finally the high-elevation species as the
290 most recent diversification events (*P. huantensis, P. brittoniana, and P. antioquensis*). Statistical
291 support for the inferred relationships was assessed using bootstrap support (RAxML), posterior
292 probabilities (ExaBayes), and Quartet Sampling. While the tree nodes are generally well-
293 supported (all posterior probabilities = 1, most bootstrap values > 75; Figure 2), Quartet
294 Sampling indicated that low bootstrap support values are not likely the result of insufficient
295 phylogenetic signal, as the proportion of informative quartets (QI) ranged from 80–100%. The
296 lack of a strong skew in the proportions of discordant phylogenetic trees at most nodes (QD >
297 0.5) suggests that incomplete lineage sorting (ILS) is more likely than introgression. The branch
298 ancestral to *P. huantensis, P. brittoniana*, and *P. antioquensis* had moderate concordance
299 (QC=0.21) and discordant skew (QD=0.22), indicating a possible introgressive event (Figure
300 S2). Together, these phylogenetic analyses point towards a history of diversification from the
301 lowlands into the highlands within Andean *Prunus*, with some evidence of incomplete lineage
302 sorting (ILS) and/or introgression.

303 *Divergence time estimation*

304 The time-calibrated phylogeny generated in BEAST recovered the same tree topology as
305 RAxML and ExaBayes with similar branch support (Figure 3). The estimated divergence time
306 *for P. myrtifolia* and the ancestor of the Amazonian and Andean species is ~27.3 Ma (95%
307 highest posterior density [HPD] 22.4–31.7). The Andean species began diverging at the start of
308 the Miocene, with the low-elevation *P. debilis*+*detrita* lineage branching off ~23.5 Ma (95%

309 HPD 19.4-28.1), and from each other ~17.6 Ma (95% HPD 12.8-22.6). The cloud forest species
310 followed, with *P. pleiantha* diverging ~20.4 Ma (95% HPD 16.3-24.4), and *P. integrifolia* ~18.0
311 Ma (95% HPD 14.2-21.7). The remaining lineages emerged in the mid to late-Miocene (15.9–
312 9.3) with the highest elevation clade diverging last, with *P. huantensis* ~11.6 Ma (95% HPD 8.6-
313 14.9) and *P. antioquensis*+ *brittoniana* ~9.3 Ma (95% HPD 6.2-12.3). In sum, these estimated
314 divergence times indicate that Andean *Prunus* diversified from the Amazonian lowlands into the
315 Andean highlands over the Miocene.

316 *Tests of historical introgression and recent admixture*

317 *D*-statistics indicated significant historical introgression events between *P. pleiantha* and
318 both the ancestors *P. debilis*+*detrita*, with more limited evidence suggesting introgression with *P.*
319 *debilis* and *P. detrita* separately (Figure 2, Table 1). A second significant introgression event was
320 detected between *P. pleiantha* and the *P. huantensis*+*antioquensis* ancestor, which is further
321 supported by Quartet Sampling (Figure S2). Finally, significant *D*-statistics supporting
322 introgression between *P. debilis* and *P. antioquensis* were also detected. Overall, introgression is
323 widespread and does not seem to be correlated with ecological or evolutionary distance.

324 STRUCTURE analysis found K=9 to be the most likely number of distinct genetic
325 clusters within the dataset, determined by mean log probability and DeltaK values (Figures 5,
326 S3). Individuals largely clustered according to their morphologically assigned species; however,
327 the number of detected clusters was fewer than the expected number of named taxonomic species
328 in the analysis (*n*=10). *P. integrifolia* and *P. amplifolia* are largely reproductively isolated, though
329 low levels of admixture were detected in *P. integrifolia* and only one sample was included for *P.*
330 *amplifolia.* Admixture is widespread amongst all other species, particularly *P. pleiantha*, *P.*

stipulata, *P. herthae*, *P. debilis, P. huantensis, P. brittoniana and P. antioquensis,* and like the introgression analyses, is unrelated to evolutionary or ecological distance.

Estimates of genomic diversity

Within each species, the individuals with the highest observed heterozygosity (H_o) were also the most admixed in the STRUCTURE results (Tables 3 and 4, Figure 4), and this was consistent with different amounts of missing data. For example, in *P. pleiantha*, individuals 163, 164, and 165 showed high levels of admixture from multiple groups and had the highest H_o estimates (50%: 0.18 – 0.22, 70%: 0.19 – 0.25; Table 3). Across species and filtering thresholds, mean pairwise nucleotide diversity (π) was high (50%: 0.41 – 0.64, 70%: 0.37 – 0.51) compared to H_o, π tracked with H_e as expected, and H_o (50%: 0.06 – 0.15, 70%: 0.06 – 0.17) was far below H_e (50%: 0.41 – 0.64; 70%: 0.37 – 0.51; Table 2). However, the estimates for *P. herthae* and the *P. debilis*+*detrita* group should be interpreted cautiously, given the low sample sizes for each. These statistics corroborate the findings of the STRUCTURE analysis and further indicate a history of interspecific gene flow among co-occurring *Prunus* species.

DISCUSSION

In this study, we aimed to investigate the patterns, timing, and extent of divergence among partially sympatric congeneric trees in the tropical Andes. The phylogenomic data presented here suggest that *Prunus* species known to co-occur as elevational replacements diversified upslope from the Amazonian lowlands into the Andean highlands during the Miocene, in a pattern consistent with adaptive diversification (lower montane lineage ~23.5 Ma, cloud forest species ~20.4–13.4, high-elevation clade ~11.6–9.3 Ma). However, despite these ancient diversification events, species do not appear to be reproductively isolated, since

substantial introgressive gene flow is detectable among species through time and is not correlated with evolutionary or ecological distance. Together, these findings suggest that during adaptive radiations, observable ecological and morphological differences may emerge despite ongoing gene flow and long before reproductive isolation is complete.

