TITLE: UPSLOPE DIVERSIFICATION AND GENE FLOW AMONG CONGENERIC ANDEAN TREES

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ABSTRACT

The high biodiversity of tropical Andean forests is characterized by many closely related species which turnover rapidly across elevation and environmental gradients. Yet, the eco-evolutionary relationships among these congeneric species remain 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 use the genus *Prunus* (cherries) as a model to investigate these processes by (1) reconstructing the phylogenetic relationships and timing of divergence among sympatric species and (2) assessing inter-specific gene flow and its correspondence to evolutionary and ecological distance. Extensive population-level sampling was conducted for species which co-occur along an elevational gradient in Manu National Park, Peru. Additional samples were included for species which co-occur across a gradient in Madidi National Park, Bolivia. Phylogenetic relationships reconstructed a pattern of upslope adaptive diversification concurrent with Andean orogeny during the Miocene. However, despite these ancient diversification events, gene flow analyses found species are not reproductively isolated, and gene flow has occurred through time. Additionally, gene flow is widespread and does not appear to be constrained by evolutionary or ecological distance. Together, these findings provide insight into the processes that have shaped tree diversity in the tropical Andes and suggest that during adaptive radiations, observable ecological, and morphological differences may emerge long before complete phylogenomic isolation, even amid ongoing gene flow.

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

INTRODUCTION

Most ecological theory surrounding diversity and coexistence assumes 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 a key driver of diversification in 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). 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.

The tendency for inter-specific gene flow and hybridization to occur amongst closely related, sympatric trees has been widely documented in temperate zones (e.g. oaks – Cavender-Bares et al. 2018, spruce – De La Torre et al. 2014, birch – Tsuda et al. 2017, poplar – Chhatre et al. 2018, pine – 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 inter-specific gene flow), determined by population densities and pollinator abundance (Hamrick and Murawski 1990; Ward et al. 2005; Cannon and Lerdau 2015). When present, conspecific

62 pollen can outcompete heterospecific pollen, reinforcing assortative mating (Williams et al.
63 1999). However, incomplete reproductive isolation and the ability to accept heterospecific pollen
64 may become adaptively beneficial when conspecific pollen is rare, as the benefit of avoiding
65 local extinction overrides potential negative consequences of hybridization such as reduced
66 hybrid fitness (Barton 2001; Wu 2001; Cannon and Lerdau 2015). Hybridization may be
67 particularly beneficial in times of rapid environmental change, as it can produce novel gene
68 combinations within a single generation, increasing adaptive potential and population resilience
69 (Anderson and Stebbins 1954; Baskett and Gomulkiewicz 2011; Suarez-Gonzalez 2018; Brauer
70 et al. 2023; Kulmuni et al. 2024). Such density-dependent mating has been reported in oaks
71 (Lepais et al. 2009), and may be particularly relevant to tropical trees which maintain extremely
72 low local population densities (Pires et al. 1953; Pitman et al. 1999; ter Steege et al. 2013).

73 Most tropical forest trees are highly outcrossed (Bawa et al. 1985a, b; Hamrick and
74 Murawski 1990; Ward et al. 2005), though individuals retain variable capacities for different
75 mating behaviors, even within populations and across flowering events (Murawski and Hamrick
76 1991; Nason and Hamrick 1997). Self-fertilization has been shown to be inversely correlated
77 with population density (Hamrick and Murawski 1990; Ward et al. 2005), but few studies have
78 assessed the extent of inter-specific gene flow. Those that have (e.g. Schley et al. 2020 –
79 *Brownea*, Larson et al. 2021 – *Eschweilera,* Schley et al. 2025 – *Inga*), detected wide-spread
80 admixture. Evolutionary distance may be one important constraint on inter-specific gene flow
81 among congeners in sympatry, as hybridization is thought to decrease with increasing divergence
82 due to the accumulation of pre- and post-zygotic barriers through time (Mendelson et al. 2004;
83 Levin 2012; Comeault and Matute 2018). Linan et al. (2021) found evidence of this in the
84 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 step-wise 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 which 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 true across taxa (e.g. birds – Terborgh 1977; fungi – Meier et al. 2010; microbes – Nottingham et al. 2018; mammals – Patterson et al. 2003, Mena and Pacheco 2020), and is especially true for forest trees (e.g. Jankowski et al. 2012). While many tree species have elevation ranges of <500 m, the distributions of entire genera are often much broader (Griffiths et al. 2020), with congeneric species occurring in partial sympatry, especially at range margins. 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. Melastomataceae – Dudley 1978, *Espletia* – Pouchon et al. 2018, lupines - Hughes and Eastwood 2006, Nevado et al. 2016, bellflowers – Lagomarsino et al. 2016, *Hypericums* - Nörk et al. 2013, orchids - Pérez-Escobar et al. 2017, and bromeliads – Givnish et al. 2011, Silvestro et al. 2014). 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 to occur 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 tree-line (~3800 m) into the lowlands (<500 m), though the ranges of individual species are typically 500-1000 m or less (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 (Hodel et al. 2021), the exclusively South American species form a strongly supported clade nested within the tropical Racemose group (Hodel et al. 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. x *P. cerasifera* Ehrh. – 'black apricots,' *P. salicina* x *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*.

Here, we use *Prunus* as a model to 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 reproductively isolated, and, if not, (3) is inter-specific gene flow related to evolutionary or ecological distance*. To do this, we reconstructed the phylogenetic relationships among sympatric species, dated the timing of divergence, and tested for historical

154 introgression and recent admixture. The results of these analyses expand current understanding
155 of the origin and maintenance of tropical Andean tree diversity, and provide important context
156 for predicting how these species may respond to rapid environmental change.

157 MATERIALS AND METHODS

158 *Study site and sample collection*

159 Sampling for this study primarily focused on species which co-occur across the Andes
160 Biodiversity and Ecosystem Research Group (ABERG; andesbiodiversity.org) plot network in
161 Manu National Park, Peru (Figure 2). This site is located on the eastern slope of the tropical
162 Andes in southeastern Peru and is a 3.5 km elevational gradient from the Andean highlands to
163 the Amazonian lowlands. With 23-1 ha and 33-0.1 ha forest plots along a series of descending
164 ridges, this is the most continuous transect in the tropical Andes. To date, ~19,000 individual
165 trees have been documented in these plots from 100 families, 292 genera, and 1255 species
166 (Farfan-Rios 2019).

