Vines in the Neotropics: Phylogenomics, biogeography and systematics in passion flowers (*Passiflora* subgenus *Decaloba* section *Decaloba*).

By

Serena Isabel Acha Macias

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Advisory Committee

Nathan Muchhala Ph.D.

Chairperson

Robert Ricklefs Ph.D.

Christine Edwards Ph.D.

John MacDougal Ph.D.

Abstract

With 600 species, *Passiflora* is a large, morphologically complex and broadly distributed genus in Passifloraceae that represents a major challenge to scientists interested in understanding the evolutionary history of tropical vines. *Passiflora* has been divided into subgenera, super sections and sections. One of the most enigmatic and species-rich (~120 spp.) groups in *Passiflora* is section *Decaloba*, which occurs in the Neotropics and is particularly diverse in Andean montane forests. In this study, we used phylogenomic and population genomic approaches to investigate the evolutionary history, biologeography, species boundaries, and taxonomy of *Passiflora* section *Decaloba*. We sampled herbarium specimens, extracted DNA, and employed a high-throughput DNA sequencing technique called 2b-RAD to generate DNA sequence data for phylogenetic and population genetic analyses. In the first chapter, we reconstructed the phylogeny and biogeographic history for this section and found that section *Decaloba* originated in Central America around 10.4 Ma, and then dispersed to South America across the Panama isthmus, where it diversified and later colonized the Caribbean and lowland South America. In the second chapter we reconstructed the phylogeny and analyzed the population genetic structure and morphology of a challenging clade in section *Decaloba*, the "alnifolia group," to understand how the lineages correspond to the current taxonomy of the group. We discovered that the "alnifolia group" likely represents a rapid radiation, that patterns of genetic variation do not correspond to described species, and that several species need a new circumscription. The third chapter used the results from the previous two chapters as the basis for a taxonomic treatment that included nomenclatural changes and new species circumscriptions. Among the most important taxonomic changes was the

synonymy of 4 species from the “alnifolia group”. Overall, the use of herbarium specimens and a rad-seq approach provided a well-resolved phylogeny that allowed us to generate the first phylogenetic framework for *Passiflora* section *Decaloba*, helped elucidate major biogeographic patterns in the genus, as well as patterns of population genetic structure and morphological variation. This approach may be useful for future studies that aim to elucidate the evolutionary relationships among broadly distributed, highly diverse, and poorly accessible groups of plants.

Keywords: 2b-RAD, Phylogenomics, species complex, Andes, Taxonomy

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Chapter I: Phylogenomics and biogeography of *Passiflora* section *Decaloba* (Passifloraceae)

Abstract

Because of their extraordinary flower morphology and highly variable leaf shape, passion flowers (Passifloraceae) have been of interest to naturalists since their discovery. With 600 species, *Passiflora* is the largest genus in the family and represents a major challenge to scientists interested in understanding evolutionary history and relationships in tropical vines. *Passiflora* has been divided in subgenera, super sections and sections. One of the most enigmatic and species-rich (120 spp.) groups in *Passiflora* is section *Decaloba,* which occurs in the Neotropics and is particularly diverse in Andean montane forests. A recent study of the phylogeny of Passifloraceae recovered the monophyly of section *Decaloba*; however, despite the use of multiple DNA regions, the evolutionary relationships and biogeography of species within the clade remained unresolved. The goal of this study was to elucidate the phylogeny and biogeography of Section *Decaloba*. We sampled leaf tissue from the Missouri Botanical Garden herbarium collection, including several accessions of most species. We used a high-throughput DNA sequencing technique called 2b-RAD to sequence a large number of DNA characters from throughout the genome of each accession and the resulting data was processed using iPyrad. We reconstructed the phylogeny based on a data set containing 11,778 loci, using RAxML and BEAST. Additionally, we reconstructed the ancestral area of all the species in this section using RASP. Both of our phylogenetic methods recovered predominantly well-supported trees in which relationships strongly corresponded to geography. We identified two main clades: 1) the Central American clade, within which the majority of

nodes well supported were and species were monophyletic and 2) the South America, that was the biggest clade, but also included some of the least supported nodes and polyphyletic species. RASP analysis showed that section *Decaloba* originated in Central America around 10.4 Ma, and then dispersed to South America across the Panama isthmus and later to the Greater Antilles and the Bahamas. Section *Decaloba* also diversified in the Northern Andes and then dispersed to the rest of South America, including the Lesser Antilles. Results also showed several species complexes that will be the focus of future research. This study to our knowledge, is one of few to have included almost exclusively herbarium samples using a 2b-RAD sequencing approach. We found encouraging the fact that we are recovering supported phylogenetic relationships from species that are only represented in herbarium material and whose field collections would be unfeasible.

Keywords: 2b-RAD, vines, passion flowers, Neotropics, Andes

1. Introduction

Historical processes related to plate tectonics, continental drift, changing climate, together with events like the uplift of the Andes and the Great American Biotic Interchange, have led the Neotropics to become one of the most diverse ecosystems in the world. In total, 15 of the 25 biodiversity hotspots identified by Myers et al. (2000) occur in the Neotropics, and it is the biogeographic region with the highest plant diversity (Gentry, 1982; Ulloa-Ulloa et al., 2017). Because of the high rates of plant diversity and endemism, as well as the relative remoteness and difficulty in studying many Neotropical ecosystems, the region is home to a large concentration of relatively poorly known plant species. Furthermore, although the species-level diversity of herbaceous plants and vines is comparable to that of trees and epiphytes in many Neotropical ecosystems (Linares-Palomino et al., 2009), species with a non-woody habit are often even less understood (Cicuzza et al., 2013), as many large biodiversity inventory studies in the Neotropics have focused primarily on woody tree species (e.g. Simon et al., 2009; Banda et al., 2016). Here, we focus on the evolutionary history of a highly diverse group of vines in the passion flower family Passifloraceae as a model system for improving our understanding of the evolutionary history of vines in the Neotropics.

Passifloraceae, the passion flower family, is one the largest families of vines (Gentry, 1991) and the 64th largest angiosperm family (Christenhusz & Byng, 2016). With more than 600 species, the genus *Passiflora* L. is among the 20 most species-rich genera of vascular plants in the New World (Frodin, 2004; MacDougal & Feuillet, 2015-2019). Most previous studies of *Passiflora* have focused on understanding the extraordinary morphological variation present in the group and advancing alpha taxonomy through new

species descriptions (e.g. Masters, 1872; Killip, 1938). However, the taxonomy of the group has been highly labile in the past, with previous workers dividing the genus into at least 4 and up to 22 subgenera (Killip, 1938; Feuillet & MacDougal, 2004). At present, six subgenera are widely accepted, including *Passiflora, Deidamioides* (Harms) Killip, *Astrophea* (DC.) Mast, *Tryphostemmatoides* (Harms) Killip, *Tetrapathea* (DC.) P.S. Green, and *Decaloba* (DC.) Rchb. (Feuillet & MacDougal, 2004; Krosnick et al. 2013, Buitrago et al. 2018). All six of these subgenera have been supported as monophyletic groups by previous phylogenetic analyses using traditional Sanger sequencing of small numbers of nuclear DNA and plastid DNA markers (Krosnick et al. 2013; Sader et al. 2019). *Passiflora* subgenera have been further subdivided into multiple ranks (supersections, sections, subsection, and series), some of which are supported by morphological, geographical, or molecular data (Kay, 2003; Muschner et al.,2012; Krosnick et al., 2013).

Passiflora subgenus *Decaloba* is currently divided into seven supersections. Supersection *Decaloba* (DC.) J.M. MacDougal & Feuillet is estimated to be one of the youngest in the subgenus and forms a large, monophyletic group containing 127 species (Krosnick et al. 2013; Sader et al., 2019). Two key morphological synapomorphies of supersection *Decaloba* are the presence of cernuous (drooping) shoot tips and loss of petiolar nectaries. Plants in this clade also have a strong tendency toward having leaves with the central vein equal to or shorter than the lateral veins (Krosnick et al., 2013). Supersection *Decaloba* is currently divided in two sections: section *Xerogona* (Raf.) *s. lat*. Killip (~32 species) and section *Decaloba* DC (~120 species). Section *Decaloba* is

differentiated from section *Xerogona* by the presence of nectaries on the leaf blade located exclusively between the primary veins (Krosnick et al., 2013).

Krosnick et al. (2013) recognized ca. 95 species in section *Decaloba*, but we recognize ~120 species including presently unpublished new species and several putative species complexes. In this study, we focused on clarifying the evolutionary relationships among species within *Passiflora* section *Decaloba*, a group that contains around 20% of all *Passiflora* species. Although the geographical center of species diversity in section *Decaloba* occurs in the Northern Andes, this group is widely distributed throughout humid montane forest habitats of the Neotropics, from Mexico to the Andean region of Bolivia, with some species occurring in the Greater and Lesser Antilles, the Bahamas, the Amazon, the Cerrado, and the Brazilian Atlantic forest. Several species in the section also occur in subtropical regions such as the southern United States and northern Argentina (Tropicos®, 2019). The range of elevations occupied by plants in section *Decaloba* is equally wide, spanning from sea level (i.e., in the Caribbean) to more than 3000 m in *Polylepis* forests of the Andes mountains, although most species are thought to occur at elevations between 1000 and 2500 m.

Despite its relatively high diversity, the phylogeny, taxonomy, and species limits of section *Decaloba* remain poorly understood. In a previous study that focused on understanding the broader relationships among the major groups within the genus *Passiflora*, Krosnick et al. (2013) achieved the most comprehensive sampling of the section *Decaloba* to date, yielding a molecular phylogeny that included 44 terminal taxa. Although this supported the monophyly of section *Decaloba* and found potential synapormorphies for it and some of its constituent subclades, nearly all relationships

among species within the section were poorly resolved. Furthermore, because their study did not sample multiple individuals per species, Krosnick et al. (2013) provided little insight into phylogeographic patterns and processes within species and did not provide formal tests of the monophyly of described species.

The timing of divergences and biogeography within section *Decaloba* are also almost completely unknown. At deeper nodes, Muschner et al. (2012) proposed that the subgenus *Decaloba* originated in South America, diverged from its sister subgenus *Deidamioides* 36.8 Million years ago (Ma), and began diversifying 29 Ma (crown age), whereas Abrahamczyk et al. (2014) proposed a more recent date for the divergence of subgenus *Decaloba* at 24.2 Ma. Abrahamczyk (2014) inferred a phylogenetic split between section *Decaloba* and section *Xerogona* at 11.06 Ma. However, because of limited sampling, poor resolution, and a lack of dating in most previous phylogenies that focused on section *Decaloba*, its geographic origin and the forces that have led to its diversification are poorly known. Although Abrahamczyk (2014) showed that subgenus *Decaloba* likely originated in South America, it is unknown whether section *Decaloba* also originated in South America and subsequently colonized Central America, North America, and the Caribbean, or instead whether it originated elsewhere before colonizing the Andes and other parts of South America. The primary forces that have affected diversification in the group are also unknown. Given that the northern Andes are arguably the center of diversity in section *Decaloba,* one hypothesis is that diversification in section *Decaloba* may have occurred predominantly in response to the uplift of the northern Andes, which is thought to have created new niches and ecological opportunities as well as geographic barriers that promoted adaptive radiations and allopatric speciation

(Lagomarsino et al., 2016; Perez-Escobar et al. 2017). Uplift in the Northern Andes is thought to have occurred mainly since the late Miocene, with around 60% of their total elevation obtained over the last 10 Ma (Gregory-Wodzicki, 2000), and this corresponds well with previously estimated stem ages of section *Decaloba*: 6.5 Ma (Kozak et al., 2015) and 11.06 Ma (Abrahamczyk et al., 2014). Another hypothesis for the origin of diversity in section *Decaloba* is that diversification in the group occurred more recently as the result of climate fluctuations during the Pleistocene, a series of glacial cycles that began ~2.6 Ma ago and lasted until about 11,700 years ago. A well-resolved, dated phylogeny with adequate taxon sampling of section *Decaloba* is needed to test these biogeographic hypotheses, as well as to achieve a broader understanding of the historical biogeographical processes shaping the diversification of Neotropical vines occupying mid-elevations, which are especially poorly understood (e.g. von Hagen & Kadereit, 2001; Bell & Donoghue, 2005; Hughes & Eastwood, 2006; Givnish et al., 2014).

In this study, we investigated the phylogeny and biogeography of *Passiflora* section *Decaloba.* To achieve the greatest taxon sampling and phylogenetic resolution possible, we predominantly attained DNA samples from herbarium specimens across the whole geographic range of the section. To generate DNA sequence data, we employed 2b RAD sequencing, a high-throughput, reduced representation DNA sequencing technique suitable for non-model organisms (Wang et al., 2012; Aglyamova & Matz, 2014), and used the resulting genome-wide data to infer a time-calibrated phylogeny of the group which was also used to reconstruct the historical biogeography of the group, using ancestral range reconstructions. The two main goals of our study were 1) to elucidate the evolutionary history of the group, including identifying major clades and testing the

monophyly of species, and 2) to reconstruct the biogeography of section *Decaloba*, including analyzing its geographic origin, path of colonization, and major processes affecting diversification (e.g., uplift of the Andes and/or fluctuations in climate during the Pleistocene). Our results provide the first well-resolved phylogeny of the group and shed light on a historical biogeographical scenario by which species of section *Decaloba* achieved their present-day distributions across the Neotropics. Our findings provide both a baseline for future evolutionary and ecological research in Passifloraceae as well as an improved understanding of the spatial and temporal patterns of evolutionary history in Neotropical vines.