Upslope diversification and biogeographic implications

Phylogenomic reconstructions and divergence time dating support the hypothesis that *Prunus* diversified upslope, likely in response to novel habitat opportunities as has been observed in other plant clades (e.g. Melastomataceae – Dudley 1978, *Espletia* – Pouchon et al. 2018, lupines - Hughes and Eastwood 2006, Nevado et al. 2016, bellflowers – Lagomarsino et al. 2016, *Hypericums* - Nürk et al. 2013, wild tomato – Pease et al. 2016, orchids - Pérez-Escobar et al. 2017, and bromeliads – Givnish et al. 2011, 2014, Silvestro et al. 2014, Males 2018). Though this does not exclude the possibility that some species may have adapted elsewhere and immigrated in once their preferred habitat became available, the predominant pattern reconstructed from the phylogenomic analyses is one of stepwise, upslope adaptive diversification. In agreement with prior studies, we found *P. myrtifolia* (endemic to the Caribbean, Central America, and lowland South America) to be ancestral to the rest of the Andean species included in this analysis, supporting the hypothesis that this lineage or its ancestor colonized South America via migration through the Caribbean and Central America (Chin et al. 2014; Hodel et al. 2023). While our divergence time estimate for *P. myrtifolia* of ~27 Ma is slightly later than the Neotropical colonization of ~34 Ma predicted by Chin et al. (2014), it is still within the climatic shift from warmhouse to transitional state that coincided with an onset of glaciation in Antarctica (~33.9 – 27.29 Ma; Judd et al. 2024) and the estimated end of the boreotropical forests in the Northern Hemisphere.

In our analyses, diversification of the Andean species occurred throughout the Miocene, beginning with the lineage that includes two of the lowest elevation species (*P. debilis* and *P. detrita*) ~23.5 Ma and ending with the high-elevation clade ~11.6 Ma (*P. huantensis*), ~9.3 Ma (*P. antioquensis* and *P. brittoniana*). This is consistent with the timing of major uplift events and estimated paleo-elevations in the North-Central and Central Andes (Boschman 2021). It also supports the findings of Hodel et al. (2023), which detected a long period of stasis followed by rapid diversification of Neotropical *Prunus* during the Miocene. Although the exact timing, pace, and sequence of Andean orogeny is a subject of ongoing study and is spatially heterogeneous, the late Oligocene and Miocene (~25-5 Ma) are known to have been a period of intense and accelerated uplift in the Eastern Cordillera of the North-Central and Central Andes (Boschman et al. 2021; Pérez-Escobar et al. 2022).

The lower montane cloud forest species in our analysis (median elevation ranges 1200–2500 m; *P. pleiantha, P. integrifolia, P. herthae, P. stipulata*) diverged during the early to mid-Miocene. This aligns with the predicted origination of cloud forests (Sempere et al. 2005) and the estimated divergence times for other cloud forest lineages (Luebert and Weigend 2014). Andean cloud forests are distinct habitats characterized by persistent cloud cover and immersion that occurs from ~1200 m to the upper forest limits at ~3200–3800 m (depending on latitude). Although they are the most speciose habitats in the Andes, persistence in these environments often requires unique adaptations to cope with the reduced light availability, high precipitation, and humidity such as thick, pachyphyllic leaves, low growth rates, and ability to withstand heavy accumulations of epiphytes (Grubb 1977; Rapp and Silman 2012; Fahey et al. 2016; Pérez-Escobar 2022). The highest elevation species in our study (median elevation ranges ≥3000 m; *P. huantensis, P. antioquensis, P. brittoniana*) diverged during the late Miocene, as the final uplift

399 phase began in the North-Central and Central Andes (Boschman et al. 2021). Species in this
400 elevation zone are exposed to unique high-elevation stressors such as night frost, with
401 temperatures dipping below 0ºC, requiring further physiological specialization (Pérez-Escobar
402 2022).

403 *Gene flow through time*

404 Our genomic analyses show that Andean *Prunus* species remain porous lineages. Despite
405 deep Miocene divergences, we detect both ancient introgression and recent admixture among
406 partially sympatric taxa, with gene flow not obviously constrained by either evolutionary or
407 elevational distance. *D*-statistics detected at least five significant ancient introgression events.
408 Two of these events occurred among lineages separated by great evolutionary and ecological
409 distance (*P. pleiantha* × *P. huantensis*+ *antioquensis*, and *P. debilis* × *P. antioquensis).*
410 Introgression was also detected between *P. pleiantha* and the ancestor of *P. debilis*+ *detrita* (*P.*
411 *pleiantha* × *P. debilis*+ *detrita*) as well as among *P. pleiantha* × *P. debilis* and *P. pleiantha* × *P.*
412 *detrita* individually. Although difficult to disentangle, especially with limited sampling and
413 reduced-representation sequencing data, these patterns are most consistent with repeated
414 interspecific hybridization that occurred among the ancestors of these lineages and has continued
415 as elevationally segregated lineages diverged.

416 Historical introgression among species separated in ecological space across the elevation
417 gradient is plausible for several reasons. While the medians of their elevation ranges may be
418 quite different, they are found in close geographic proximity, and their elevational ranges overlap
419 (or are much closer) at the range margins. *Prunus* species are thought to be obligate outcrossers
420 with generalist pollinators, as is common in tropical trees (Ashton 1969; Bawa 1974, 1979). The
421 extent of self-incompatibility across the genus and potential for breakdown after polyploidization

422 are still unclear (Hauck et al. 2006, Tao et al. 2010, Aguiar et al. 2015). Generalist pollinators can
423 forage over kilometers, providing opportunity for introgression, especially given that many
424 tropical species flower semi-synchronously during the dry season (Bawa et al. 1985; Van Schaik
425 et al. 1993).

426 Even where known species ranges are separate at present, they have not necessarily
427 always been so. Pollen records from Lago Consuelo (1360 m a.s.l., located in southeastern Peru
428 adjacent to the Bolivian border) show that during the last full glacial period (43.5–22 kyr BP),
429 individual cloud forest species migrated downslope at least 1000–1700 vertical m in response to
430 lower cloud base and cooler temperatures (-5ºC; Urrego et al. 2005). Today, these species ranges
431 are separated as far apart as they have likely been during the Quaternary, and historical
432 introgression events may be signatures of past ecological overlap.