167 Intensive field sampling was conducted for *Prunus* species along this transect, with
168 botanists searching in lateral transects centered on the 1-ha plots (approximately every 250 m)
169 for up to 6 hrs. Leaf tissue was field collected and stored in silica following the Smithsonian
170 Institution guidelines for collecting vouchers and tissues intended for genomic work (Funk et al.
171 2017). Samples were also included for species known to co-occur across an additional plot
172 network spanning 4 km of elevation in the Madidi region of Bolivia known as the Madidi
173 Project, and from at least one external population when possible. These samples were collected
174 from herbarium specimens previously not treated with alcohol or fresh tissue stored in silica
175 collected from individuals in the Cotapata region of Bolivia (Table 1, Figure 2). In total, we
176 sampled 158 individuals from 13 species including two outgroups (*P. myrtifolia* and *P.*

caroliniana) and a reference (Table 1). See Appendix for species descriptions adapted from Pérez-Zabala 2022 and Achá 2013.

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

Reads were assembled into orthologous loci using reference-based assembly in ipyrad. Default settings were used to trim and filter reads and the clustering threshold was set to 0.85 as recommended by the ipyrad manual and McCartney-Melstad et al. (2019) for phylogenetic analyses. For the reference-based assembly, reads were mapped to the *Prunus persica* (peach) genome (GCF_000346465.2; The International Peach Genome Initiative et al. 2013, Verde et al. 2017). The proportion of mapped reads varied across samples (mean = 67%, s.d. = 13%), but variation in the quality of sample preservation and DNA extractions likely explain much of the variation. When calling consensus alleles, we allowed for up to 4 alleles per sample following Donoghue et al. (2022), as all *Prunus* species in the tropical Racemose group studied to date are tetraploids (2n=32; Hodel et al. 2023), but ploidy is not known for most of the species in our study. The final reference-mapped dataset comprised 35,099 loci, representing non-overlapping

genomic regions where data from at least 4 samples passed filtering. Further downstream analyses were performed using vcftools and the ipyrad-analysis toolkit, which allows for filtering and subsampling loci, genomic windows, and/or SNPs for phylogenetic inference and D-statistics.

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 tree 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 tree under the rapid hill-climbing algorithm, with support values from 100 bootstrap replicates. The tree 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 tree (n=13). The samples selected grouped with other

223 members of their species on the individual-level tree and had the most data present. Again, we
224 used the ipa.window_extracter module in the ipyrad-analysis toolkit to extract data from the
225 chromosomes and filter sites, selecting those shared by at least 25% of all species (after testing
226 multiple filtering thresholds). The resulting sequence matrix is 4.2 Mb with 62,262 SNPs and
227 63.6% missing data. To reconstruct the phylogenetic relationships, we used both maximum
228 likelihood (RAxML) and Bayesian (ExaBayes) analyses. We ran RAxML using GTR+GAMMA
229 to infer a best tree under the rapid hill-climbing algorithm with support values from 100
230 bootstrap replicates. For ExaBayes, we ran two Metropolis-coupling replicates with four
231 coupled-chains (each with three heated chains) for $1x10^6$ MCMC generations, sampled every
232 500 generations. Estimated sample size (ESS) for all parameters and branch lengths were
233 summarized with the ProcParam tool and confirmed as sufficiently sampled. The majority-rule
234 consensus tree was generated with the ExaBayes 'consense' tool with a 25% burn-in.

235 To assess the robustness of the resolved relationships and distinguish between conflicted
236 and poorly informed branches, we used Quartet Sampling (Pease et al. 2018;
237 https://www.github.com/ fephyfofum/quartetsampling). This method randomly samples groups
238 of four taxa from the provided tree and then evaluates each of the three possible unrooted
239 topologies to determine which best fits the data. The frequency at which each of these possible
240 topologies appears is recorded and then aggregated across quartets to determine whether they are
241 concordant or discordant with the larger tree topology. Discordance is quantified with four
242 metrics – quartet concordance (QC), quartet differential (QD), quartet informativeness (QI), and
243 quartet frequency (QF). We performed Quartet Sampling on the resulting RAxML tree with the
244 full concatenated alignment, allowing 100 replicates per branch (Figure S2).

245 *Divergence time dating*

To assess the timing of divergence among species, we generated a time-calibrated phylogeny using BEAST v. 2.7.7 (Bouckaert et al., 2019) with the same alignment used for the species-level phylogenies. We used an optimized relaxed molecular clock and Yule speciation process with the GTR+GAMMA substitution model. A nodal calibration was set for the crown age of *Prunus* using a normal prior with an offset of 61.5 Mya and a standard deviation of 3.0 Mya (Chin et al. 2014; Su et al. 2023). The crown age of the racemose group was calibrated from a staminate flower of *Prunus hirsutipetala* D.D. Sokoloff, Remizowa et Nuraliev preserved in the late Eocene (Priabonian) Rovno amber from northwestern Ukraine (Sokoloff et al. 2018), using a normal prior with an offset of 35.0 Mya and standard deviation of 2.0 Mya (following Su et al. 2023). To ensure a more rapid convergence of MCMC runs, we used our RAxML topology as our starting tree. The Markov chain Monte Carlo (MCMC) run was conducted for 20,000,000 generations and trees sampled every 2000 generations producing a total of 10,000 trees. The resulting log files were checked in Tracer v.1.5 (Rambaut et al. 2018) and we used TreeAnnotator v.1.10.4 to generate the maximum clade credibility tree discarding 25% of trees as burn-in before visualizing the results in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Tests of historical introgression

To test for historical introgression, we used the four-taxon D-statistic (ABBA-BABA), which quantifies allele frequency patterns that are incongruent with the hypothesized species tree topology (Durand et al. 2011). Sub-sampling alleles across sets of three species and an outgroup [((P1, P2), P3) O], the ancestral allele is denoted as “A” and the derived allele as “B”. With this topology there are two parsimony-informative patterns of discordant sites, of which the pattern “ABBA” represents sites where P2 and P3 share the derived allele and the pattern “BABA” represents sites where P1 and P3 share the derived allele (Hibbins and Hahn 2022). The

frequencies of ABBA and BABA site patterns are then summed across many genomic loci. Under incomplete lineage sorting (ILS) these discordant patterns are expected to be equal (D=0), and if the number of ABBA and BABA sites differ significantly this asymmetry is inferred as evidence of introgression between the species sharing the derived state ("B") more frequently (Hibbins and Hahn 2022). Here, we ran the ABBA-BABA tests using the ipa.baba tool as implemented in ipyrad, which is specifically designed for analysis of RAD-seq datasets. We used the species-level RAxML tree as our starting tree hypothesis and constrained the tests setting P4 to *P. myrtifolia*, so all tests would focus on ingroup species. To assess significance, we performed 1000 bootstrap replicates for each test with loci resampled with replacement. Z-scores were then calculated as the number of bootstrap standard deviations that the D-stat deviates from zero (Table 2). Z-scores > 2.5 were considered significant.