2. Methods

2.1. Sample selection

Most material used in the study was derived from the *Passiflora* collections at the herbarium of the Missouri Botanical Garden (MO), as well as loans of *Passiflora* from >20 herbaria as part of a project focused on the systematics of subgenus *Decaloba* (Krosnick et al., 2013). We reviewed close to 2000 herbarium specimens representing nearly all of the ~120 species considered to be part of section *Decaloba s. str.* (clade W of Krosnick et al., 2013; MacDougal & Feuillet, 2015-2019). We aimed to sample all taxa known to be in section *Decaloba*, including both identified and undetermined specimens. We re-evaluated the identifications of all specimens; some samples corresponded to accepted species descriptions, whereas other samples were unclear or appeared to be misidentified, in which case they were assigned a tentative name to be tested using the phylogeny. From these collections, we sampled the sheets with enough material to obtain ~2 cm^2 of leaf tissue, and in some cases flower or stem tissue,

following the destructive sampling policy from MO and other institutions. Whenever possible, we sampled several specimens representing the geographical and morphological diversity for each species, targeting around 5 individuals per species. We prioritized sampling herbarium specimens with an age of collection of less than 20 years, with the assumption that DNA degrades over time. The outgroups used in this study included samples from three species (*P. goniosperma*, *P. lutea*, *P. sexflora*) in section *Xerogona* s. lat., the sister clade to section *Decaloba* (Krosnick et al., 2013). We also included one species from supersection *Auriculatae,* a more distant outgroup in subgenus *Decaloba*. (sample P.aff.auriculata332).

2.2. DNA extraction

All lab work was conducted in the Conservation Genetics Laboratory at Missouri Botanical Garden. We extracted whole genomic DNA from 779 samples using the CTAB DNA extraction protocol for plants (Doyle & Doyle, 1987). Subsequently, we quantified the DNA concentrations in each sample using a Qubit™ fluorometer (ThermoFisher) and we cleaned the samples using a GENECLEAN® turbo kit (MP Biomedicals). As expected for herbarium specimens, the quantity and quality of DNA varied among samples, with only 542 samples containing more than 200ng of DNA minimum needed to successfully complete 2b-RAD library preparation.

2.3. 2b-RAD Seq protocol

To quickly obtain high-quality DNA sequence data across the genome at a relatively low cost, we employed 2b-RAD Seq (Wang et al. 2012). We followed the protocol described by Aglyamova and Matz (2014; available at: https://github.com/z0on/2bRAD_denovo), with some modifications. A total of 150-250

ng of DNA was digested using the restriction enzyme BcgI (New England Biolabs), which excises 36 bp long fragments of DNA throughout the genome. Digested DNA was arranged in 96-well plates and then each of 12 unique double-stranded adaptors was ligated to samples in each column. Ligations were then subjected to an amplification test, where each sample was amplified using high fidelity Phusion® PCR mix (New England Biolabs) for 14 PCR cycles. Amplified samples were checked by running them on 2% agarose gels to confirm the success of digestion and ligation. Of the original 542 samples that met our minimum DNA concentration, only 219 (Appendix) produced positive results in the amplification test.

For each plate, the uniquely barcoded samples across a row were pooled and amplified using one of eight uniquely barcoded PCR primers. Thus, when combined with the unique adaptors used for each column, this produced up to 96 uniquely barcoded samples per 96-well plate. PCRs were run for 13-15 amplification cycles and then subjected to agarose gel electrophoresis. The resulting 170 bp bands were excised from the gel and purified using a MinElute Gel Extraction Kit (QIAGEN). We quantified each PCR using a Qubit fluorometer, pooled the eight PCR reactions at a concentration of 10nM, and sequenced them for 1x50 cycles on an Illumina HiSeq 2500 or 4000 sequencer (2017-2018) at Duke University. After including technical replicates, we sequenced a total of 225 samples, which were evenly and randomly divided across three sequencing runs.

2.4. Data analysis

2.4.1. Sequencing quality control, assembly of loci, and SNP calling

We applied the 2bRAD_denovo script written by M. Matz (available at: https://github.com/z0on/2bRAD_denovo) to demultiplex the sequencing reads (sorting them into individuals), remove barcodes and Illumina adapters. We conducted default quality control procedures with FastQC (Babraham Bioinformatics) and used FastX toolkit (available at: http://hannonlab.cshl.edu/fastx_toolkit/) to remove low quality sequences. In FastX toolkit, we kept reads that had at least 90% (p) of the bases with a minimum quality score of 20 (q) and an input quality ASCII offset of 33 (Q).

We used iPyrad v0.7.28 (Eaton and Overcast, 2016) with the parameters described in Supplementary Data table S1 to assemble loci *de novo*. To optimize the number and quality of called loci, the assembly pipeline was run several times, varying the minimum number of samples in which a locus must be present to be called (4, 8, 12, 16, 20 and 22). We found the optimal resolution and bootstrap support when a locus was present in a minimum of 12 samples (analysis not shown), and this value was employed for all subsequent analyses. We then calculated the percentage of missing data per sample and discarded any sample with more than 95% missing data (supplementary material S2). Additionally, we employed a more restrictive filter, discarding samples with greater than 50% missing data, but obtained lower resolution and support for nodes in the phylogenies based on this data set (data not shown).

2.4.2. Phylogenetic analyses

We initially used the full dataset containing all 219 samples to reconstruct the phylogeny using RAxML v8.2.10 (Stamatakis, 2014) with the GTRCAT model with 1000 rapid bootstraps. We also employed the transfer bootstrap expectation (TBE) approach proposed by Lemoine et al. (2018) to quantify support for nodes in the RAxML

gene trees. The main advantage of TBE is that it allows degrees of uncertainty or instability to the nodes, calculating a transfer index per tip and node. In contrast, Felsenstein's bootstrap proportions (FBP) discards nodes with some conflict in placement, even those that are "mostly correct". TBE has been shown to recover high support where FBP fails: medium depth and deep nodes. To calculate the TBE values for our RAxML phylogeny, we uploaded our bootstrap trees file and our best tree file to an online tool for TBE fast estimation (https://booster.pasteur.fr/, Lemoine et al. 2018). We considered high support values to be those >85% for TBE and >75% for FBP.

2.5 Divergence time estimation and historical biogeographical reconstruction.

Because all subsequent analyses required the inclusion of only one individual per species, we reduced the number of accessions in the datasets. We inspected phylogenies based on the full data set (see results) and selected the individual from each monophyletic species that had less missing information in the "complete" dataset to be retained in the analysis. When species were non-monophyletic, we retained one randomly selected individual from each uniquely placed group.

We employed BEAUti and BEAST v2.5.2 (Bouckaert et al. 2014) to generate a time-calibrated phylogeny using the "reduced" assembly, with 100 million generations and a GTR substitution model, gamma rate, and relaxed log normal clock model. Additional priors included a Yule speciation model and two calibration dates: 1) the Galapagos Santa Cruz island date (0.7 – 1.5 Ma, Hickman & Lipps, 1985), which we parameterized using a uniform distribution and, 2) a secondary calibration from Abrahamczyk et al. (2014) based on their estimated age for section *Decaloba* of 11.06 Ma, which we parameterized with a normal distribution Additionally, we used as our

starting tree a chronogram of the "reduced" dataset produced using treePL v1 (Smith & O'meara, 2012), while using the same calibration points described above. We explored the BEAST log results in Tracer v1.7.1, we summarized posterior trees in TreeAnnotator v.2.5.2 (Rambaut et al. 2018) and we visualized the phylogenic tree using Figtree v1.4.2.

For our historical biogeographical reconstruction, we divided the range of sect. *Decaloba* into 11 geographical units (Fig. 1) according to the ecoregions proposed by Griffith et al (1998): A) North America, including northern Mexico. B) Mainland Central America (units 13, 14 and 15 in Griffith et al., 1998). C) Greater Antilles (unit 16.2). D) Bahamas (unit 16.1). E) Lesser Antilles (unit 16.3). F) Guianas region (units 17.2, 20.1, 20.3 and 21.1). G) Northern Andes (unit 17.3). H) Choco (unit 17.1). I) Central–South Andes and the dry Chaco (units 18.3 and 22.1). J) the Amazonas region plus the Brazilian Cerrado dry biome combined (units 20.2, 20.4, 20.5 and 21.2). K) the Brazilian Atlantic Forest plus the humid Chaco biome combined (units 21.40, 22.2 and 23.1). The corresponding base maps and additional metadata can be found at the following websites: https://www.epa.gov/eco-research/ecoregions-north-america and http://ecologicalregions.info/.

To assign the current distributions of species to the 11 areas of endemism, we collated information on species geographical ranges using several available data sources. We mapped accessions in the Tropicos® database using QGIS (QGIS Development Team, 2019). The Tropicos® database also provided us with elevation and collection information that was used in further interpretation of the results. We also used collection information from non-georeferenced specimens, as well as description of the species' ranges from the relevant taxonomic literature.

We conducted ancestral range reconstruction analysis using the program RASP v4 (Reconstruct Ancestral State in Phylogenies; Yu et al., 2015), which incorporates the following modules: Lagrange (Dispersal-Extinction-Cladogenesis model) (Ree & Smith, 2008; Massana et al. 2015), S-DIVA (Statistical-Dispersal Vicariance Analysis) (Yu et al., 2010), BayArea (Landis et al., 2013), BioGeoBEARS (Matzke 2013a, 2013b, 2014 and R Core Team, 2017), and other tools included in the program (Ronquist & Huelsenbeck, 2003; Beaulieu et al., 2013).). We ran our ancestral range reconstruction analyses using more than one model in order to improve our ability to detect erroneous reconstructions. We interpreted congruent ancestral range reconstruction results for a given node across different models as indicative of high support for that node's reconstruction state(s). In our RASP analyses, we used the maximum clade credibility chronogram generated in BEAST. We ran the non-stratified ancestral range reconstruction analyses in RASP using a maximum of 2–5 areas per node and 250 bootstrap pseudoreplicates. Additionally, we excluded range combinations that included disjunct distributions and considered the node reconstructions to be well supported when they reached a probability greater than or equal to 75%. We used the model likelihood-ratio test included in RASP to select the best model based on comparisons of Akaike information criterion (AIC) values using standard information-theoretic approaches (Burnham & Anderson 2004).

3. Results

3.1. Locus assembly and SNP calling

After removing accessions with poor sequence quality, the full data set included 209 unique samples (appendix), including sequences from: 1) 7 accessions representing 4

outgroup taxa, 2) 202 accessions representing 82 of the ~120 known species in section *Decaloba*, including both species with accepted published names as well as unpublished names, and 3) 15 specimens with an ambiguous determination ("sp.", "cf." or "aff." designations) that included putative new and undescribed new species or specimens with insufficient information to be identified (appendix).

In the "complete" dataset, the total number of loci that passed the initial sequencing quality control procedures was 513,321, each of which was 36 bp in length. After applying quality control filters in iPyrad as described in Supplementary Table S1, we obtained a final filtered data set comprised of 11,778 concatenated loci, with locus 36bp in length, resulting in a total alignment of 424,008 bp, which included both variant and invariant sites. Missing data per sample was highly variable among our final RAD loci, ranging from 48–98% (Supplementary table S2), with most samples having between 62–92% missing information.

3.2. Results of phylogenetic analyses

The maximum likelihood (ML) tree is presented in Fig. 2. Because of the large number of accessions included in this phylogeny, we collapsed samples corresponding to the same species if they formed monophyletic groups, but indicated the number of samples included in the terminal in parentheses. Hereafter, all support values will refer to the TBE support if not specified otherwise. TBE instability index per taxa is listed in supplementary table S3.

Relative to the outgroups representing supersection *Auriculatae* (sample P.aff.auriculata332) and section *Xerogona* s. lat. *(P. goniosperma, P. lutea, P. sexflora),* section *Decaloba* was strongly supported as monophyletic with 100% support (Fig. 2),

consistent with previous results reported by Krosnick et al. (2013). All taxa in section *Decaloba* were grouped into two large, well supported clades: a predominately Central American clade (92% support) comprising 72 accessions representing 28 species mostly from Central America but also from South America (Fig. 2A) and a predominately South American clade (100% support), comprising 129 accessions in 67 species (Figs. 2B and 2C).