433 Tests of recent admixture also found gene flow to be widespread among Andean *Prunus*
434 species. *P. integrifolia* and *P. amplifolia* are largely genomically isolated, but gene flow appears
435 rampant among all other species. The isolation of *P. integrifolia* is particularly interesting as this
436 species has the widest elevational range and is one of the most abundant species in the tropical
437 Andes. Gene flow was especially high among *P. pleiantha, P. stipulata*, and *P. herthae*, all cloud
438 forest species with largely overlapping elevation ranges. However, individuals of these species
439 mostly form monophyletic groups, indicating they have developed specific genetic
440 distinctiveness despite gene flow (Figure S1). As *P. pleiantha* was identified in most of the
441 historical introgression events we can infer that this species readily hybridizes when in contact
442 with close relatives. Further investigation will be needed to test this hypothesis, identify the
443 directionality of such gene flow, and determine whether physiological, ecological, or genetic
444 factors drive this weaker reproductive isolation. Ploidy variation may also underlie some of these

445 ambiguous genomic patterns, and an important next step will be to characterize ploidy across
446 these species and assess its influence on inferred gene flow and lineage distinctiveness.
447 STRUCTURE did not identify *P. debilis* and *P. detrita* as distinct genetic clusters, possibly due
448 to how closely related these species are, introgression, and the low number of individuals for
449 both species in the dataset. Our genomic data also support the most recent taxonomic revision by
450 Pérez-Zabala (2022) which considered *P. brittoniana* and *P. huantensis* to be the same species
451 given their morphological and ecological similarities (Figure S1). Taken together, these results
452 indicate that gene flow has been a persistent and complex feature of Andean *Prunus*
453 diversification, occurring across both evolutionary time and ecological space despite ancient
454 divergence times and emergence of species-level distinctiveness.

455

456 *Conclusions*

457 Overall, these results suggest that adaptive diversification following the emergence of
458 novel gradients has been important for within-genus diversification of Andean trees, particularly
459 in the genus *Prunus*, while immigration and sorting of pre-adapted clades may be the dominant
460 mechanism shaping community assembly at higher-level taxonomic levels (Segovia et al. 2020,
461 Griffiths et al. 2020, Linan et al. 2021). For most of the Neogene, *Prunus* diversified with the
462 rise of the Andes, developing sequential elevational replacements with narrow ranges, specially
463 adapted to live in radically different environmental conditions from the Amazonian lowlands to
464 Andean tree line. This has important implications in the context of climate change, as species are
465 now shifting their ranges to track their preferred temperatures (Feeley et al. 2011; Fadrique et al.
466 2018). Though most species will not be able to migrate fast enough to keep pace with climatic
467 shifts and are instead experiencing range retractions at the warmer-end, any upslope range shift

468 may increasingly result in range overlap among congeneric species and potential gene flow
469 (Duque et al. 2015; Farfan-Rios et al. 2025; Quinlan et al. 2025)
470 Here, we found evidence of both historical introgression, possibly in response to
471 Quaternary range shifts, and widespread admixture among co-occurring *Prunus* species. Yet
472 despite gene flow, these species have maintained distinct evolutionary lineages with identifiable
473 ecological and morphological differences since the Miocene. These results align with the
474 syngameon model, wherein species maintain incomplete reproductive barriers but primarily
475 experience divergent selection (Lotsy 1925; Grant 1971). Though oaks are the classic example
476 (e.g. Cannon and Petit 2020), such genomic mutualism may be an important yet under-
477 appreciated mechanism in the maintenance of diversity in hyper-diverse tropical forests, where
478 individuals predominantly outcross but retain some capacity for self-fertilization and
479 interspecific hybridization at low population densities (Wu 2001; Cannon and Lerdau 2015).
480 Further work is required to understand the prevalence of syngameons in tropical forests and their
481 role in species' responses to rapid environmental change, namely resilience and avoidance of
482 local extinction. Incorporating these complex dynamics into demographic models may be
483 important for accurately predicting the futures of Andean forests and effective conservation and
484 management under current climate change.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

EJQ, MRS, and JBP initially conceived of the study. EJQ acquired funding, led research design, data collection, data analysis and interpretation, and wrote the manuscript. JSB, AFF, and WFR assisted with data collection and WFR additionally contributed to manuscript editing and translated the summary. JBP assisted with data analysis and manuscript review and editing. MRS provided supervision, acquired funding, and contributed to research design and manuscript writing.

DATA AVAILABILITY

The genomic data generated for this study are available on NCBI's GenBank under submission ID SUB15831409 and corresponding herbarium vouchers are deposited in Herbario Vargas (CUZ) at the Universidad Nacional de San Antonio Abad del Cusco (UNSAAC) and Herbario Nacional de Bolivia (LPB).

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FIGURES AND TABLES

Table 1. Summary of significant D-statistic tests, with *P. myrtifolia* set to P4.

P1	P2	P3	dstat	Z	ABBA	BABA	nloci
P. antioquensis	*P. stipulata*	*P. debilis*	-0.69	4.75	8.38	45.13	152
P. stipulata	*P. antioquensis*	*P. debilis*	0.69	4.60	45.13	8.38	152
P. amplifolia, P. stipulata	*P. antioquensis*	*P. debilis*	0.66	4.16	43.19	8.94	163
P. antioquensis	*P. amplifolia, P. stipulata*	*P. debilis*	-0.66	4.01	8.94	43.19	163
P. stipulata	*P. pleiantha*	*P. detrita, P. debilis*	0.13	2.94	793	605.44	3968
P. pleiantha	*P. stipulata*	*P. detrita, P. debilis*	-0.13	2.87	605.44	793	3968
P. herthae, P. huantensis	*P. pleiantha*	*P. detrita, P. debilis*	0.11	2.81	864.08	686.51	4485
P. stipulata	*P. huantensis, P. antioquensis*	*P. pleiantha*	0.09	2.71	1190.20	989.74	6218
P. huantensis, P. antioquensis	*P. stipulata*	*P. pleiantha*	-0.09	2.71	989.74	1190.2	6218
P. pleiantha	*P. herthae, P. huantensis*	*P. detrita, P. debilis*	-0.11	2.67	686.51	864.08	4485
P. stipulata	*P. pleiantha*	*P. detrita*	0.13	2.66	744.38	578.5	3725
P. pleiantha	*P. antioquensis*	*P. debilis*	0.51	2.63	20.25	6.5	142
P. pleiantha	*P. stipulata*	*P. detrita*	-0.13	2.62	578.5	744.38	3725
P. antioquensis	*P. pleiantha*	*P. debilis*	-0.51	2.58	6.5	20.25	142
P. pleiantha	*P. stipulata*	*P. debilis*	-0.27	2.53	68.5	119.25	576
P. herthae, P. huantensis, P. antioquensis, P. brittoniana	*P. pleiantha*	*P. detrita*	0.11	2.52	813.45	654.78	4203

Table 2. Summary of within species genomic diversity statistics.