Tests of recent admixture

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

292 Genetic diversity was estimated within individuals and species to compare to the
293 admixture results. In VCFtools v.1.16 (Danecek et al. 2011), sequences were filtered using two
294 different missing data thresholds, retaining loci present within at least 50% or 70% of the
295 samples for each species. Sites were also filtered if they had a minor allele frequency less than
296 0.05. We calculated nucleotide diversity (π) for each species – the average pairwise nucleotide
297 differences between sequences in a sample at a site as well as observed (H_o) and expected
298 heterozygosity (H_e) – the proportion of loci where an individual is heterozygous vs. the
299 proportion expected by Hardy-Weinberg equilibrium – within samples and averaged across the
300 species. For these calculations, *P. debilis* and *P. detrita* samples were grouped together as well as
301 *P. huantensis* and *P. brittoniana,* given that STRUCTURE (Figure 5) did not detect any distinct
302 genetic clusters among these species' pairs. Species with <3 samples were excluded from these
303 analyses given the lack of statistical power from such a small sample size.

304 RESULTS

305 *Phylogenomic relationships and elevational niche diversification*

306 Both the species-level maximum likelihood (RAxML) and Bayesian (ExaBayes)
307 phylogenetic analyses reconstructed the same evolutionary relationships among the focal Andean
308 *Prunus* species and their outgroups (Figure 3). The trees were rooted to the reference (*P.*
309 *persica*), with *P. caroliniana* diverging before the tropical species and *P. myrtifolia* sister to the
310 Andean species, supporting the results of prior studies (Chin et al. 2014; Hodel et al. 2023).
311 Among these partially sympatric Andean species, diversification generally occurred from the
312 lowlands to the highlands, with the lineage including *P. debilis* and *P. detrita* diverging first,
313 followed by the mid-elevation cloud-forest species (*P. herthae, P. pleiantha, P. stipulata, P.*
314 *integrifolia*) and finally the high-elevation species as the most recent diversification events (*P.*

315 *huantensis, P. brittoniana, and P. antioquensis*). Statistical support for the inferred relationships
316 was assessed using bootstrap support (RAxML), posterior probabilities (ExaBayes) and Quartet
317 Sampling. While the tree nodes are generally well-supported (all posterior probabilities = 1, most
318 bootstrap values >75; Figure 3), Quartet Sampling (Pease et al. 2018) indicated that low
319 bootstrap support values are not likely the result of insufficient phylogenetic signal, as QI values
320 ranged from 0.8 – 1.0. Moreover, the lack a strong skew in discordance at most nodes (QD > 0.5)
321 suggests that incomplete lineage sorting (ILS) may instead be responsible (Pease et al. 2018).
322 One node resulted in both weak support and a discordant skew (0.21/0.22/1, *P. huantensis*, *P.*
323 *brittoniana*, *P. antioquensis; Figure S2*), indicating a possible secondary evolutionary history
324 (Pease et al. 2018).

325 *Divergence time estimation*

326 The time-calibrated phylogeny generated in BEAST recovered the same tree topology as
327 RAxML and ExaBayes with similar branch support (Figure 4). The estimated divergence time
328 *for P. myrtifolia* and the ancestor of the Amazonian and Andean species is 27.3 Ma (95% highest
329 posterior density [HPD] 22.4-31.7). The Andean species began diverging at the start of the
330 Miocene, with the low-elevation *P. debilis*/*P. detrita* lineage branching off 23.5 Ma (95% HPD
331 19.4-28.1), and from each other ~17.6 Ma (95% HPD 12.8-22.6). The cloud forest species
332 followed, with *P. pleiantha* diverging ~20.4 Ma (95% HPD 16.3-24.4), and *P. integrifolia* ~18.0
333 Ma (95% HPD 14.2-21.7). The remaining lineages emerged in the mid to late-Miocene (15.9-
334 9.3) with the highest elevation clade diverging last, with *P. huantensis* ~11.6 Ma (95% HPD 8.6-
335 14.9) and *P. antioquensis*/*P. brittoniana* ~9.3 Ma (95% HPD 6.2-12.3).

336 *Tests of historical introgression and recent admixture*

D-statistics indicated significant historical introgression events between *P. pleiantha* x *P. debilis/P. detrita*, *P. pleiantha* x *P. huantensis/P. antioquensis*, and *P. debilis* x *P. antioquensis* (Figure 3, Table 2). There is also evidence of introgression between *P. pleiantha* x *P. debilis* and *P. pleiantha* x *P. detrita*, although it is difficult to disentangle this from introgression between *P. pleiantha* and the ancestor of *P. debilis*/*P. detrita*. The introgression event detected between *P. pleiantha* x *P. huantensis/ P. antioquensis* is further supported by the results of Quartet Sampling (Figure S2).

STRUCTURE analysis found K=9 to be the most likely number of distinct genetic clusters within the dataset, determined by mean log probability and DeltaK values (Figures 5, S3). Individuals largely clustered according to their morphologically assigned species; however, the number of detected clusters was fewer than the expected number of named taxonomic species in the analysis (n=10). *P. integrifolia* and *P. amplifolia* are largely reproductively isolated, though low levels of admixture were detected in *P. integrifolia* and only one sample was included for *P. 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 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 5), 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 4). Across species and filtering thresholds,

359 nucleotide diversity (π) was high (50%: 0.41 – 0.64, 70%: 0.37 – 0.51) compared to H_o, π
360 tracked with H_e as expected, and H_o (50%: 0.06 – 0.15, 70%: 0.06 – 0.17) was far below H_e
361 (50%: 0.41 – 0.64; 70%: 0.37 – 0.51; Table 3). However, the estimates for *P. herthae* and the *P.*
362 *debilis/detrita* group should be interpreted cautiously given the low sample sizes for each.