3.2.1. Central American clades (CA1 and CA2)

The Central American clade was divided into two strongly supported clades, referred to as clades "CA1" and "CA2" (Fig. 2A, and Supplementary Figure S1). Both CA1 and CA2 clades also showed medium to high values of support in most of their internal branches. The CA1 clade (85% TBE and 35% FBP) contained 26 accessions from 9 taxa (*P. standleyi*, *P. lancearia*, *P. boenderi*, *P. jorullensis* var. *jorullensis*, *P.* aff. *mexicana*, *P. jorullensis* var. *salvadorensis*, *P. ilamo*, *P. gilbertiana* and *P. apetala*) that are distributed from the southwest USA and west coast of Mexico southward to the Isthmus of Panama. The internal branches within clade CA1 also showed high values of support, and most species represented by multiple accessions were monophyletic except the two varieties of *P. jorullensis*, which did not form a monophyletic group.

The CA2 clade (100% TBE and 90% FBP) contained 46 accessions from 19 taxa (*P. affinis*, *P. nubicola*, *P. yucatanensis*, *P. biflora*, *P talamacensis*, *P. subfertilis*, *P. insolita*, *P. helleri*, *P. cupraea*, *P. coronapapillata*, *P. penduliflora*, *P.calcicola*, *P. oblongata*, P. sp. 612, *P. bicornis*, *P. cubensis*, *P. tulae*, *P. orbiculata* and P. *murucuja*). Members of this clade can be found from the Edwards plateau in Texas, United States to

the Andean region of Colombia, as well as the Bahamas, the Antilles, and northern Venezuela. In CA2, all species were monophyletic except *P. biflora* and *P. penduliflora*.

3.2.2. South American clades (SA1-SA8)

In the large South American clade (Figs. 2B, 2C and Supplementary Figure S1), five small subclades were placed as successive sisters to the remainder of the SA clade. The first clade, deemed "SA1", had 100% support and was comprised of a pair of accessions (*P. lyra* and *P.* cf. *lyra*) from Colombia and Northwest Ecuador (400–900 m) (Fig. 2B), that was placed as sister to a strongly supported group (99%) containing the remaining accessions in the SA clade. This clade was divided into two groups, the first (SA2) was weakly supported (58%) and contained six accessions representing four species (*P. vespertilio*, *P. anfracta*, *P. micropetala* and *P. rotundifolia*) all from lowland areas of Ecuador, Peru and the Lesser Antilles. The second group was strongly supported (97%) and contained clades SA3–SA8. Within this group, a strongly supported (100%) small clade, "SA3" contained four accessions representing two species: *P. sandrae,* from the central-eastern regions of Panama and *P. occidentalis* from the central region of Panama to the Northernmost Ecuadorian coast (Fig. 2B).

Next, clade "SA4" (Fig. 2B; 73%) included 9 accessions representing 6 species (*P. caduca*, *P. panamensis*, *P.* sp., *P. misera*, *P. punctata* and *P. colinvauxii*) distributed from the eastern region of Panama south to the Northern Andes in Colombia, along the Ecuadorian coast, and the Galapagos Islands. Two species in clade SA4 were not monophyletic: *P. colinvauxii* was nested with a clade containing the two *P. punctata* accessions (P.punctata056 and P.punctata057), rendering *P. punctata* as paraphyletic

(Fig. 2B), and P.misera135 from Paraguay was not grouped with the remaining *P. misera* samples, which were placed in the "SA7" clade (Fig.2C and Supplementary Figure S1) .

The next South American clade was a strongly supported (94%) group that contained clades SA5–SA8. Within this group, a well-supported (91%) clade, "SA5" (Fig. 2B), contained 7 accessions representing 5 species (*P. andersonii, P. stenosepala, P. tuberosa, P. yucatanensis* and *P. tricuspis*) and was placed as sister to a strongly supported group (95%) containing clades SA6-SA8. The species in clade SA5 are mostly distributed from the Lesser Antilles (*P. andersonii* and *P. stenosepala*) to Trinidad and the Venezuelan Andean and Costa regions (*P. tuberosa*). Two species placed in clade SA5 were not monophyletic: a P.yucatanensis478 sample was not grouped with the remaining accessions of *P. yucatanensis* in clade CA2, and the three accessions of *P. tuberosa* were all placed in clade SA5 but did not form a monophyletic group.

The next clade to diverge was well-supported and contained clades SA6-SA8 (95%). This clade was divided into two groups: the strongly supported (94%) "alnifolia" or clade "SA6" (Fig. 2B) that contained 29 accessions from 15 taxa (*P.* aff. *tribolophylla, P.pilosissima, P. mollis, P. cuspidifolia, P. bogotensis, P. hyacinthiflora, P. kalbreyeri, P.* cf. *cuneata, P. bucaramangensis, P. micrantha, P. trinervia, P. andreana, P. alnifolia, P. chelidonea* and *P. tribolophylla*), and the strongly supported (95%) *P. magdalenae* + SA7 + SA8 clade that contained 73 accessions and 45 taxa. The species in the clade SA6 typically occupy Andean humid montane forest ranging from Colombia to Bolivia, but can also be found in Venezuela. Most of the species represented by more than one sample placed in this clade were not monophyletic, except for *P. bogotensis, P. kalbreyeri, P.trinervia* and *P. hyacinthiflora.*

The *P. magdalenae* + SA7 + SA8 clade (Fig. 2B-C) included taxa distributed across South America, including taxa found in the Andes, coastal regions, and Amazon. In this group, the single accession of *P. magdalenae* was placed as a strongly supported sister (95%) to two strongly supported groups, the ‘”SA7” clade (98%) and the SA8 clade (91%). *P. magdalenae* is found in the central inter-Andean Valleys of Colombia.

The SA7 clade comprised 35 accessions representing 23 taxa (*P. heptantha*, *P. tatei*, *P. urnifolia*, *P. pardifolia*, *P. ichthyura*, *P. tricuspis*, *P*. cf. *hexadenia*, *P. indecora*, *P. hexadenia*, *P. ketura*, *P. punctata*, *P. pascoensis*, *P*.cf. *pilpintu*, *P. cana*, *P. nana*, *P. caduca*, *P. chrysosepala*, *P. viridescens*, *P. hirtiflora*, *P. carnosisepala*, *P. telesiphe*, *P. quadriflora* and *P. rotundifolia*), and it contains mostly well-supported internal branches. Species in this clade are distributed from the Andean region of Ecuador to central Bolivian mountain forests and Southern Brazil. Clade SA7 is divided into two groups that correspond to geography, with the smaller clade (7 spp., 96% TBE) ranging from the eastern slopes of the Andes to the Amazonas basin and the Atlantic forest, and the larger clade (17 spp., 95%.TBE) occurring only in the Andes.

Most of species included in clade SA7 were supported as monophyletic except for *P. telesiphe* and *P. indecora*. Additionally, two accessions were unexpectedly included in the SA7 clade: the sample P.punctata192 was placed in a different clade than other *P. punctata* accessions in SA4 (Figs.2A and B), and P.rotundifolia700 was placed in this clade whereas the rest of *P. rotundifolia* accessions were found in SA3 (Fig. 2B).
The “SA8” clade contains 37 accessions and 22 taxa (*P. candollei*, *P. leptoclada*, *P. tricuspis*, *P. vespertilio*, *P*. cf. *cuspidifolia*, *P. smilacifolia*, *P*. aff. *micropetala*, *P. micropetala*, *P*. cf. *telesiphe*, *P. jeannetteae*, *P*. aff. *pohlii*, *P. saxicola*, *P*. sp. *nov*., *P*.aff.

trifasciata, *P. occidentalis*, *P. urnifolia*?, *P. trifasciata*, *P.* aff. *tricuspis*, *P. misera*, *P. poeppigii*, *P. amalocarpa* and *P. leptoclada*). This clade's distribution is the broadest of all section *Decaloba*, with species distributed from eastern Panama to the Andean region, extending to the Guianas, Brazil, Paraguay and part of Argentina. Clade SA8 also contains 8 nodes with low support (<80%) as well as six apparently non-monophyletic species (*P. leptoclada*, *P. trifasciata*, *P. misera*, *P. amalocarpa*, *P. vespertilio* and *P. tricuspis*) (Fig. 2C & Fig.3).

3.3. Divergence time estimation and biogeographic analysis

After removing duplicate accessions of monophyletic species as identified through phylogenetic analyses of the full data set, the "reduced" data set contained 109 unique samples, including: 1) 4 accessions representing 4 outgroup taxa, 2) 89 accessions representing monophyletic species in section *Decaloba*, including both accepted published names and unpublished names, and 3) 16 specimens with an ambiguous determination (sp., cf. or aff. designations) that include potential new species or specimens with insufficient information to be identified. The total number of loci (each 36bp in length) that passed the sequencing quality control was 301,700, resulting in total concatenated dataset of 9,994,212 bp. After applying the filters in iPyrad as described in supplementary table S1, we obtained a final data set comprised of 7,299 loci and 263,227 total bp in length.

Results of BEAST analyses of the reduced data set are shown in Fig. 3. We recovered section *Decaloba* as monophyletic (posterior probability=1). Although half the nodes had a posterior probability (PP) ≥0.95, relationships in this tree showed lower resolution and support than the RAxML tree. The BEAST phylogeny differed somewhat

from the phylogeny generated using RAxML, but the major clades were similar. Both reconstructions recovered the main two clades: Central America (CA) and South America (SA). Inside the CA clade, we recovered a very similar topology in both trees, with accessions forming two smaller clades with strongly supported nodes that mostly correspond to CA1 and CA2 as seen in the RAxML tree. In contrast, the SA clade, despite being recovered in both trees, showed significant differences between the two topologies. RAxML SA1-SA5 clades were not recovered in the BEAST tree, instead BEAST placed these samples in other large clades. Both RAxML and BEAST topologies recovered the SA6 clade; RAxML showed significantly higher support values for this clade (94% TBE) and its internal nodes and were more resolved (67-100%) than BEAST, which showed lower PP support values (49% clade, 46-100% internal nodes) and some differences in topology. Clades SA7 and SA8 from the the RAxML tree were not recovered in BEAST, which instead placed the samples into one large clade, with relationships that had lower support than the RAxML tree.

We used BEAST together with RASP results to conduct divergence time estimation with two calibration points, and the resulting chronogram was used for ancestral range reconstructions of section *Decaloba* lineages. Based on AIC model selection results, we found that the best-supported model for our dataset was the DEC + J model in all four RASP analyses (Supplementary table S4). The overall pattern did not differ significantly across analyses when maximum number of areas allowed per node/tip were set to values ranging from 2 to 5 (S. Acha, unpublished results), except for an increase in uncertainty in some nodes as we increased the maximum number of areas allowed; we therefore present only the ancestral range reconstruction that allowed a

maximum of two areas per node (Figs. 1, 4 and Supplementary material S4 and S5). Globally, RASP showed evidence for 88 dispersal, 40 vicariance and 2 extinction events. The most common dispersal pattern was that inferred between the Central–Southern Andes mountains (I) and the Northern Andes mountains (G), and these were also the areas with the highest numbers of speciation events (I:9, G:34), along with Central America (B) (15).

Analyses showed that most recent common ancestor of all section *Decaloba* diverged from all other passion flowers around 10.4 Ma (95% HPD: 6.5, 13.8 Ma) in the late Miocene and that its range most likely occurred in mainland Central America (Fig. 4, node 215, 0.38). The Central American clade (CA) and the South American Clade (SA) diverged 7.8 Ma (95% HPD: 5, 10.9) in the late Miocene–Pliocene. The common ancestor of the CA clade (Fig. 4, node 136) showed a highly supported (0.93) origin in mainland Central America around 5.7 Ma (95% HPD: 3.7, 7.7), and most of the early diverging nodes in the CA clade (i.e., nodes 132-136) showed ancestral ranges in Central America. Range reconstructions showed the following cases of range shifts: 1) a range expansion of *P*. aff. *mexicana* into North America (i.e., from B to AB; Fig 4) around 0.58 Ma (95% HPD: 0.2-1.3 Ma), 3) an early dispersal into North America (i.e., from B to A; Fig 1) around 4.3 Ma (95% HPD: 2.7-6.5 Ma), giving rise to *P. affinis*, 3) a range expansion at node 131 into the Major Antilles around 2.8 Ma (95% HPD: 1.6-4.1 Ma), resulting in two subsequent clades with ranges solely in the Major Antilles (i.e., nodes 121-128; Fig. 4) and, and 4) a dispersal at node 129 from the Major Antilles to the Bahamas around 1.8 Ma (95% HPD: 0.8, 2.6 Ma), giving rise to the *P. cupraea* Bahamas populations.