Species	# of samples	Filter	# of loci	Average π	Average H_o	Average H_e
P. integrifolia	10	50	53574	0.42 ± 0.13	0.15 ± 0.064	0.42 ± 0.0025
		70	17592	0.41 ± 0.14	0.17 ± 0.073	0.40 ±0.0015
P. huantensis/ brittoniana	12	50	24457	0.41 ± 0.13	0.12 ± 0.078	0.41 ± 0.0012
		70	1960	0.38 ± 0.15	0.16 ± 0.10	0.37 ± 0.0031
P. stipulata	7	50	24457	0.45 ± 0.11	0.095 ± 0.096	0.45 ± 0.0024
				0.42 ± 0.13	0.11 ± 0.11	0.42 ± 0.0064
P. pleiantha	7	50	23299	0.45 ± 0.11	0.12 ± 0.081	0.44 ± 0.0015
		70	7295	0.43 ± 0.12	0.13 ± 0.091	0.42 ± 0.0013
P. herthae	3	50	6251	0.62 ± 0.077	0.085 ± 0.03	0.61 ± 0.0067
		70	699	0.51 ± 0.073	0.064 ± 0.0072	0.51 ± 0
P. debilis/detrita	3	50	1747	0.64 ± 0.061	0.064 ± 0.058	0.064 ± 0.0009

Table 3. Summary of within-individual heterozygosity estimates.

Species	Individual	H_o (50% filter)	H_e (50% filter)	H_o (70% filter)	H_e (70% filter)
P. integrifolia	139_integrifolia	0.11	0.42	0.12	0.41
	147_integrifolia	0.31	0.42	0.36	0.4
	148_integrifolia	0.14	0.42	0.16	0.4
	149_integrifolia	0.18	0.42	0.21	0.4
	151_integrifolia	0.10	0.42	0.12	0.4
	155_integrifolia	0.16	0.42	0.19	0.4
	156_integrifolia	0.12	0.42	0.14	0.4
	157_integrifolia	0.17	0.42	0.2	0.4
	212_integrifolia	0.09	0.41	0.1	0.4
	253_integrifolia	0.12	0.42	0.14	0.41
P. huantensis /brittoniana	108_huantensis	0.16	0.41	0.23	0.37
	115_huantensis	0.1	0.41	0.13	0.38
	116_huantensis	0.22	0.41	0.29	0.37
	117_huantensis	0.044	0.4	0.07	0.37
	119_huantensis	0.072	0.41	0.093	0.37
	123_huantensis	0.069	0.4	0.12	0.37
	124_huantensis	0.2	0.4	0.25	0.37
	130_huantensis	0.05	0.41	0.079	0.38
	131_huantensis	0.15	0.41	0.22	0.37
	132_huantensis	0.26	0.41	0.36	0.37
	179_brittoniana	0.064	0.41	0.08	0.37
	210_brittoniana	0.03	0.4	0.05	0.38
P. stipulata	171_stipulata	0.28	0.45	0.32	0.42
	173_stipulata	0.17	0.45	0.18	0.42
	175_stipulata	0.07	0.45	0.07	0.41
	285_stipulata	0.058	0.45	0.1	0.42
	286_stipulata	0.03	0.45	0.041	0.41
	312_stipulata	0.037	0.45	0.032	0.42
	332_stipulata	0.02	0.45	0.021	0.43
P. pleiantha	162_pleiantha	0.12	0.44	0.13	0.43
	163_pleiantha	0.22	0.44	0.25	0.43
	164_pleiantha	0.18	0.44	0.19	0.42
	165_pleiantha	0.2	0.44	0.22	0.42
	166_pleiantha	0.023	0.44	0.021	0.42
	167_pleiantha	0.05	0.44	0.052	0.42
	169_pleiantha	0.051	0.44	0.056	0.42
P. herthae	226_herthae	0.087	0.62	0.057	0.51
	236_herthae	0.054	0.61	0.063	0.51
	237_herthae	0.11	0.62	0.072	0.51
P. debilis/ detrita	180_debilis	0.13	0.64	NA	NA
	225_debilis	0.036	0.64	NA	NA
	107_detrita	0.026	0.64	NA	NA