363 DISCUSSION

364 The phylogenomic data presented here suggest that congeneric *Prunus* species known to
365 co-occur as elevational replacements in the tropical Andes diversified upslope from the lowlands
366 into the highlands during the Miocene (lower montane lineage ~23.5 Ma, cloud forest species
367 ~20.4 – 13.4, high-elevation clade ~11.6 – 9.3 Ma) in a pattern consistent with adaptive
368 diversification. However, despite the ancient diversification events, species are not
369 reproductively isolated, as gene flow has occurred among species through time and is not related
370 to evolutionary or ecological distance. Together, these findings provide insight into the processes
371 that have shaped tree diversity across elevation gradients in the tropical Andes, and suggest that
372 during adaptive radiations, observable ecological and morphological differences may emerge
373 long before complete genomic isolation, even in the presence of ongoing gene flow.

374 *Upslope diversification and biogeographic implications*

375 Phylogenomic reconstructions and divergence time dating support the hypothesis that *Prunus*
376 diversified upslope, likely in response to novel habitat opportunities as has been observed in
377 other plant clades (e.g. Melastomataceae – Dudley 1978, *Espletia* – Pouchon et al. 2018, lupines
378 - Hughes and Eastwood 2006, Nevado et al. 2016, bellflowers – Lagomarsino et al. 2016,
379 *Hypericums* - Nürk et al. 2013, orchids - Pérez-Escobar et al. 2017, and bromeliads – Givnish et
380 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 step-wise, 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 idea 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 heterogeneous across space, 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).

Andean cloud forests are distinct habitats that occur from ~1200 m to the upper forest limits at ~3200-3800 m (depending on latitude), and are characterized by persistent cloud cover and immersion. Although they are the most speciose habitats in the Andes (Pérez-Escobar 2022), 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). 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). 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 phase began in the North-Central and Central Andes (Boschman et al. 2021; Pérez-Escobar 2022). Species in this elevation zone are exposed to unique high-elevation stressors such as night frost, with temperatures dipping below 0°C, requiring further physiological specialization (Pérez-Escobar 2022).

Gene flow through time

Analyses also indicated that these partially sympatric, congeneric trees are not reproductively isolated. Introgression and admixture have occurred throughout the evolutionary history of the Andean *Prunus* clade. D-statistics detected at least five significant ancient introgression events. Two of these events occurred among lineages separated by great evolutionary and ecological distance (*P. pleiantha* x *P. huantensis/P. antioquensis*, and *P. debilis* x *P. antioquensis).* Introgression was also detected between *P. pleiantha* and the ancestor of *P.*

426 *debilis/P. detrita* (*P. pleiantha* x *P. debilis/P. detrita*) as well as among *P. pleiantha* x *P. debilis*
427 and *P. pleiantha* x *P. detrita* individually. Although difficult to disentangle, especially with few
428 samples and loci, it suggests that interspecific hybridization occurred among the ancestors of
429 these lineages and has continued as they diverged.

430 Historical introgression among species separated in ecological space across the elevation
431 gradient is plausible for several reasons. While the medians of their elevation ranges may be
432 quite different, they are found in close geographic proximity, and their ranges do overlap (or are
433 much closer) at the range margins. *Prunus* species are thought to be obligate outcrossers with
434 generalist pollinators, as is common in tropical trees (Ashton 1969; Bawa 1974, 1979), though
435 the extent of self-incompatibility across the genus and its potential breakdown with
436 polyploidization is still unclear (Hauck et al. 2006, Tao et al. 2010, Aguiar et al. 2015). As some
437 generalist pollinators forage over kilometers (Bawa et al. 1985), opportunity for introgression is
438 possible, especially given that many Andean species flower semi-synchronously during the dry
439 season. Additionally, species ranges are not stagnant through time. Pollen records from Lago
440 Consuelo (1360 m a.s.l., located in southeastern Peru adjacent to the Bolivian border) show that
441 during the last full glacial period (43.5 – 22,000 yr BP), individual cloud forest species migrated
442 downslope at least 1000 – 1700 vertical m in response to lower cloud base and cooler
443 temperatures (-5ºC; Urrego et al. 2005). Today, these species are as far apart as they have likely
444 been during the Quaternary, thus these historical introgression events may be signatures of past
445 ecological overlap.

446 Tests of recent admixture also found gene flow to be widespread among Andean *Prunus*
447 species. Though *P. integrifolia* and *P. amplifolia* are (to an extent) genomically isolated, gene
448 flow is rampant among all other species. The isolation of *P. integrifolia* is particularly interesting

as this species has the widest elevational range and is one of the most abundant species in the tropical Andes. Gene flow was especially high among *P. pleiantha, P. stipulata*, and *P. herthae*, all cloud forest species with largely overlapping elevation ranges. However, these species did mostly form monophyletic groups in our individual-level phylogeny (Figure S1), indicating that despite gene flow, these are distinct evolutionary lineages. Given that *P. pleiantha* was identified in most of the historical introgression events and those events are further supported by the genetic clusters detected by STRUCTURE, it seems this species readily hybridizes when in contact with close relatives. Further investigation is needed to test this and identify the directionality of such gene flow. The algorithm did not distinguish *P. debilis* and *P. detrita* as distinct genetic clusters, possibly due to how closely related these species are, introgression, and the low number of individuals for both species in the dataset. Similarly, *P. huantensis* and *P. brittoniana* are indistinguishable, which is unsurprising given that *P. brittoniana* was nested within *P. huantensis* in the individual-level phylogeny (Figure S1). This supports the most recent taxonomic revision by Pérez-Zabala (2022) which considered *P. brittoniana* and *P. huantensis* to be the same species given their morphological and ecological similarities. Finally, the genetic cluster indicated in orange (Figure 5) is the only cluster that does not appear to be dominant in any one species, possibly representative of a co-occurring species not captured in our sampling or a ghost ancestral lineage (Ottenburghs 2020; Tricou et al. 2022).

Estimates of genomic diversity further support this evidence of recent admixture, as the individuals in each species that appear the most admixed in STRUCTURE also have the highest observed heterozygosity (H_o). *P. integrifolia* also shows moderate H_o but it is more consistent across individuals, likely a result of its larger population sizes. Additionally, nucleotide diversity (π) is moderate to high and expected heterozygosity exceeds observed ($H_e >> H_o$) across species,

472 a signal of a possible historical bottleneck. This is because during bottlenecks, H_o declines much
473 faster than π and recovers much slower, as H_o is more sensitive to allele frequency changes (Nei
474 et al. 1975; Chakroborty and Nei 1977). Future work will aim to test this.