The common ancestor of the SA clade was reconstructed as having diverged around 7.2 Ma (95% HPD: 4.9, 9.8). The first early diverging lineages in this clade (nodes 212–214) showed ranges that were reconstructed as occurring either in Central America or the North Andes. Several dispersal events or range expansions were reconstructed in these early diverging lineages of the SA clade, including a colonization of the Choco (node 137, giving rise to *P. occidentalis* and *P. sandrae),* and a dispersal to the Guianas and the Lesser Antilles (nodes 138 and 139). After the first three equivocal nodes in the SA clade, the next divergence (node 211) occurred around 6 Ma (95% HPD: 3.9, 8.9) and had an ancestral range occurring in the North Andes. This clade diverged into two major clades (nodes169 and 210), also with ancestral ranges in the North Andes. Within the first clade beginning at node 169, most nodes had ancestral ranges in the north Andes, but we observed several subsequent range shifts, all of which occurred in the last 2 Ma: 1) two independent dispersal events to the Choco/Galapagos region (H) (141 and 145), 2) two independent dispersal events to the Lesser Antilles (nodes 144 and 150), 3) a colonization event into the Guianas (node 150), and 4) one recolonization of Central America by the ancestor of *P. insolita* around 2.3 Ma (95% HPD: 0.06,0.5)

The other SA clade corresponded mostly to the accessions in clades SA7-8 from the RaxML tree. We discovered several main range shifts in this group: 1) dispersal from the northern to the central and southern Andes that occurred 4.5 Ma (2.9, 6.4), which gave rise to several clades with ranges reconstructed predominantly in the southern Andes, and 2) a colonization of Amazonas and Cerrado around 3 Ma (1.9, 4.3) (node 205). Finally, we found two or more instances of the transition to the Atlantic forest region: one that occurred less than 1 Ma, giving rise to *P. pardifolia* (node 181), and

several possible dispersal events from Amazonas into the Atlantic forest around 2.5 Ma (1.6, 3.5) (nodes 191, 193 and 195).

4. Discussion

In this study, we reconstructed the phylogeny of *Passiflora* section *Decaloba* (Passifloraceae) using samples obtained almost exclusively from herbarium specimens, employing a recently developed restriction-associated DNA sequencing approach, 2b-RAD (Wang et al., 2012). The use of herbarium specimens allowed us to achieve nearly complete taxon sampling of the ~120 species in section *Decaloba*, or around one fifth of all the species in Passifloraceae. Further, the 2b-RAD approach employed in this study provided a remarkably well-supported and well-resolved phylogeny of the group, despite the fact that section *Decaloba* represents a relatively rapid radiation (i.e., ~120 species evolving in only ~7.8 Ma), in which conventional data previously failed to resolve relationships. Furthermore, we obtained well resolved phylogenies despite many samples having a high percentage of missing data, and like other like other previous studies (e.g. Tripp et al. 2017), found that the resolution of the phylogenies increased to a certain point as the percentage of missing data increased. The use of TBE (Lemoine et al. 2018) provided support for phylogenetic relationships that FBP failed to provide. Although RAD-seq approaches have been used successfully to reconstruct patterns of evolution in groups even older than 60 Ma (Ree and Hipp, 2015), our results indicate that the 2b-RAD approach is particularly useful for clarifying relationships in rapid radiations. This approach may be useful for future studies that aim to elucidate the evolutionary relationships among broadly distributed, highly diverse, and poorly accessible groups of plants.

The goals of our study were to elucidate the evolutionary history of section *Decaloba*, including identifying major clades and testing the monophyly of species, and to reconstruct the biogeography of the group, including analyzing its geographic origin, path of colonization, and major processes affecting diversification (i.e., the uplift of the Andes and fluctuations in climate during the Pleistocene). Although the center of diversity of Section *Decaloba* is located in the Northern Andes and previous analyses suggested a South American origin to the larger group subgenus *Decaloba*, our analyses revealed a Central American origin to the clade. We do not discard completely the possibility that section *Decaloba* originated in North America with a progressive dispersal southward, but additional sampling of outgroups and a more in-depth analysis of the supersection or subgenus *Decaloba* is necessary to test these hypotheses. From its origin in Central America, Section *Decaloba* then diverged into two major clades (the SA and CA clades); we discuss important subclades, biogeography and monophyly of species in each of these clades below.

4.1. Central America

The CA clade originated in Central America and then diverged into two main groups: clades CA1 and CA2, both of which diversified primarily in Central America. Species in the CA1 clade are distributed in Central America and occupy montane forest (around~1500 m), except for *P. lancearia*, which can occur at lower elevations. All species in this clade have disc shaped-flowers with white, yellow or red corona elements and bilobed leaves, except for *P. lancearia*, which is early diverging within the clade and has elliptic leaves.

The CA2 clade originated in Central America and subsequently dispersed to the Bahamas, the Greater and Lesser Antilles, and in an exceptional case, to the Peruvian Andes (*P. coronapapillata*). The species in the Greater Antilles likely originated from two separate dispersal events and likely had one reversion back to the Central American mainland (*P. bicornis*). Fascinatingly, the species in this clade are more closely related to those on different islands than to those that co-occur on the same island. The phylogenetic relationships and pollination biology of the clades occupying the Greater Antilles have been studied in depth (Kay, 2003), and we obtained a similar topology to the one proposed previously but with higher support values. Several of these species were recognized by Killip (1938) as forming part of three distinct subgenera that correspond to red/pink elongated or green tubular flowers with hummingbird or bat pollination syndromes. In addition to the species with the specialized floral morphology, eight taxa have the more typical *Decaloba* disc-shaped flower.

4.2 South America

Our analyses suggest that the SA clade experienced an early range expansion into the Andes prior to the formation of the isthmus of Panama (Bacon et al., 2015; O'Dea et al., 2016), supporting the hypothesis that the Great American Biotic Interchange occurred before the closing of the isthmus in several pulses, one of which coincides with the date of the dispersal of the SA clade into South America (Bacon et al., 2015). Our results strongly suggest that the colonization of the Northern Andes occurred once, giving rise to a rapid radiation that likely diversified both in response to the uplift of the Andes as well as to fluctuations in environmental conditions during the Pleistocene, with subsequent dispersal into other areas of South America. Surprisingly, the adjacent Choco region is

home to only five species in section *Decaloba* and was colonized several times from the Andes and the Amazon. The Amazon and Brazilian Atlantic Forest ecoregions were colonized recently from the Andes, lending support to the theory that many Amazonian taxa originated through dispersal from the Andes (Gentry, 1982; Upham et al., 2013). The Lesser Antilles were also colonized from South America, following a similar pattern found previously for other organisms occupying these islands (Valentin and Olmstead, 2004; Maunder et al. 2011), such as the modern colonization events of the Lesser Antilles observed in birds (Ricklefs & Bermingham 2007).

Within the SA clade, all the five early diverging subclades (SA1-5) share the common morphological characteristics of having white disc shape flowers, some with more greenish tonalities and other with more purple. The corona filaments are highly variable in size, number, color and shape among species. In subclades (SA1-5), we observed a range of relationships among accessions that had varying support, many of which have taxonomic implications. The SA1 clade includes two specimens identified as *P. lyra* and *P.* cf. *lyra*. Both specimens occupy lowland areas and are morphologically similar. Although *P. lyra* was described originally from the Caribbean region of Venezuela, the *P. cf. lyra* sample is from Ecuador, suggesting that the range of *P. lyra* is broader than originally thought, extending into Colombia and Ecuador. In clade SA2, we suspect that the placement of many taxa in this clade may be an artifact, as it is composed primarily of samples with high to moderate transfer index values such as P.micropetala512, which has the greatest transfer index value of all samples. In particular, the placement of P.micropetala512 and *P. rotundifolia* in the SA2 has low support; we suspect that *P. rotundifolia* is closely related to *P. kalbreyeri* in the SA6

clade based on their similar morphology and distribution from the Lesser Antilles to the Venezuelan coast, respectively. Clade SA3, which is composed of *P. sandrae* and three accessions of *P. occidentalis*, contains what we currently identify as the true *P. occidentalis.* The other sample identified as *P. occidentalis* is placed in clade SA8; we suspect this is another case of morphological convergence that merits further study to determine whether it represents a new species. Clade SA4 includes the greatest concentration of poorly known species, which are distributed from eastern Panama to the western slope of the Andes and the Galapagos Islands (*P. colinvauxii*). The Galapagos-endemic species is nested within a variable species, *P. punctata*, which occurs in the Choco. *P. punctata* is a Linnaean species described from Peruvian specimens, and based on our results, it is polyphyletic, as another accession identified as this species placed in clade SA7. Additional research on *P. punctata* is necessary, as it is likely that the name may have been applied to more than one species.

In the SA5 clade, we found two cases of paraphyly for the species *P. yucatanensis* and *P. tricuspis*. Most accessions of *P. yucatanensis* were placed in the CA2 clade; if the accession P.yucatanensis478 (which was collected in Quintana Roo, Mexico) is correctly placed, then it would involve a long-distance dispersal event from the Venezuelan coast to Quintana Roo-Mexico, although a possible incorrect placement could be also supported by its relative high transfer index. One accession of the polyphyletic species P.tricuspis514 was also placed in this clade, as well as in three different places in clades SA7 and SA8. Additional research focusing on *P. tricuspis* is needed, as we suspect that it currently encompasses several independent lineages that have been lumped due to

morphological similarities, which is in part supported by the fact that the species has previously been divided into three varieties (Killip, 1938; Zuloaga et al., 2008).

In the larger South America clades (SA6, SA7 and SA8), the number of species keeps growing and the ambiguous taxonomy of some groups makes the systematics for most of these species a challenge. Clade SA6 is distributed along both slopes of the Andean mountain chain from Venezuela to Ecuador and is distinguished morphologically by having leaves that tend to be longer than they are wide and disc-shaped flowers are small, white, with some traces of purple, except two species, *P. hyacinthiflora* and *P. trinervia,* which both have tubular pink flowers suggesting adaptation to hummingbird pollination (Ocampo et al., 2017). However, despite the strong geographic and morphological characters uniting this clade, most species within the clade are highly polyphyletic; extensive additional research is necessary to clarify species limits.

Most species in the SA7 clade are monophyletic except *P. telesiphe, P. cana* and *P. indecora*. Given that this is one of youngest clades, one explanation for these paraphyletic patterns could be incomplete linage sorting. The distribution of these species also overlaps with members of clade SA6, and we suspect that some degree of hybridization may be possible. Future research is needed to evaluate the relative effects of these processes in this clade, as well the possibility that taxonomic changes are necessary. The SA8 clade included five polyphyletic taxa, the highest number found in this study, as well as five polyphyletic species that were placed in different clades. These results could be the product of a young diversification in the Amazon and adjacent lowlands regions, such that few morphological characters may be sufficiently variable to

differentiate species. Additional research is also needed in this clade to evaluate this as well as to test whether these patterns may be in part related to incomplete lineage sorting.

5. Conclusions

The use of herbarium specimens and a 2b-RAD approach succeeded in providing the most comprehensive and well-resolved phylogeny of *Passiflora* section *decaloba* to date. The phylogeny has uncovered some new relationships that were not known previously, confirmed some relationships that were previously proposed, and resolved many important questions that arose through previous morphological and taxonomic studies. However, we also identified several groups that will require more extensive taxon sampling and additional phylogenetic analyses to clarify their phylogeny and biogeography. The results of this study further highlight the need for a modern, comprehensive taxonomic treatment for section *Decaloba,* which will undoubtedly be facilitated by the phylogenetic framework developed in the present study.

The phylogeny of *Passiflora* section *Decaloba* also allowed us to generate the first hypothesis of the biogeographic history of the group. Our analyses indicate that section *Decaloba* originated in Central America, then diverged into two major clades (the SA and CA clades). The CA clade subsequently diversified mostly in Central America, with subsequent dispersal events into North America, the Bahamas, and the Lesser and Greater Antilles, with the species in the Greater Antilles likely originating from two (or more) separate dispersal events. The SA clade likely originated from a single colonization of the Andes, apparently prior the formation of the isthmus of Panama, that gave rise to a rapid radiation that likely diversified both in response to the uplift of the Andes as well as to fluctuations in environmental conditions during the Pleistocene,

establishing a diversity hotspot in the North Andean mountain forest. The group then progressively colonized the remaining regions of South America, including the Choco, the Galapagos, the Amazon, and the Brazilian Atlantic forest. These results highlight the importance of the Andean region as a biodiversity hotspot that has given rise to a species distributed throughout South America.

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Appendix

List of samples and collection information.

Accession	Institution	Collection		Country	Locality
P.sp.884	MO	Vanderplank	sn	Cult.	Cult.
P.aff.mexicana310	MO	Boyle	632	Mexico	Oaxaca
P.aff.mexicana311	MO	Tajia Yocupicio	MOID: 2238530	Mexico,	Sinaloa
P.micropetala510	MO	Grandez	5801	Peru	Loreto
P.aff.pohlii460	MO	Basualdo	6410	Paraguay	Amambay
P.aff.tribolophylla199	MO	Fonnegra	4960	Colombia	Antioquia
P.aff.trifasciata105	MO	Schunke	7642	Peru	San Martín
P.affinis304	MO,F,US	Webster	11193	Mexico	Nuevo Leon
P.affinis363a & b	MO,TEX	Lott	4393	United States	Texas
P.affinis447	MO	Servin	1179	Mexico	Queterato
P.alnifolia41	MO	Croat	96520	Ecuador	Pichincha
P.alnifolia424	MO	Jorgensen	2475	Ecuador	Napo
P.alnifolia820	MO	Dodson	10887	Ecuador	Pichincha
P.alnifolia832	US	Drew	E-265	Ecuador	Imbabura
P.amalocarpa604	MO	MacDougal	6337	Cult.	Cult.