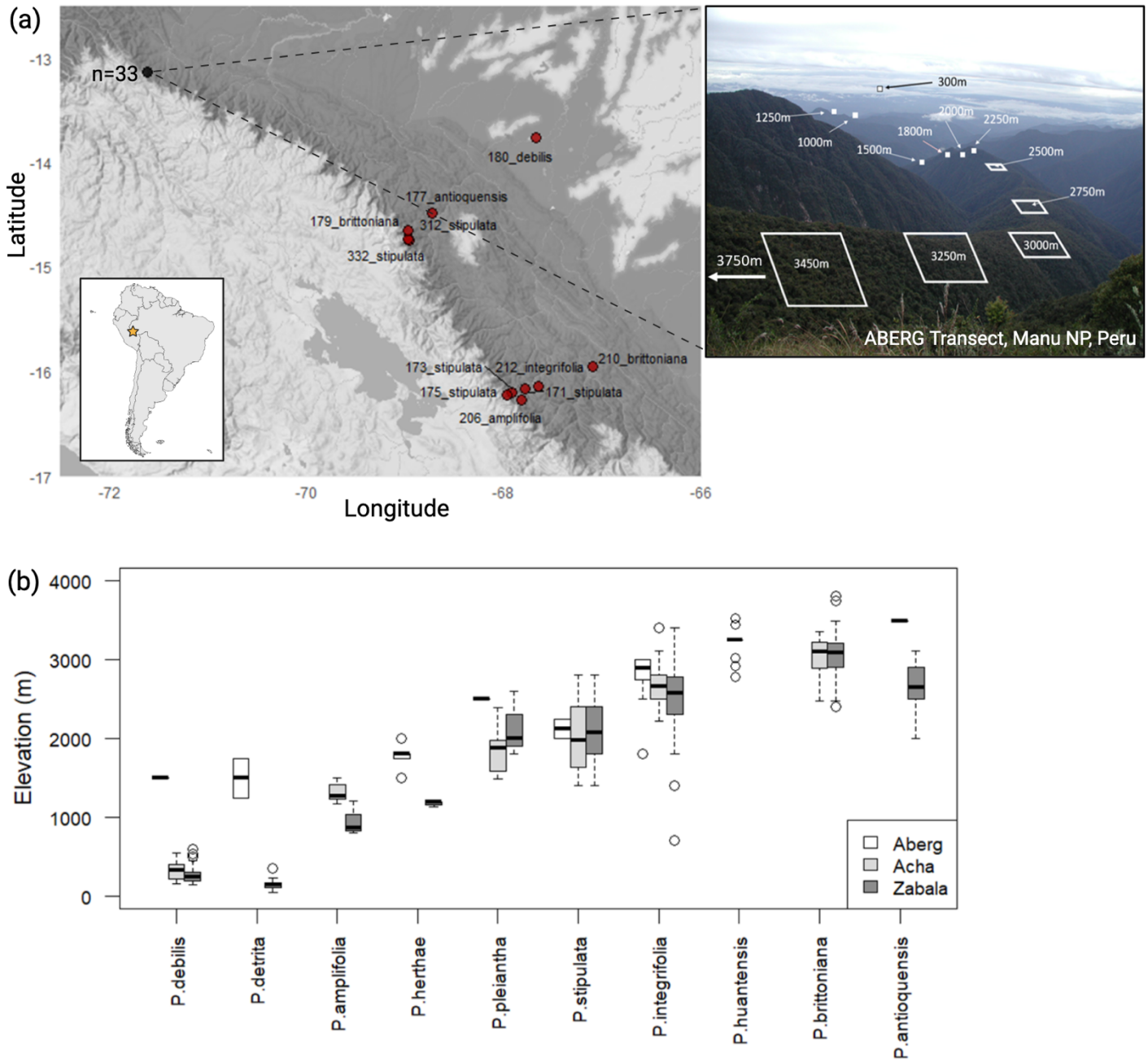

Figure 1. (a) Map of samples included in this study. Black dot indicates the location of the ABERG transect in Manu National Park, Peru where most samples (n=33) were collected, also shown in picture to the right. Red points indicate locations of additional samples included in the study. Further details on individual samples provided in Table S1. (b) Box plots showing elevational distributions of the in-group Andean species across three different sources. “Aberg” includes all known individuals along the ABERG transect >10 dbh over 20 years. “Acha” includes all Bolivian material reviewed by Achá (2013) and “Zabala” includes all material

reviewed by Perez-Zabala (2022) from Venezuela to Bolivia. Perez-Zabala considered *P. huantensis* to be *P. brittoniana* so those samples are grouped together on the figure under *P. brittoniana*. Figure does not include new "varieties" of species proposed by either Achá or Perez-Zabala.

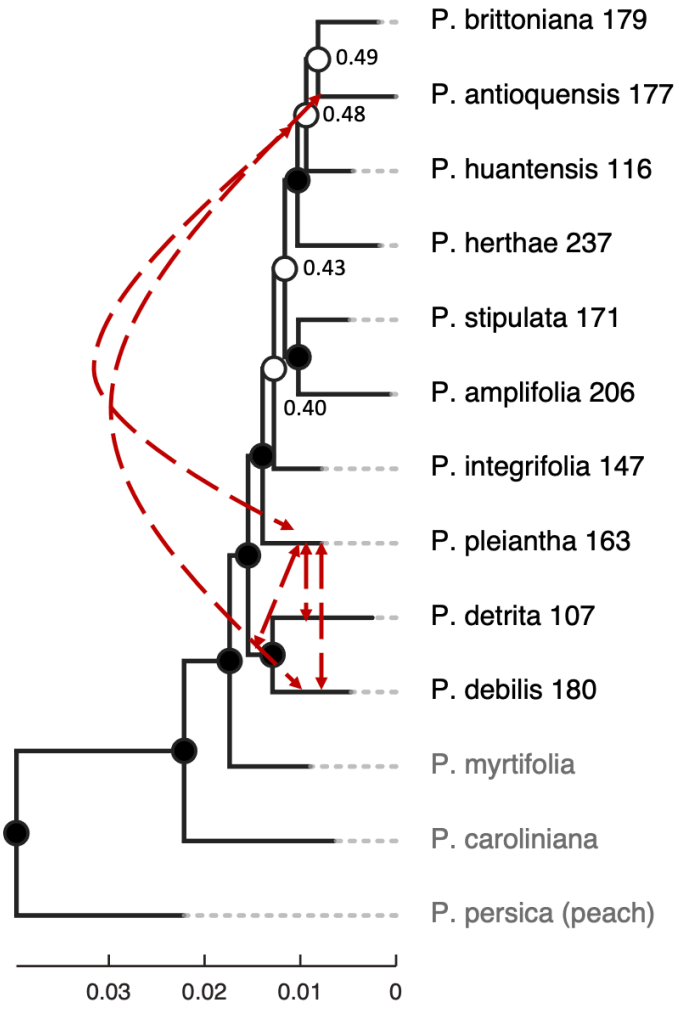

Figure 2. Species-level topology resulting from RAxML and ExaBayes phylogenetic analyses. Nodes show RAxML bootstrap support values where black circles indicate values > 75 and values < 75 are shown on white nodes. All Bayesian posterior probabilities were equal to 1. The tree is rooted to the reference (*P. persica*) and all three outgroup species are shown in grey. Dashed arrows summarize patterns of historical admixture inferred from D-statistics.