475 *Conclusions*

476 Overall, these results suggest that adaptive diversification following the emergence of
477 novel gradients has been important for within-genus diversification of Andean trees, particularly
478 in the genus *Prunus*, while immigration and sorting of pre-adapted clades may be the dominant
479 mechanism shaping community assembly at higher-level taxonomic levels (Segovia et al. 2020,
480 Griffiths et al. 2020, Linan et al. 2021). For most of the Neogene, *Prunus* diversified with the
481 rise of the Andes, developing sequential elevational replacements with narrow ranges, specially
482 adapted to live in radically different environmental conditions from the Amazonian lowlands to
483 Andean tree line. This has important implications in the context of climate change, as species are
484 now shifting their ranges to track their preferred temperatures (Feeley et al. 2011; Fadrique et al.
485 2018). Though most species will not be able to migrate fast enough to keep pace with climatic
486 shifts and are instead experiencing range retractions at the warmer-end (Duque et al. 2015;
487 Farfan-Rios et al. 2025 – *in review*), any upslope range shift may increasingly result in range
488 overlap among congeneric species and potential gene flow (e.g. Quinlan et al. 2025)

489 Here, we found evidence of both historical introgression (possibly in response to
490 Quaternary range shifts) and widespread admixture among co-occurring *Prunus* species. Yet
491 despite gene flow, these species have maintained distinct evolutionary lineages with identifiable
492 ecological and morphological differences since the Miocene. This aligns with the syngameon
493 model, wherein species maintain incomplete reproductive barriers but primarily experience
494 divergent selection (Lotsy 1925; Grant 1971). Though oaks are the classic example (e.g. Cannon

and Petit 2020), such genomic mutualism may be an important yet under-appreciated mechanism in the maintenance of diversity in hyper-diverse tropical forests, where individuals predominantly outcross but retain some capacity for self-fertilization and interspecific hybridization at low population densities (Wu 2001; Cannon and Lerdau 2015). Further work is required to understand the prevalence of syngameons in tropical forests and their role in species' responses to rapid environmental change, namely resilience and avoidance of local extinction. Incorporating these complex dynamics into demographic models may be important for accurately predicting the futures of Andean forests and effective conservation and management under current climate change.

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FIGURES AND TABLES

Table 1. 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**

Table 2. 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 3. 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 4. 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

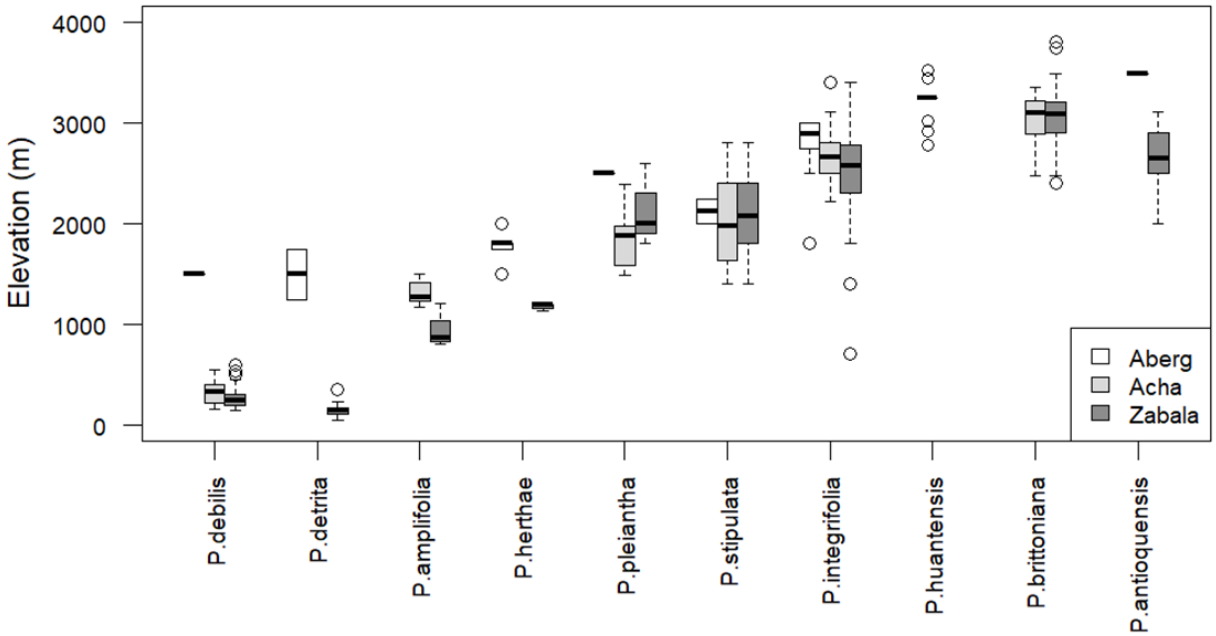

Figure 1. Box plots showing elevational distributions of the in-group Andean species across three different sources. “Aberg” includes all known individuals along the ABERG transect >10 dbh over 20 years. “Acha” includes all Bolivian material reviewed by Achá (2013) and “Zabala” includes all material 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.