P.amalocarpa703	MO	Silveira	1185	Brazil	Acre
P.andersonii630	DUKE	Webster	13379	Dominica	NA
P.andreana049	MO	Jorgensen	2476	Ecuador	Carchi
P.andreana102	MO	Jorgensen	2478	Ecuador	Carchi
P.andreana256	MO	Jorgensen	2477	Ecuador	Carchi
P.anfracta280	MO	Dodson	6673	Ecuador	Los Ríos
P.anfracta286	MO	Dodson	14452	Ecuador	Los Ríos
P.apetala632	F,MO	Rodriguez	1583	Costa Rica	San José
P.apetala633	MO	Morales	2180	Costa Rica,	San José
P.apetala887	MO	Grayum	8085	Costa Rica,	Cartago
P.apetala914	MO	Fernandez	1472	Costa Rica,	Heredia
P.bicornis141	MO	Coronado	4866	Nicaragua	León
P.bicornis359	MO	Gonzalez	385	El Salvador	La Libertad
P.bicornis695	NY	Thorne	7216	United States	Hawaii
P.bicornis696	NY	Albert de Escobar	3482	Colombia	Magdalena
P.biflora052	MO	Avila	3717	Guatemala	Izabal
P.biflora244	MO	Morales	2997	Guatemala	Izabal
P.biflora288	MO	Pascual	999	Mexico	Oaxaca
P.biflora423	MO	MacDougal	3458GR	Honduras	Atlandtida
P.biflora613	MO	Kay	197	Costa Rica	Heredia
P.apetala594	MO	Kay	194	Costa Rica	Heredia
P.boenderi597	MO	Kay	196	Costa Rica	Heredia
P.bogotensis435	MO	Krosnick	405	Cult.	Cult.
P.bogotensis439	MO	Krosnick	383	Cult.	Cult.
P.bogotensis441	MO	Krosnick	503	Cult.	Cult.
P.bucaramangensis641	NY,US	Killip	17046	Colombia	Santander
P.caduca885	MO	Vanderplank	2398/17	Cult.	Cult.
P.calcicola600 & P.calcicolaa582	MO	Kay	131	Jamaica	Clarendon
P.calcicola610	MO	Kay	105	Jamaica	Trelawny
P.cana558	MO	Gentry	23238	Peru	Amazonas
P.cana643	F	Weigend	98/374a	Peru	Amazonas
P.cana886	MO	Vanderplank	2449/18	Cult.	Cult.
P.candollei646	MO,US	Betancur	2836	Colombia	Amazonas
P.candollei782	MO	Nunez	14616	Peru	Madre de Dios
P.cf.cuneata074	MO	Ramos	3588	Colombia	Valle del Cauca
P.cf.cuneata901	US	Daniel (Hermano)	147	Colombia	Antioquia
P.cf.cuspidifolia872	MO	Krosnick	367	Cult.	Cult.
P.cf.lyra366	MO	Clark	4920	Ecuador	Esmeraldas
P.cf.pilpintu270	MO	Raurau	91	Peru	Cusco
P.cf.tatei164	MO	Fuentes	8025	Bolivia	La Paz
P.cf.telesiphe88	MO	Campos	6273	Peru	Cajamarca
P.aff.tricuspis152	MO	Fuentes	4395	Bolivia	La Paz
P.chelidonea811	MO	Knapp	6204	Ecuador	Napo
P.chelidonea812	MO	Jorgensen	61638	Ecuador	Pichincha
P.chrysosepala106	MO	Alvarez	1982	Ecuador	Napo
P.chrysosepala107	MO	Schwerdtfeger	95022134	Ecuador	Sucumbíos
P.colinvauxii878	MO	Krosnick	539	Ecuador	Galapagos
P.cubensis585	MO	Kay	233	Cuba	Camaguay
P.cubensis586	MO	Kay	231	Cuba	Santiago de Cuba
P.cubensis601	MO	Kay	232	Cuba	Santiago de Cuba
P.cupraea584	MO	Kay	227	Cuba	Las Tunas
P.cuspidifolia122	MO	Stein	3686	Colombia	Cundinamarca
P.gilbertiana780	MO,US	Hammel	18530	Costa Rica	San José
P.goniosperma325	MO	Lott	3785	Mexico,	Jalisco
P.helleri108	MO	Mendoza	1382	Mexico	Puebla
P.helleri143	MO	Sevilla DJS	1033	Mexico	Veracruz
P.helleri144	MO	Cornejo tenorio	2525	Mexico	Puebla
P.helleri509	MO	Ventura	19556	Mexico	Veracruz
P.heptantha328	MO	Rojas	3955	Peru	Pasco

P.hexadenia565	MO	Vasquez	28889	Peru	Pasco
P.hirtiflora714	MO	Perea	2982	Peru	Cajamarca
P.hyacinthiflora285	MO	Hernandez	195	Colombia	Santander
P.ichthyura099	MO	Nee	36203	Bolivia	Santa Cruz
P. insolita415a;	MO	MacDougal	6213	Guatemala	Baja Verapaz
P. insolita839	MO	Vanderplank	sn	Mexico	Chiapas
P.ilamo407a & b	MO	MacDougal	6201	Guatemala	Solola
P.ilamo409	MO	MacDougal	6203	Guatemala	Solola
P.indecora282	MO	Lewis	2413	Ecuador	Loja
P.indecora562	MO	Jorgensen	1136	Ecuador	Loja
P.jeannettae469	MO	Giraldo Canas	593	Colombia	Antioquia
P.jeannettae720	MO	MacDougal	4160	Colombia	Antioquia
P.jorullensis.sal660	MO	Sandoval	112	El Salvador	Ahuachapán
P.jorullensis.sal661	MO	Fidel Lopez	MOID: 2243361	El Salvador	Ahuachapán
P.jorullensis.sal663	MO	Toledo	1	El Salvador	Ahuachapán
P.jorullensis.sal891	MO	Breedlove	27627	Mexico	Chiapas
P.jorullensis.jor781	MO	Vazquez	1227	Mexico	Jalisco
P.kalbreyeri283	MO	Davidse	21150	Venezuela	Lara
P.kalbreyeri553	MO	Porter-Utley	415	Cult.	Cult.
P.kalbreyeri846	NY,US	Weitzman	112	Venezuela	Aragua
P.ketura330	MO,US	Woytkowski	7804	Peru	Amazonas
P.ketura710	MO	de Cevasco	MOID: 2877363	Peru	Amazonas
P.lancearia114	MO	MacDougal	6276	Panama	Colón
P.lancearia115	MO	MacDougal	6268	Panama	Coclé
P.lancearia251	MO	MacDougal	6263	Panama	Coclé
P.lancearia399	MO	Morales	4078	Costa Rica	Heredia
P.leptoclada442	MO	Krosnick	491	Cult.	Cult.
P.leptoclada665	F,US	Williams	5252	Peru	Loreto
P.leptoclada666	US	Williams	2737	Peru	Loreto
P.lutea319	MO	Thomas	150563	United States	Mississippi
P.lutea320	MO	Stone	1532	United States,	North Carolina
P.lutea322	MO	Christy	MOID:34151736	United States	Arkansas
P.lyra119	MO	Miller	5884	Colombia:	Antioquia
P.magdalenae669	NY,US	Uribe	2568	Colombia	Tolima
P.micrantha683	NY	Fosberg	22018	Colombia	Cundinamarca
P.micropetala385	MO	Bass	377	Ecuador	Napo
P.micropetala512	MO	Jaramillo	1335	Peru	Amazonas
P.micropetala721	MO	MacDougal	4982	Ecuador	Napo
P.misera070	MO	MacDougal	6281	Panama	Canal Area
P.misera135	MO	Zardini	60751	Paraguay	Canindeyú
P.misera257	MO	Beck	3292A	Bolivia	Beni
P.misera501	MO	Zardini	31610	Paraguay	Central
P.misera503	MO	Zardini	34670	Paraguay	Central
P.misera504	MO	Zardini	36019	Paraguay	Central
P.mollis455	MO	Gentry	48035	Colombia	Valle del Cauca
P.mollis788	TEX	Escobar	420	Colombia	Caldas
P.murucuja592	MO	Kay	217	Dominican Republic	Distrito Nacional
P.murucuja617	MO	Kay	211	Dominican Republic	Baoruco
P.murucuja618	MO	Kay	206	Dominican Republic	Independencia
P.murucuja619	MO	Kay	212	Dominican Republic	Independencia
P.nana716	MO	Campos	2921	Peru	Cajamarca
P.nubicola260	MO	MacDougal	465	Costa Rica	Heredia
P.nubicola674	DUKE	MacDougal	1244	Costa Rica	Cartago
P.nubicola676	TEX	Knapp	857	Costa Rica	Alajuela
P.oblongata587	MO	Kay	107	Jamaica	Trelawny
P.oblongata611	MO	Kay	183	Jamaica	Trelawny
P.occidentalis261	MO	MacDougal	6303	Panama	Coclé

P.occidentalis337	MO,US	MacDougal	6302	Panama	Coclé
P.occidentalis470	MO	Taylor	12192	Colombia	Valle del Cauca
P.cf.occidentalis472a & b	MO	Onore	MOID:10098783	Ecuador	Esmeraldas
P.orbiculata616	MO	Kay	214	Dominican Republic	Independencia
P.panamensis698	DUKE	MacDougal	444	Panama	Darién
P.panamensis787	MO	Zarucchi	5107	Colombia	Antioquia
P.panamensis912	MO,US	Foster	2837	Panama	Darién
P.pardifolia443	MO	Vanderplank	MOID: 3330227	NA	NA
P.pascoensis189	MO	Rodriguez	95	Peru	Pasco
P.pascoensis190	MO	Rodriguez	42	Peru	Pasco
P.penduliflora580	MO	Kay	102	Jamaica	Trelawny
P.penduliflora589	MO	Kay	230	Cuba	Santiago de Cuba
P.penduliflora595	MO	Kay	104	Jamaica	Trelawny
P.penduliflora599	MO	Kay	174	Jamaica	Claredon
P.pilosissima044	MO	Hernandez	291	Colombia	Antioquia
P.poeppigii266	MO	Boza	2139	Peru	Loreto
P.punctata056	MO	Jorgensen	2458	Ecuador	Azuay
P.punctata057	MO	Jorgensen	2457	Ecuador	El Oro
P.punctata192	MO	Weigend	98/184	Peru	Piura
P.coronapapillata421	MO	Campos	3901	Peru	Cajamarca
P.quadriflora369	MO	Galiano	6424	Peru	Cusco
P.rotundifolia700	US	Stehle	1513	Guadeloupe	NA
P.rotundifolia701	NY	Stehle	123	Caribbean,	Guadeloupe
P.rotundifolia908	US	Stehle	2585	Caribbean,	Guadeloupe
P.sandrae127	MO	MacDougal	6290	Panama	Coclé
P.saxicola603	MO	MacDougal	6336	Brazil	Cult.
P.sexflora323	MO	Hansen	9185	Puerto Rico	Patillas
P.sexflora324	MO, NY, US	Axelrod	6137	Puerto Rico	Barranquitas
P.smilacifolia444	MO	Schwerdtfeger	MOID: 2879562	Ecuador	Napo
P.smilacifolia464	MO	Krosnick	500	Ecuador	Cutl.
P.sp.nov388	MO	Valenzuela	13876	Peru	Pasco
P.sp.nov404	MO	Ferreyra	7783	Peru	San Martin
P.sp612	MO	Kay	108	Jamaica	Trelawny
P.spnov332	MO	Costa	439	Brazil,	Amazonas
P.standleyi272	MO	Breedlove	37173	Mexico	Chiapas
P.standleyi273	DUKE	MacDougal	855	Costa Rica	San Jose
P.standleyi395	MO	Castillo	ISF00812	El Salvador	Ahuachapán
P.standleyi426	MO	Renderos	410	El Salvador	La Libertad
P.standleyi431	MO	Davidse	35029	Honduras	El Paraiso
P.standleyi432	MO	Davidse	29971	Mexico	Chiapas
P.stenosepala631	US	Morton	6140	St. Vincent	NA
P.subfertilis449, & P.subfertilis263	MO,DUKE	MacDougal	597GR	Guatemala	Quetzaltenango
P.talamancensis412	MO	Kernan	120	Costa Rica	Puntaremas
P.tatei068	MO	Boza	2113	Bolivia	La Paz
P.tatei166	MO	Delanoy	398	Bolivia	La Paz
P.telesiphe173a & b	MO	Grant	08-4525	Ecuador	Zamora-Chinchipe
P.telesiphe718	MO	Knapp	9124	Ecuador	Zamora-Chinchipe
P.transversalis491	MO	Pedersen	15696	Brazil	Rio Grande do Sul
P.tribolophylla708	NY	Luteyn	12480	Colombia	Antioquia
P.tribolophylla709	NY	Lehmann	BT859	Colombia	NA
P.tribolophylla866	TEX	Albert de Escobar	1022	Colombia	Valle del Cauca
P.tricuspis150	MO	Delanoy	154	Bolivia	La Paz
P.tricuspis506	MO	Boza	2104	Bolivia	La Paz
P.tricuspis515	MO	Zardini	46427	Paraguay	Amambay
P.tricuspis516	MO	Zardini	46426	Paraguay	Amambay
P.tricuspis625a & b	NY	Nee	37485	Bolivia	Santa Cruz
P.trifasciata536	MO	Krosnick	506	Cult	Cult.
P.trifasciata537	MO	Krosnick	460	Cult	Cult.