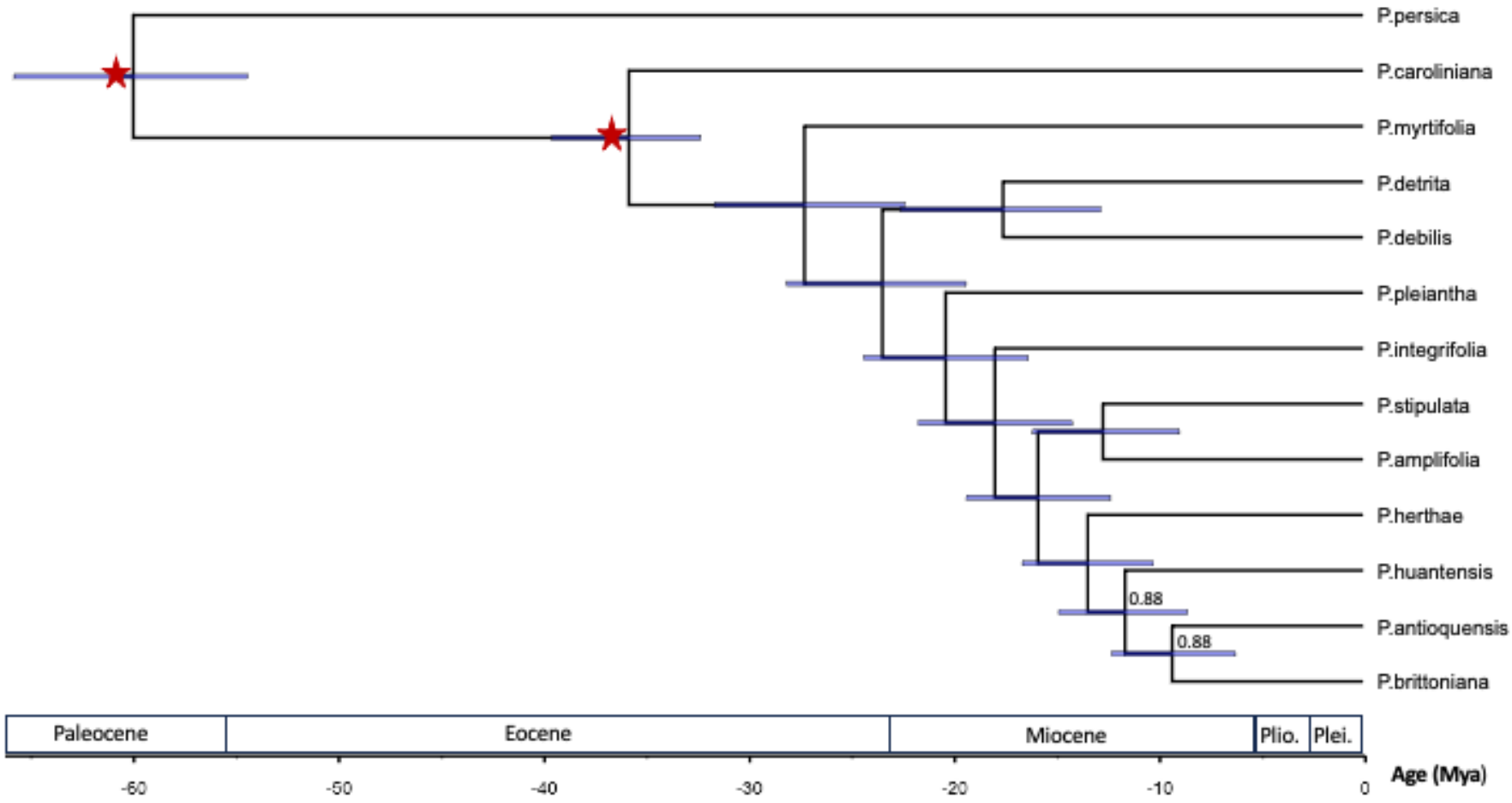

Figure 3. Time-calibrated phylogeny generated from BEAST analysis. Posterior probabilities are shown for nodes when < 1. Node bars represent the 95% highest probability density (HPD) interval for node ages. Red stars indicate the fossil calibration points.

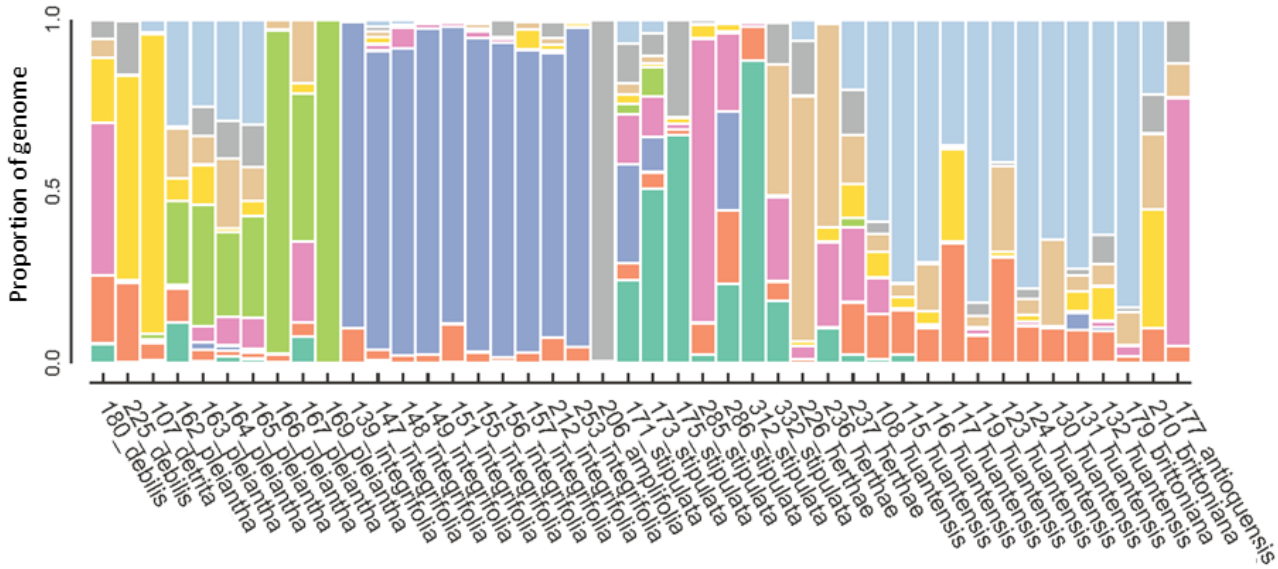

Figure 4. Patterns of genetic structure among individuals of sympatric species. Each vertical bar represents a single individual and the y-axis indicates the proportion of genetic ancestry of that individual assigned to different inferred genetic clusters, designated with different colors. STRUCTURE detected K=9 distinct genetic clusters as the most likely grouping (Figure S3).

SUPPORTING INFORMATION

Table S1. Summary of samples included in individual-level phylogeny and genetic structure analyses. Samples in bold were used to construct the species-level phylogeny.