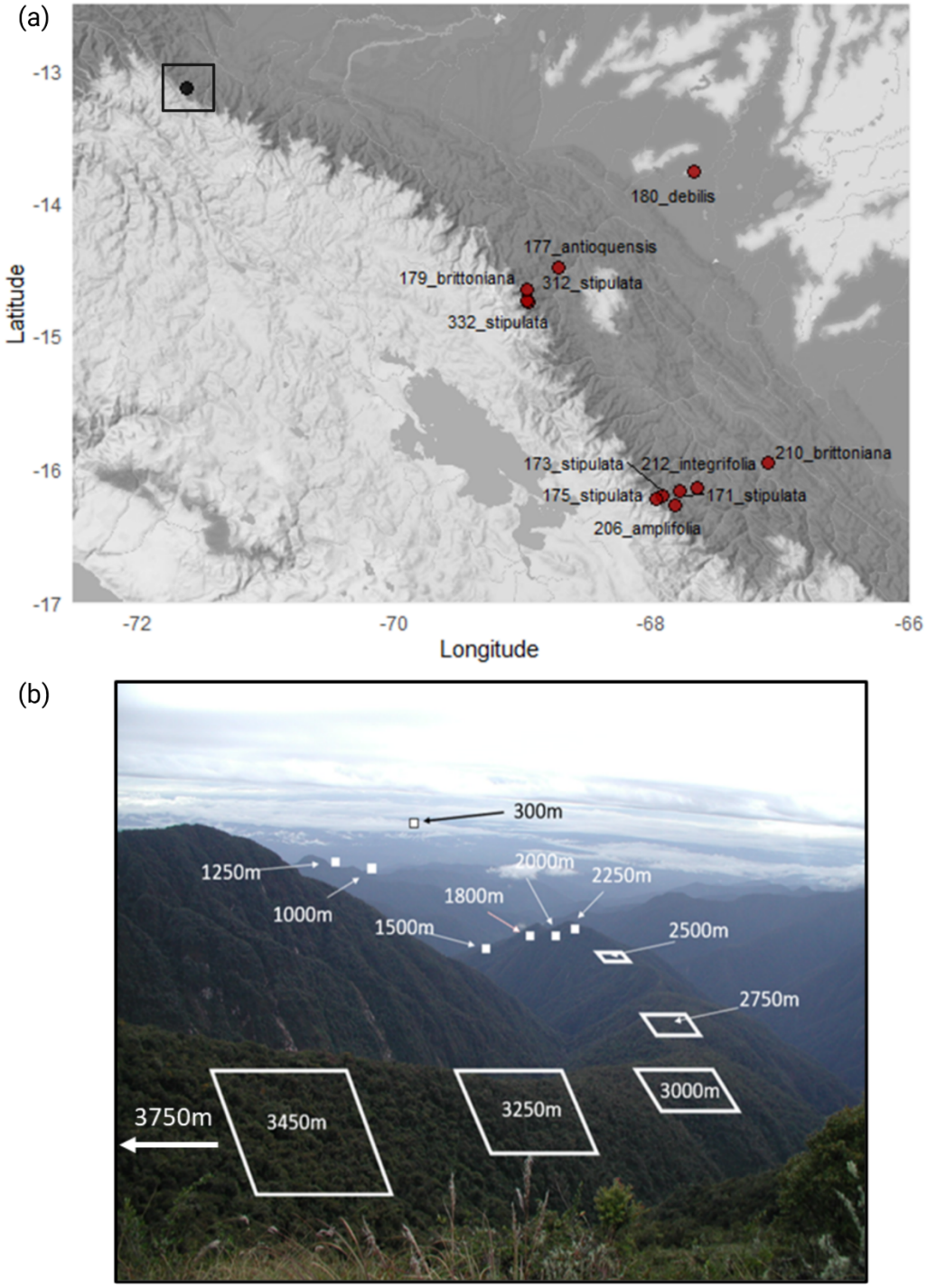

Figure 2. (a) Map of samples included in this study. Black dot indicates the location of the ABERG transect in Manu National Park, Peru where most samples were collected for this study. Red points indicate locations of additional samples included in the study. (b) Image of the ABERG transect highlighted by the box in (a), showing location of 1-ha plots. Further details on individual samples provided in Table 1.

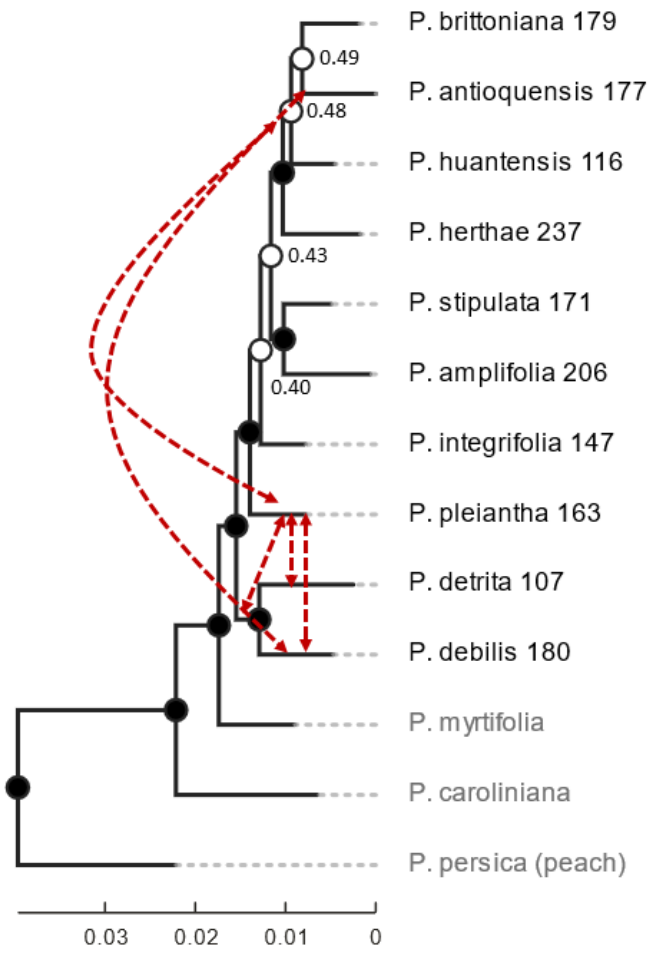

Figure 3. Species-level topology resulting from RAxML and ExaBayes phylogenetic analyses. Nodes show RAxML bootstrap support values where black circles indicate values > 75 and values < 75 are shown on white nodes. All Bayesian posterior probabilities were equal to 1. The tree is rooted to the reference (*P. persica*) and all three outgroup species are shown in grey. Dotted arrows summarize patterns of historical admixture inferred from D-statistics.