P.trinervia078	MO	Ramos	3000	Colombia	Valle del Cauca
P.tuberosa437	MO	Krosnick	484	Cult	Cult.
P.tuberosa609	MO	Kay	223	Trinidad	Cult.
P.tulae581	MO	Kay	225	Puerto Rico	Maricao
P.tulae583	MO	Kay	224	Puerto Rico	Maricao
P.tulae590	MO	MacDougal	6030	Cult	Cult.
P.tulae614	MO	Kay	202	Puerto Rico	Patillas
P.urnifolia067	MO	Delanoy	190	Bolivia	La Paz
P.urnifolia783	LPB	Beck	14905	Bolivia,	La Paz
P.aff.tricuspis514	MO	Zardini	46506	Paraguay	Amambay
P.urnifolia?405	MO	Villarroel	1494	Bolivia	
P.vespertilio128	MO	Valenzuela	2488	Peru	Madre de Dios
P.vespertilio598 & P. vespertilio549	MO	MacDougal	6022	French Guiana	NA
P.viridescens039	MO	Ulloa	2522	Ecuador	Azuay
P.viridescens040	MO	Ulloa	1887	Ecuador	Azuay
P.viridescens125	MO	Schwerdtfeger	96090602	Ecuador	Loja
P.yucatanensis478	MO	Cabrera	6470	Mexico	Quintana Roo
P.yucatanensis722	MO	MacDougal	4680	Mexico	Quintana Roo

Figures.

Figure 1 (next page). Map of the collections and areas of endemism used as biogeographical regions in this study. Black dots represent the *Passiflora* accessions used in this study. A) North America, including northern Mexico, B) Central America, C) the Greater Antilles, D) the Bahamas, E) the Lesser Antilles, F) the Guianas region of South America, G) the Northern Andes mountains range, H) Chocó), I) the Central–Southern Andes mountains and the dry Chaco, J) the Amazonas region plus the Brazilian Cerrado dry biome combined, and K) the Brazilian Atlantic Forest plus the humid Chaco biome combined. The base map shows a digital elevation model, with higher elevations indicated by progressively darker shading, and international borders.

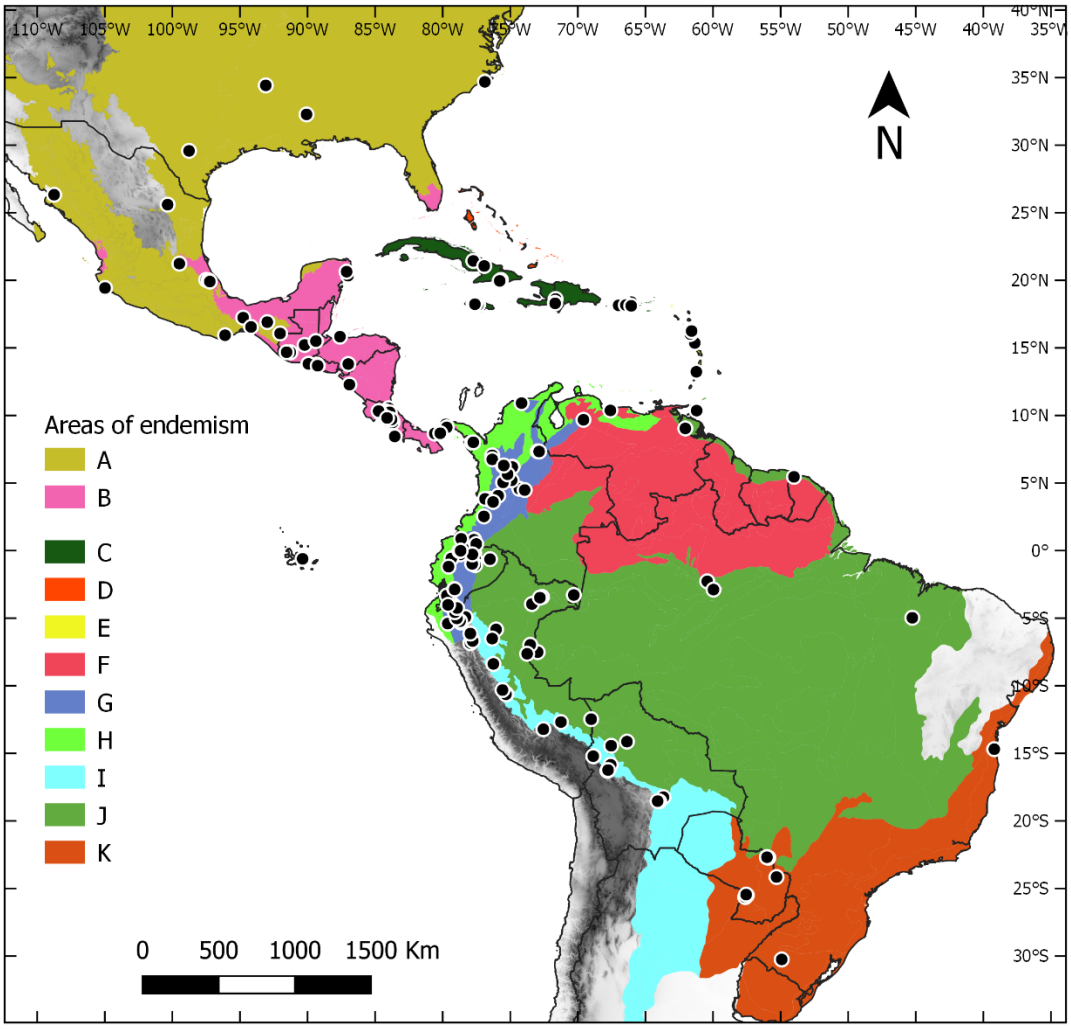
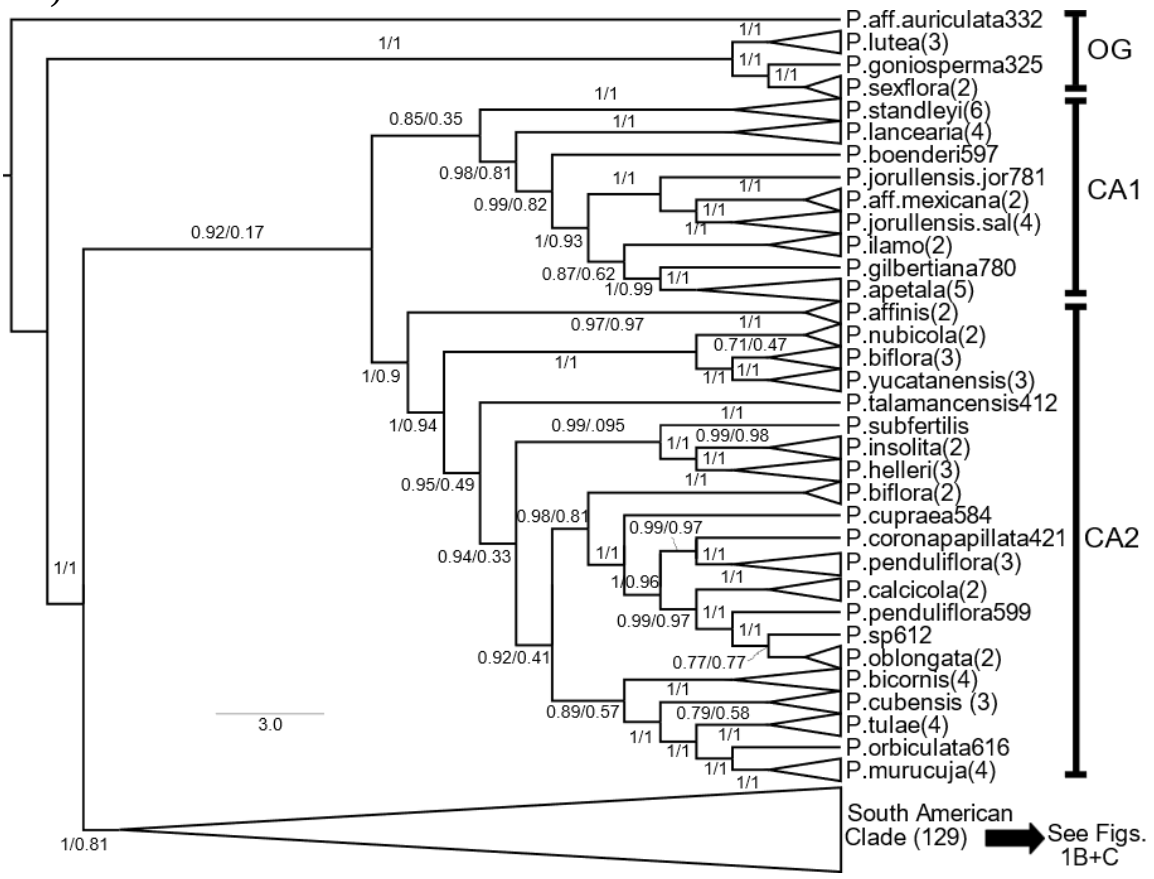
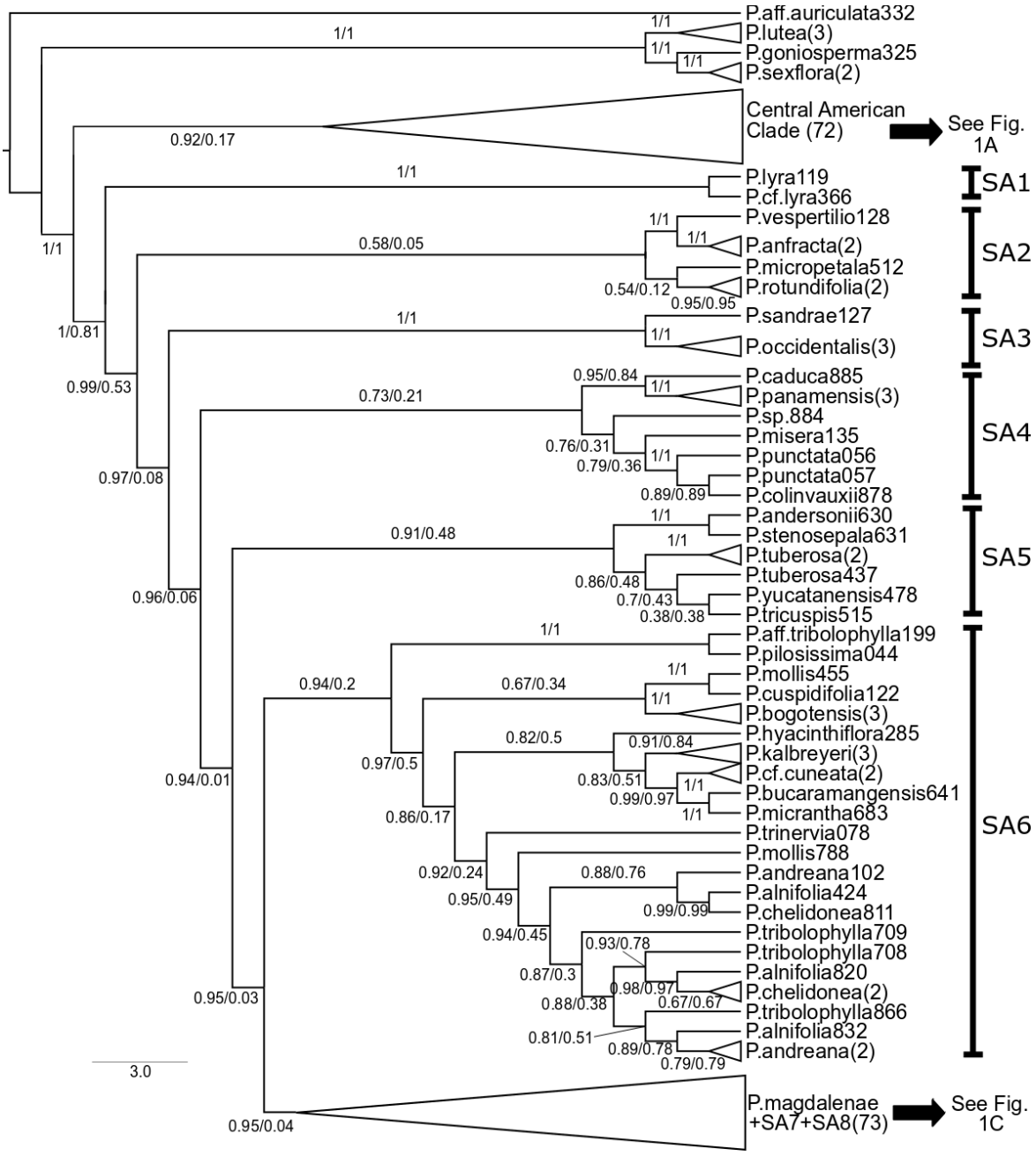


Figure 2 (next page). Phylogenetic reconstruction from the RAxML analysis. Cladograms with support values of transfer bootstrap expectation (TBE) and Felsenstein bootstrap (FBP) in the format: TBE/FBP. For practical purposes we show three variants: A) With the South American clade collapsed. B) With central America, SA7 and SA8 clades collapsed and C) With Central America and SA1-SA6 clades collapsed. Triangles show collapsed clades. OG: outgroup, CA: Central America clades, SA: South America clades. For a non-collapsed complete phylogram, refer to Supplementary material 7.