Species determination	Sample ID	Voucher (Herbarium)	Material	Sampling locality
P. detrita	**107_detrita**	**EJQ82 (CUZ)**	**Fresh**	**Manu NP, Peru**
P. debilis	**180_debilis**	**Loza et al. 388 (LPB)**	**Herbarium**	**Madidi NP, Bolivia**
P. debilis	225_debilis	JSB6352A (CUZ)	Fresh	Manu NP, Peru
P. stipulata	**171_stipulata**	**EJQ114 (LPB)**	**Fresh**	**Cotapata, Bolivia**
P. stipulata	173_stipulata	EJQ116 (LPB)	Fresh	Cotapata, Bolivia
P. stipulata	175_stipulata	EJQ118 (LPB)	Fresh	Cotapata, Bolivia
P. stipulata	285_stipulata	JSB6320 (CUZ)	Fresh	Manu NP, Peru
P. stipulata	286_stipulata	JSB6321(CUZ)	Fresh	Manu, NP, Peru
P. stipulata	312_stipulata	Cayola 3259	Herbarium	La Paz, Bolivia
P. stipulata	332_stipulata	Villalobosos 81	Herbarium	
P. integrifolia	139_integrifolia	JSB6366 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	**147_integrifolia**	**JSB6402 (CUZ)**	**Fresh**	**Manu NP, Peru**
P. integrifolia	148_integrifolia	JSB6396 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	149_integrifolia	JSB6403 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	151_integrifolia	JSB6400 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	155_integrifolia	JSB6397 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	156_integrifolia	JSB6391 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	157_integrifolia	JSB6392 (CUZ)	Fresh	Manu NP, Peru
P. integrifolia	212_integrifolia	Meneses 7535 (LPB)	Herbarium	Nor Yungas, Cotapata, Bolivia
P. integrifolia	253_integrifolia	JSB7255 (CUZ)	Fresh	Manu NP, Peru
P. pleiantha	162_pleiantha	JSB6382 (CUZ)	Fresh	Manu NP, Peru
P. pleiantha	**163_pleiantha**	**JSB6388 (CUZ)**	**Fresh**	**Manu NP, Peru**
P. pleiantha	164_pleiantha	JSB6386 (CUZ)	Fresh	Manu NP, Peru
P. pleiantha	165_pleiantha	JSB6387 (CUZ)	Fresh	Manu NP, Peru
P. pleiantha	166_pleiantha	JSB6385 (CUZ)	Fresh	Manu NP, Peru
P. pleiantha	167_pleiantha	JSB6380 (CUZ)	Fresh	Manu NP, Peru
P. pleiantha	169_pleiantha	JSB6390 (CUZ)	Fresh	Manu NP, Peru
P. herthae	226_herthae	JSB6343 (CUZ)	Fresh	Manu NP, Peru
P. herthae	236_herthae	JSB6333 (CUZ)	Fresh	Manu NP, Peru
P. herthae	**237_herthae**	**JSB6334 (CUZ)**	**Fresh**	**Manu NP, Peru**
P. amplifolia	**206_amplifolia**	**Beck 33550 (LPB)**	**Herbarium**	**La Paz, Bolivia**
P. antioquensis	**177_antioquensis**	**Araujo & Canqui 348 (LPB)**	**Herbarium**	**Madidi, Bolivia**
P. huantensis	108_huantensis	JSB6316 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	115_huantensis	JSB6310 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	**116_huantensis**	**JSB6358 (CUZ)**	**Fresh**	**Manu NP, Peru**
P. huantensis	117_huantensis	JSB6360 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	119_huantensis	JSB6362 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	123_huantensis	JSB6353 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	124_huantensis	JSB6357 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	130_huantensis	JSB6375 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	131_huantensis	JSB6311 (CUZ)	Fresh	Manu NP, Peru
P. huantensis	132_huantensis	JSB6312 (CUZ)	Fresh	Manu NP, Peru

P. brittoniana	**179_brittoniana**	**Paniagua et al. 5782A (LPB)**	**Herbarium**	**Pelechuco-Mojo, Bolivia**
P. brittoniana	210_brittoniana	Beck 35044 (LPB)	Herbarium	Sud Yungas, Cotapata, Bolivia
Outgroups:				
P. myrtifolia	**1_myrtifolia**	**Silica sample only**	**Fresh**	**Fairchild Botanical Garden, Miami, Florida, USA**
P. caroliniana	**2_caroliniana**	**Silica sample only**	**Fresh**	**Weymouth Woods, North Carolina, USA**

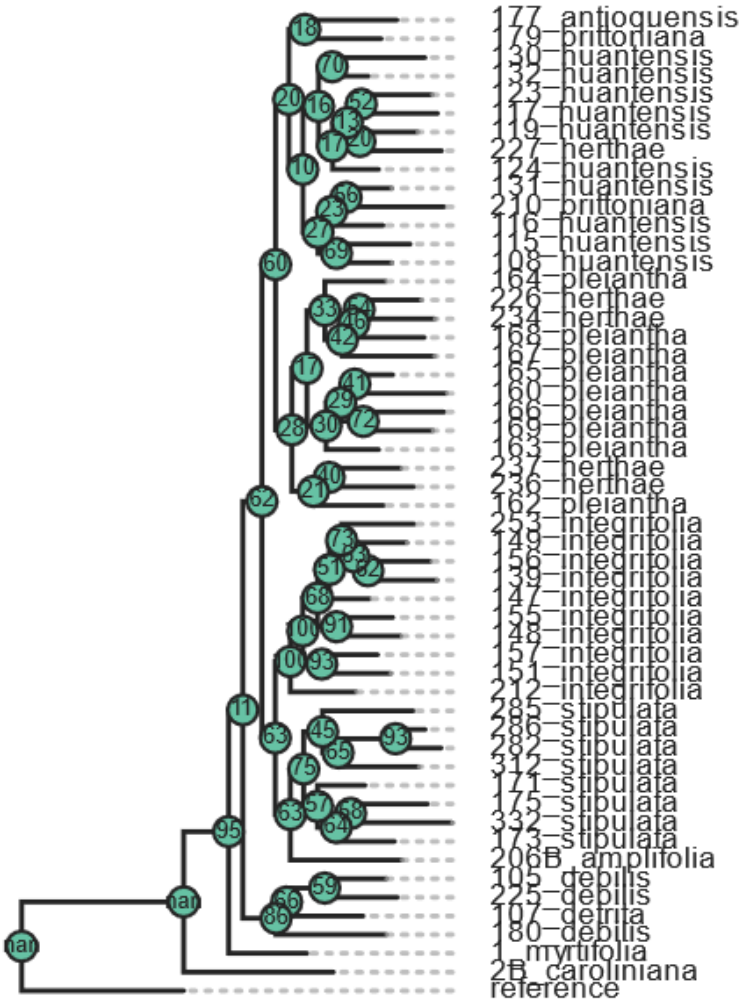

Figure S1. Maximum likelihood individual-level phylogeny reconstructed in RAxML. Individuals were selected to include those with at least 1000 loci per sample and up to 10 individuals per species. Sites were filtered to only include those with at least 25% coverage across samples. Tree was rooted to the reference (*P. persica*) and nodes show bootstrap support value.