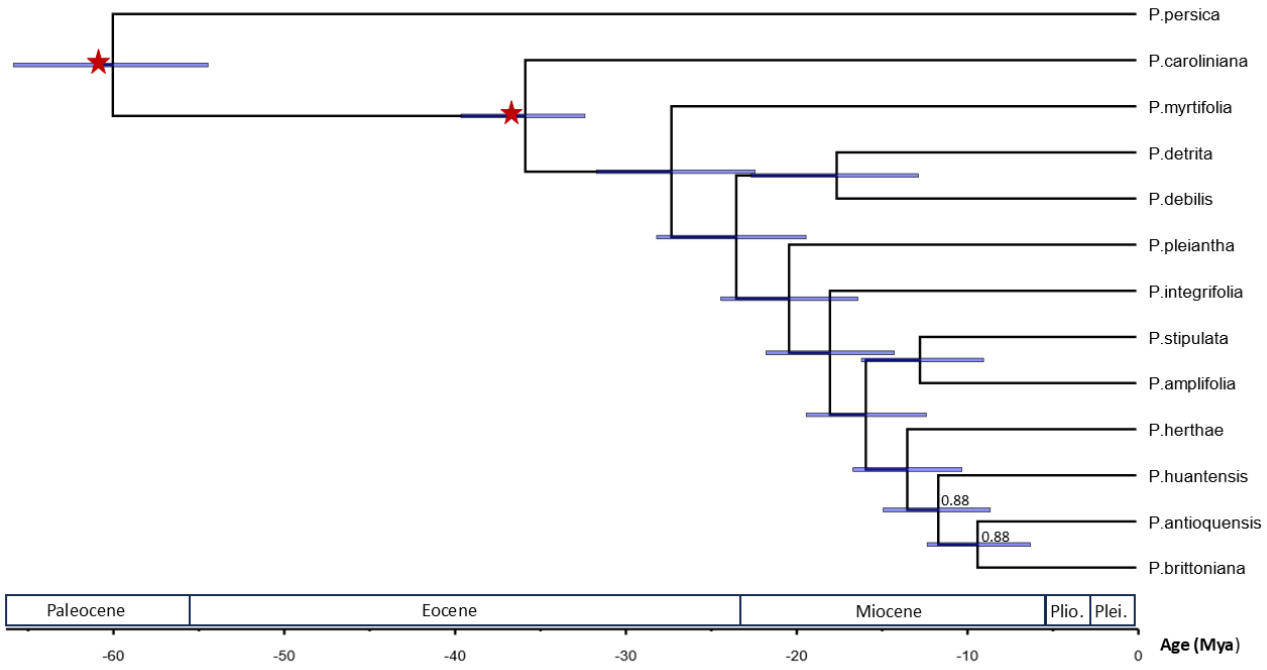

Figure 4. Time-calibrated phylogeny generated from BEAST analysis. Posterior probabilities are shown for nodes when < 1. Node bars represent the 95% highest probability density (HPD) interval for node ages. Red stars indicate the fossil calibration points.

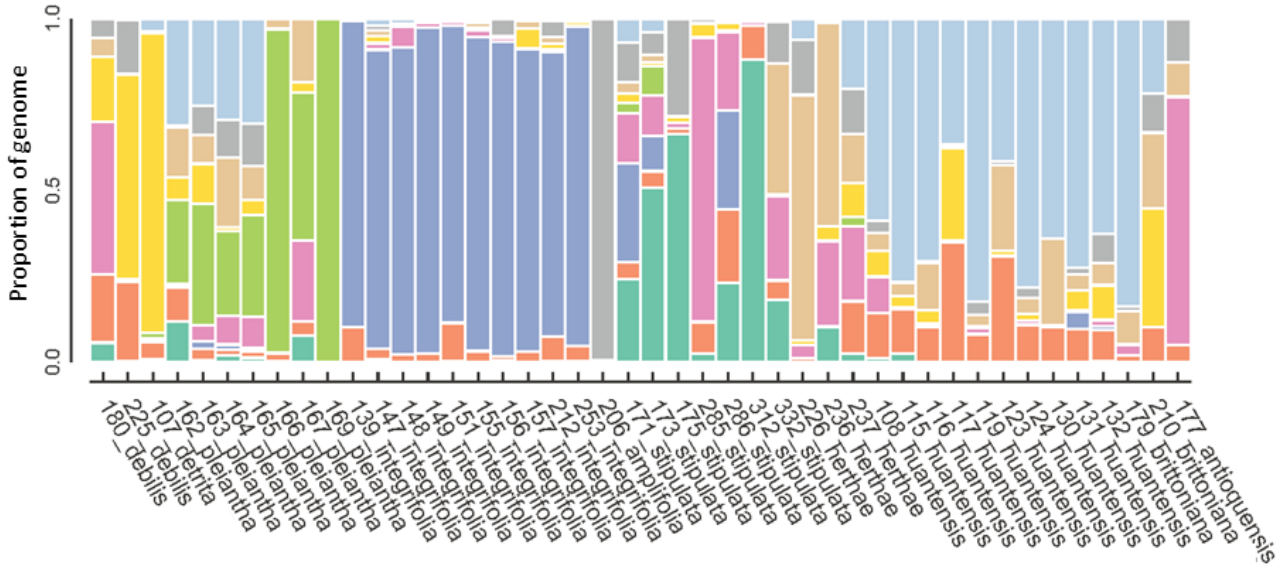

Figure 5. Patterns of genetic structure among individuals of sympatric species. Each vertical bar represents a single individual and the y-axis indicates the proportion of genetic ancestry of that individual assigned to different inferred genetic clusters, designated with different colors. STRUCTURE detected K=9 distinct genetic clusters as the most likely grouping (Figure S3).

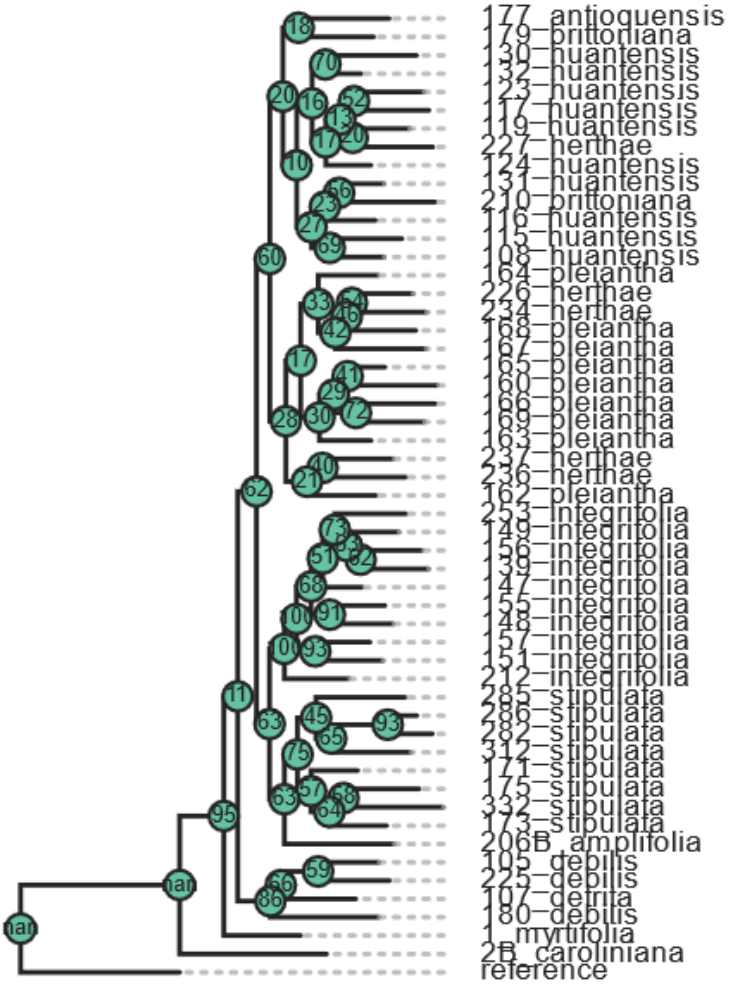

Figure S1. Maximum likelihood individual-level phylogeny reconstructed in RAxML. Individuals were selected to include those with at least 1000 loci per sample and up to 10 individuals per species. Sites were filtered to only include those with at least 25% coverage across samples. Tree was rooted to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