A)


P.aff.auriculata332
P.lutea(3)
P.goniosperma325
P.sexflora(2)
P.standleyi(6)
P.lancearia(4)
P.boenderi597
P.jorullensis.jor781
P.aff.mexicana(2)
P.jorullensis.sal(4)
P.ilamo(2)
P.gilbertiana780
P.apetala(5)
P.affinis(2)
P.nubicola(2)
P.biflora(3)
P.yucatanensis(3)
P.talamancensis412
P.subfertilis
P.insolita(2)
P.helleri(3)
P.biflora(2)
P.cupraea584
P.coronapapillata421
P.penduliflora(3)
P.calcicola(2)
P.penduliflora599
P.sp612
P.oblongata(2)
P.bicornis(4)
P.cubensis (3)
P.tulae(4)
P.orbiculata616
P.murucuja(4)
OG
CA1
CA2
South American Clade (129)
See Figs. 1B+C
3.0

B)


P.aff.auriculata332
P.lutea(3)
P.goniosperma325
P.sexflora(2)
Central American Clade (72)
See Fig. 1A
P.lyra119
P.cf.lyra366
P.vespertilio128
P.anfracta(2)
P.micropetala512
P.rotundifolia(2)
P.sandrae127
P.occidentalis(3)
P.caduca885
P.panamensis(3)
P.sp.884
P.misera135
P.punctata056
P.punctata057
P.colinvauxii878
P.andersonii630
P.stenosepala631
P.tuberosa(2)
P.tuberosa437
P.yucatanensis478
P.tricuspis515
P.aff.tribolophylla199
P.pilosissima044
P.mollis455
P.cuspidifolia122
P.bogotensis(3)
P.hyacinthiflora285
P.kalbreyeri(3)
P.cf.cuneata(2)
P.bucaramangensis641
P.micrantha683
P.trinervia078
P.mollis788
P.andreana102
P.alnifolia424
P.chelidonea811
P.tribolophylla709
P.tribolophylla708
P.alnifolia820
P.chelidonea(2)
P.tribolophylla866
P.alnifolia832
P.andreana(2)
P.magdalenae +SA7+SA8(73)
See Fig. 1C
SA1
SA2
SA3
SA4
SA5
SA6
3.0

C)

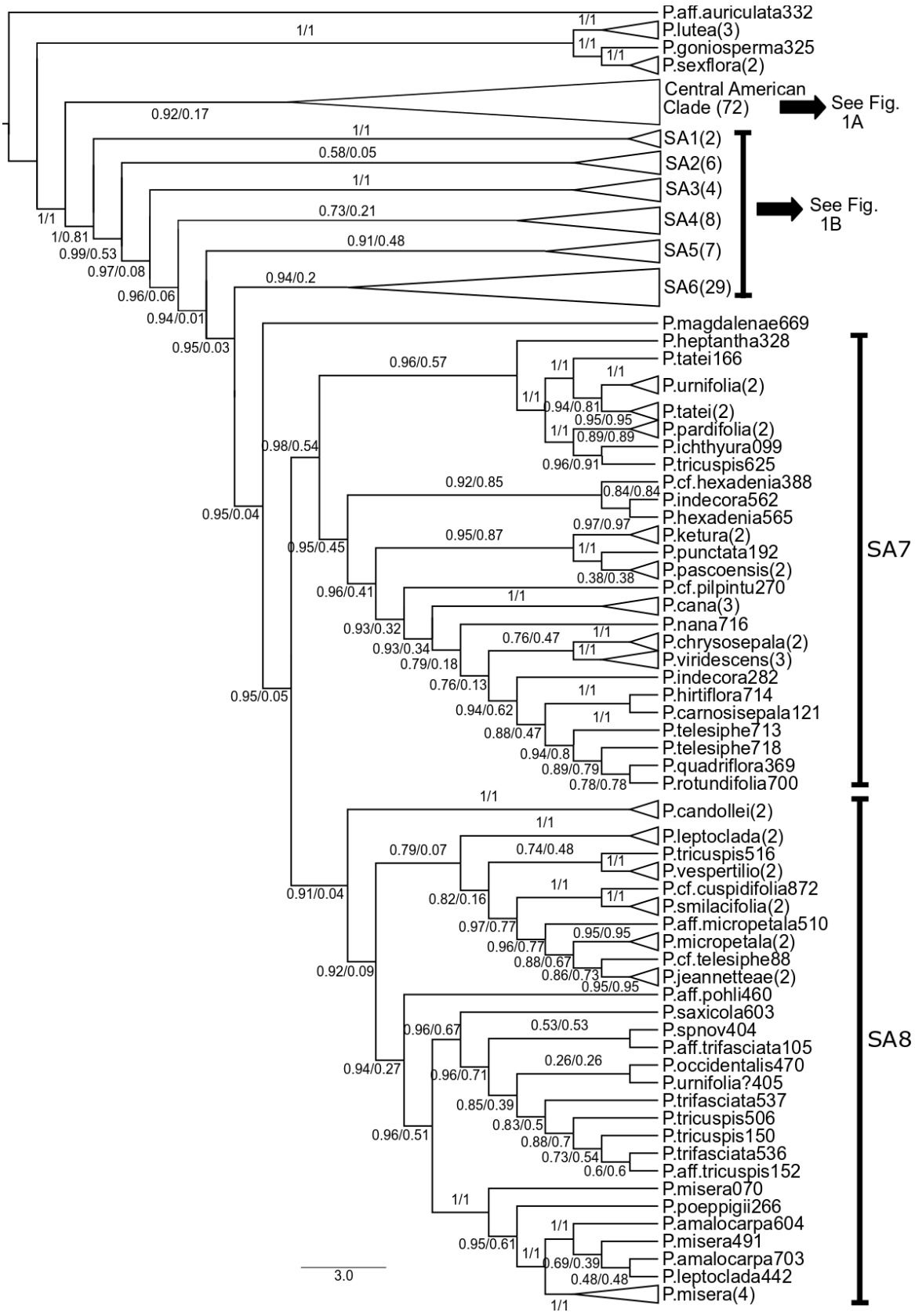
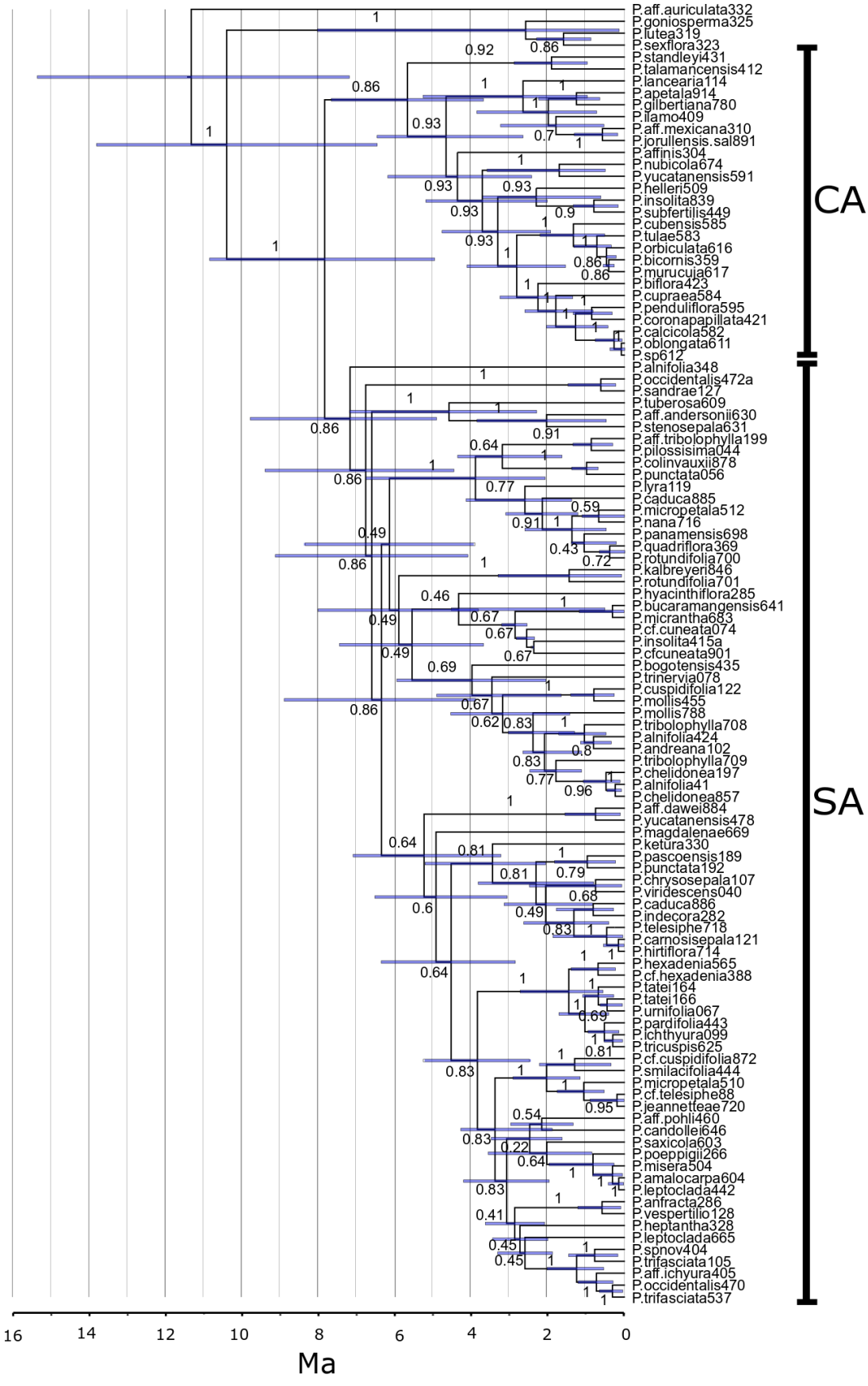

P.aff.auriculata332
P.lutea(3)
P.goniosperma325
P.sexflora(2)
Central American Clade (72)
See Fig. 1A
SA1(2)
SA2(6)
SA3(4)
SA4(8)
SA5(7)
SA6(29)
See Fig. 1B
P.magdalenae669
P.heptantha328
P.tatei166
P.urnifolia(2)
P.tatei(2)
P.pardifolia(2)
P.ichthyura099
P.tricuspis625
P.cf.hexadenia388
P.indecora562
P.hexadenia565
P.ketura(2)
P.punctata192
P.pascoensis(2)
P.cf.pilpintu270
P.cana(3)
P.nana716
P.chrysosepala(2)
P.viridescens(3)
P.indecora282
P.hirtiflora714
P.carnosisepala121
P.telesiphe713
P.telesiphe718
P.quadriflora369
P.rotundifolia700
SA7
P.candollei(2)
P.leptoclada(2)
P.tricuspis516
P.vespertilio(2)
P.cf.cuspidifolia872
P.smilacifolia(2)
P.aff.micropetala510
P.micropetala(2)
P.cf.telesiphe88
P.jeannetteae(2)
P.aff.pohli460
P.saxicola603
P.spnov404
P.aff.trifasciata105
P.occidentalis470
P.urnifolia?405
P.trifasciata537
P.tricuspis506
P.tricuspis150
P.trifasciata536
P.aff.tricuspis152
P.misera070
P.poeppigii266
P.amalocarpa604
P.misera491
P.amalocarpa703
P.leptoclada442
P.misera(4)
SA8
3.0