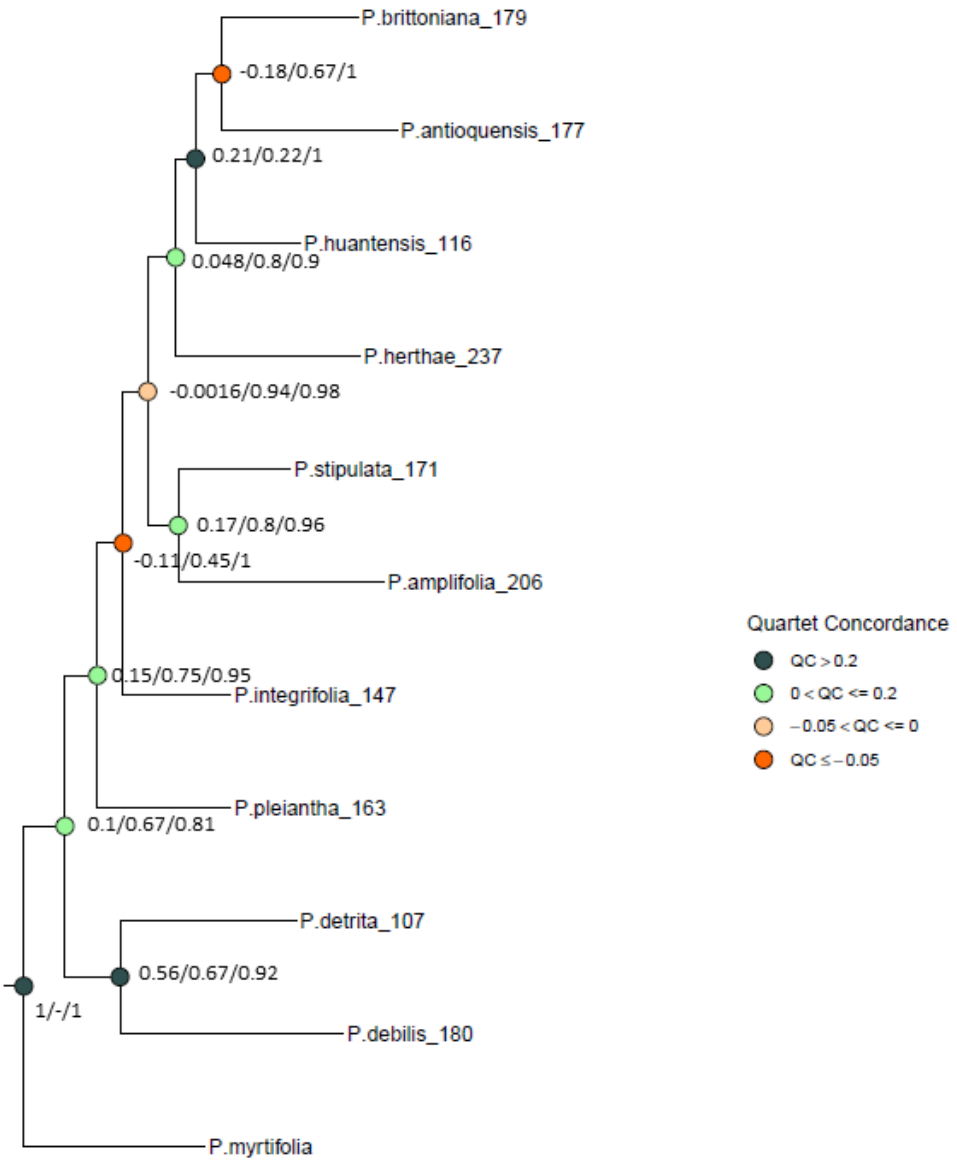

Figure S2. Quartet sampling results with quartet concordance (QC)/quartet differential (QD)/quartet informativeness (QI) indicated next to nodes. Nodes are colored according to QC scores, indicating how often the concordant quartet is inferred over both discordant quartets. Outgroup samples *P. persica* and *P. caroliniana* are removed for clarity. Figure created with https://github.com/ShuiyinLIU/QS_visualization.

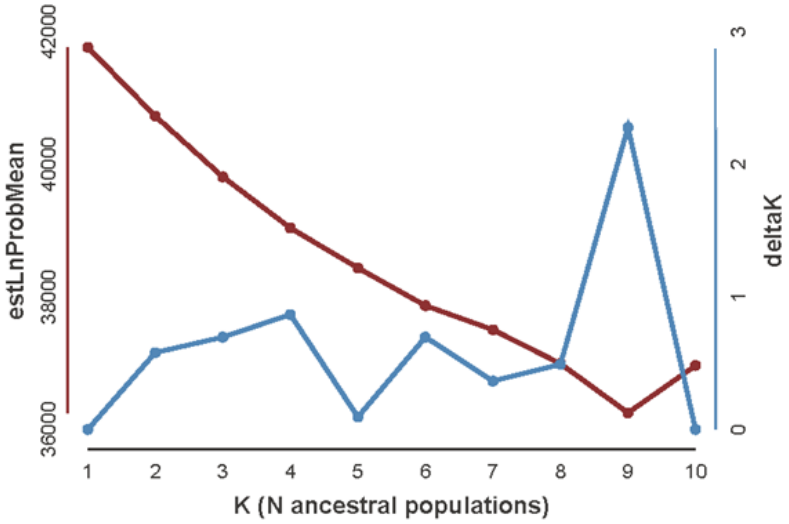

Figure S3. Plot of estimators used to select the optimal number of genetic clusters (K) in the STRUCTURE analysis. Estimated log mean probability assesses how well the model with a particular K explains the genetic data. As the log-likelihood values are negative, the best-fit K-value is the least negative. Delta K estimates the rate of change in the log probability of data between successive K values. The K value corresponding to the highest delta K and estimated log mean probability (K=9) was considered the best estimate of the number of subpopulations in this dataset.