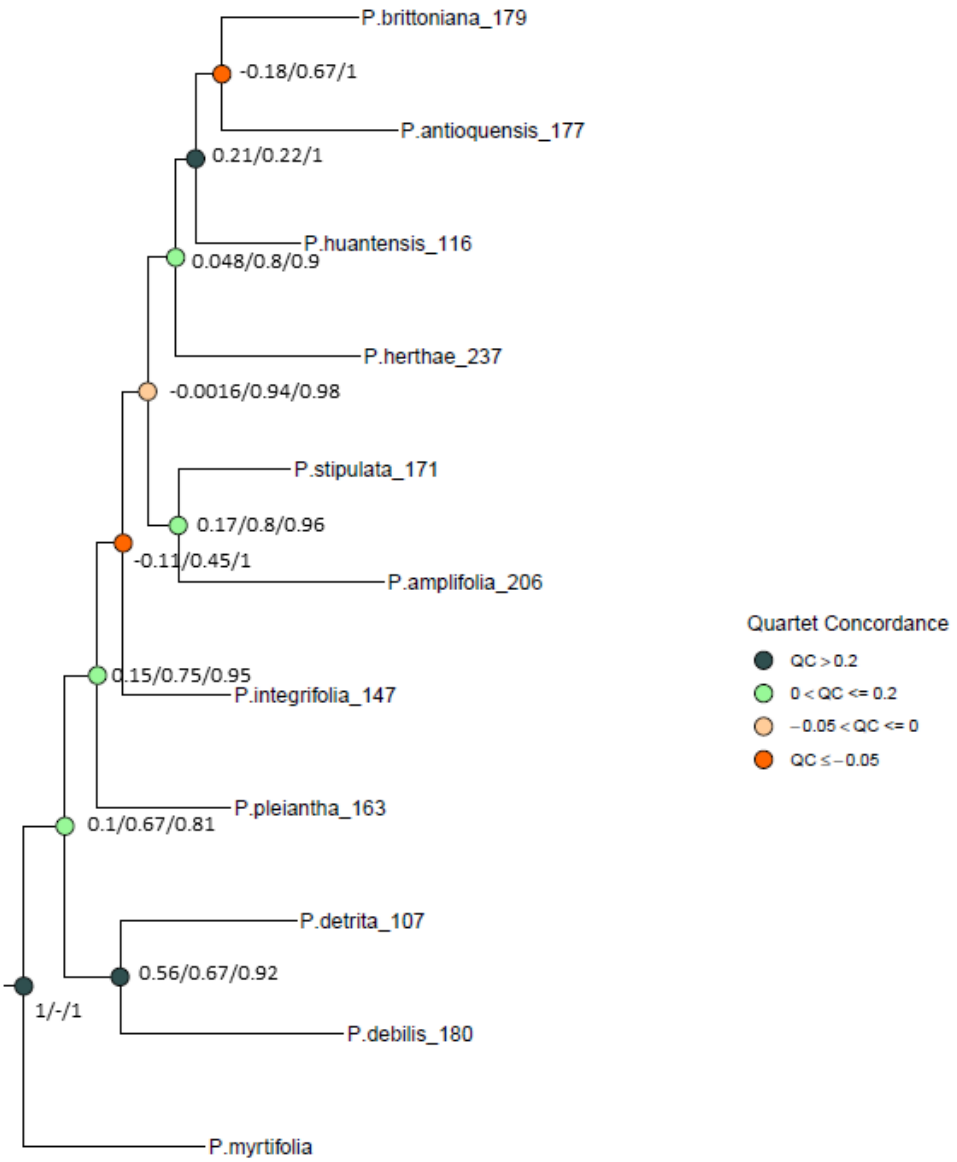

Figure S2. Quartet sampling results with quartet concordance (QC)/quartet differential (QD)/quartet informativeness (QI) indicated next to nodes. Nodes are colored according to QC scores, indicating how often the concordant quartet is inferred over both discordant quartets. Outgroup samples *P. persica*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

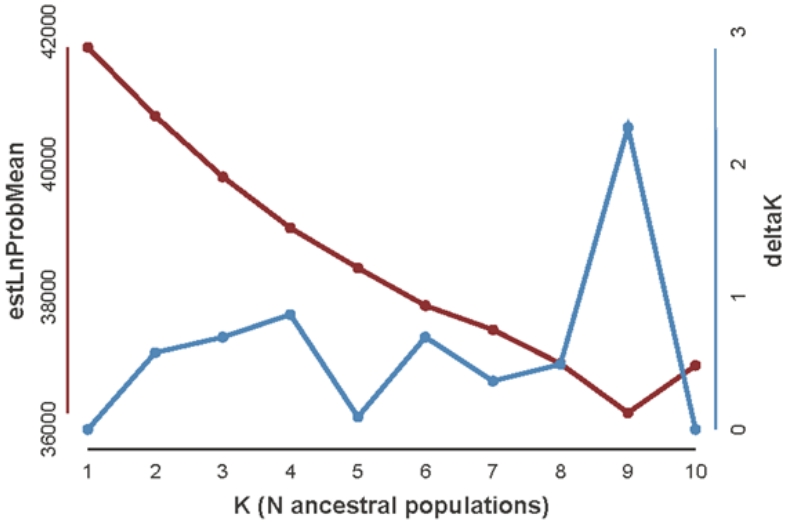

Figure S3. Plot of estimators used to select the optimal number of genetic clusters (K) in the STRUCTURE analysis. Estimated log mean probability assesses how well the model with a particular K explains the genetic data. As the log-likelihood values are negative, the best-fit K-value is the least negative. 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.