Figure 3 (next page). BEAST maximum credibility chronogram. Branch values are posterior probability, node bars represent 95% credibility interval for node age in millions of years.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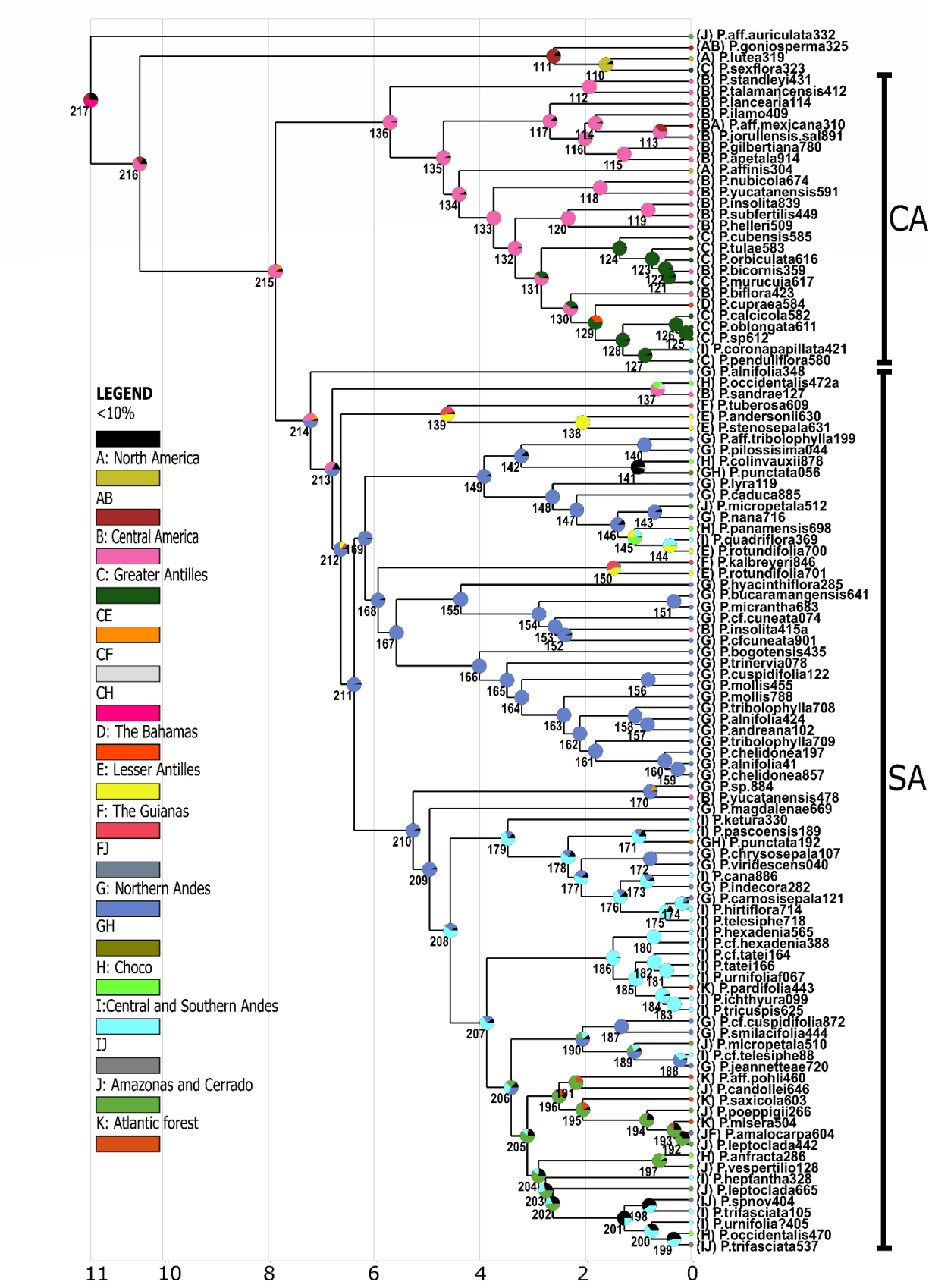
P.sexflora323
P.standleyi431
P.talamancensis412
P.lancearia114
P.apetala914
P.gilbertiana780
P.llamo409
P.aff.mexicana310
P.jorullensis.sal891
P.affinis304
P.nubicola674
P.yucatanensis591
P.helleri509
P.insolita839
P.subfertilis449
P.cubensis585
P.tulae583
P.orbiculata616
P.bicornis359
P.murucuja617
P.biflora423
P.cupraea584
P.penduliflora595
P.coronapapillata421
P.calcicola582
P.oblongata611
P.sp612
P.alnifolia348
P.occidentalis472a
P.sandrae127
P.tuberosa609
P.aff.andersonii630
P.stenosepala631
P.aff.tribolophylla199
P.pilosissima044
P.colinvauxii878
P.punctata056
P.lyra119
P.caduca885
P.micropetala512
P.nana716
P.panamensis698
P.quadriflora369
P.rotundifolia700
P.kalbreyeri846
P.rotundifolia701
P.hyacinthiflora285
P.bucaramangensis641
P.micrantha683
P.cf.cuneata074
P.insolita415a
P.cfcuneata901
P.bogotensis435
P.trinervia078
P.cuspidifolia122
P.mollis455
P.mollis788
P.tribolophylla708
P.alnifolia424
P.andreana102
P.tribolophylla709
P.chelidonea197
P.alnifolia41
P.chelidonea857
P.aff.dawei884
P.yucatanensis478
P.magdalenae669
P.ketura330
P.pascoensis189
P.punctata192
P.chrysosepala107
P.viridescens040
P.caduca886
P.indecora282
P.telesiphe718
P.carnosisepala121
P.hirtiflora714
P.hexadenia565
P.cf.hexadenia388
P.tatei164
P.tatei166
P.urnifolia067
P.pardifolia443
P.ichthyura099
P.tricuspis625
P.cf.cuspidifolia872
P.smilacifolia444
P.micropetala510
P.cf.telesiphe88
P.jeannetteae720
P.aff.pohli460
P.candollei646
P.saxicola603
P.poeppigii266
P.misera504
P.amalocarpa604
P.leptoclada442
P.anfracta286
P.vespertilio128
P.heptantha328
P.leptoclada665
P.spnov404
P.trifasciata105
P.aff.ichyura405
P.occidentalis470
P.trifasciata537
CA
SA
16
14
12
10
8
6
4
2
0
Ma

Figure 4 (next page). Ancestral area reconstruction tree from RASP. The tips contain the current area assigned to the taxa in parentheses together with a colored circle representing the assigned area. Each node pie represents the probability of an ancestral area. Colors for biogeographical areas that we modeled (areas of endemism coded as biogeographical provinces) match those in Fig. 1, except for the categories that represented a two area range combination. The * symbol and black color represent areas with <10% probability of ancestry. The *x*-axis represents time in millions of years ago (Ma).


LEGEND
<10%
A: North America
AB
B: Central America
C: Greater Antilles
CE
CF
CH
D: The Bahamas
E: Lesser Antilles
F: The Guianas
FJ
G: Northern Andes
GH
H: Choco
I:Central and Southern Andes
IJ
J: Amazonas and Cerrado
K: Atlantic forest
CA
SA
11
10
8
6
4
2
0

Supplementary material

Supplementary Table S1. Parameters used for the iPyrad pipeline

Parameter	Setting used in this study
[5] [assembly_method]: Assembly method (denovo, reference, denovo+reference, denovo-reference)	denovo
[7] [datatype]: Datatype (see docs): rad, gbs, ddrad, etc.	2brad
[9] [max_low_qual_bases]: Max low quality base calls (Q<20) in a read	5
[10] [phred_Qscore_offset]: phred Q score offset (33 is default and very standard)	33
[11] [mindepth_statistical]: Min depth for statistical base calling	6
[12] [mindepth_majrule]: Min depth for majority-rule base calling	6
[13] [maxdepth]: Max cluster depth within samples	10000
[14] [clust_threshold]: Clustering threshold for de novo assembly	0.9
[15] [max_barcode_mismatch]: Max number of allowable mismatches in barcodes	0
[16] [filter_adapters]: Filter for adapters/primers (1 or 2=stricter)	0
[17] [filter_min_trim_len]: Min length of reads after adapter trim	35
[18] [max_alleles_consens]: Max alleles per site in consensus sequences	2
[19] [max_Ns_consens]: Max N's (uncalled bases) in consensus (R1, R2)	5, 5
[20] [max_Hs_consens]: Max Hs (heterozygotes) in consensus (R1, R2)	8, 8
[21] [min_samples_locus]: Min	12
[22] [max_SNPs_locus]: Max	20, 20
[23] [max_Indels_locus]: Max	8, 8
[24] [max_shared_Hs_locus]: Max	0.5
[25] [trim_reads]: Trim raw read edges (R1>, <R1, R2>, <R2) (see docs)	0, 0, 0, 0
[26] [trim_loci]: Trim locus edges (see docs) (R1>, <R1, R2>, <R2)	0, 0, 0, 0
[28] [pop_assign_file]: Path to population assignment file	

Supplementary Table S2. Final sample stats summaryfrom iPyrad for the "complete" dataset

Sample	reads_raw	hetero_est	error_est	reads_consens	loci_in_assembly	missing _info_%
P.aff.ichyura405	951135	0.016462	0.00053	5856	2273	81
P.aff.lancearia415a	364678	0.019554	0.00144	6025	2540	78
P.aff.lancearia415b	10424809	0.009464	0.00036	15638	6052	49
P.aff.lancearia839	2292597	0.009742	0.00044	6563	1723	85
P.aff.mexicana310	1671168	0.008224	0.00038	4761	1255	89
P.aff.mexicana311	991796	0.004122	0.00032	3829	1417	88
P.aff.micropetala510a	4612414	0.011052	0.00089	41983	1480	87
P.aff.micropetala510b	4015655	0.010272	0.00127	66231	4304	63
P.aff.pohli460	1294240	0.009488	0.00055	5768	1959	83
P.aff.tribolophylla199	5772289	0.004649	0.00059	37042	4447	62
P.affinis304	1315165	0.00983	0.00137	7862	3350	72
P.affinis363	1789941	0.017973	0.00114	9806	3515	70
P.alnifolia424	3206559	0.005065	0.00041	18989	1543	87
P.alnifolia820	1616849	0.019334	0.00152	35456	1349	89
P.alnifolia832	1600980	0.012124	0.00083	27325	1456	88
P.amalocarpa604	4353544	0.007176	0.00063	11263	4737	60
P.amalocarpa703	1409950	0.019719	0.00096	22527	1252	89
P.andersonii630	607658	0.006827	0.00044	3381	1119	90
P.andreana049	866484	0.008284	0.00030	2898	1059	91
P.andreana102	3039628	0.028508	0.00255	74146	1594	86
P.andreana256	1080863	0.009133	0.00045	5712	285	98
P.anfracta280	211407	0.025689	0.00433	4621	228	98
P.anfracta286	921299	0.011173	0.00068	8257	1117	91
P.apetala632	1043238	0.018075	0.00227	30602	959	92
P.apetala633	2052849	0.015834	0.00074	18670	1674	86
P.apetala887	1159954	0.017047	0.00150	23815	1375	88
P.apetala914	2307772	0.015186	0.00079	25094	2207	81
P.bicornis141	643126	0.004118	0.00033	2538	974	92
P.bicornis359	1443973	0.02066	0.00105	20186	3348	72
P.bicornis695	1900706	0.007406	0.00036	5429	1668	86
P.bicornis696	1363666	0.00792	0.00041	4007	1358	88
P.biflora052	2012521	0.026857	0.00306	53487	1399	88
P.biflora244	324646	0.003309	0.00063	3208	641	95
P.biflora288	623709	0.008161	0.00051	2228	807	93
P.biflora423	527861	0.011523	0.00070	4047	1705	86
P.biflora613	2966773	0.009669	0.00104	11453	4659	60
P.boenderi594	5209436	0.00729	0.00050	13271	5354	55
P.boenderi597	1977967	0.008496	0.00118	17227	4240	64

P.bogotensis435	4721995	0.015303	0.00115	73054	4833	59
P.bogotensis439	438502	0.018948	0.00162	6791	2927	75
P.bogotensis441	3746297	0.024834	0.00182	66860	1613	86
P.bucaramangensis641	2020632	0.009454	0.00048	7919	1589	87
P.caduca886	645065	0.006373	0.00076	4270	1368	88
P.calcicola582	4126075	0.007851	0.00052	12830	5376	54
P.calcicola600	3375983	0.007094	0.00061	12220	5527	53
P.calcicola610	3940789	0.006341	0.00043	10027	4616	61
P.cana558	2511557	0.023825	0.00129	29186	1493	87
P.cana643	5757823	0.021249	0.00251	153230	1798	85
P.cana885	1912678	0.016178	0.00070	16225	1549	87
P.candollei646	1477963	0.019158	0.00187	40913	1248	89
P.candollei783	1189249	0.009082	0.00044	4324	1331	89
P.carnosisepala121	1415546	0.013825	0.00042	6686	1415	88
P.cf.candollei782	2024627	0.032525	0.00329	53756	1345	89
P.cf.chelidonea366	976420	0.017265	0.00108	10504	3598	69
P.cf.cuneata074	1042522	0.012906	0.00066	9904	1527	87
P.cf.cuspidifolia872	2388927	0.018054	0.00084	28241	1574	87
P.cf.tatei164	7974265	0.009671	0.00077	69222	4882	59
P.cf.telesiphe88	30166	0.018117	0.00312	966	336	97
P.cfcuneata901	2672085	0.008866	0.00062	35614	2009	83
P.chelidonea811	1280895	0.017477	0.00103	18485	1295	89
P.chelidonea812	1754910	0.015216	0.00154	21449	1710	85
P.chelidonea857	3577429	0.014672	0.00096	44127	1747	85
P.chrysosepala106	1127439	0.025958	0.00479	32247	435	96
P.chrysosepala107	599617	0.008496	0.00128	12733	722	94
P.colinvauxii878	2981695	0.008807	0.00041	5388	1913	84
P.condorita281	225093	0.021088	0.00243	5048	451	96
P.cubensis585	4622960	0.008311	0.00059	11975	5176	56
P.cubensis586	4391249	0.011	0.00047	11818	4896	58
P.cubensis601	5721147	0.009224	0.00044	12196	5028	57
P.cupraea584	6246913	0.007686	0.00046	13269	5623	52
P.cuspidifolia122	732899	0.031037	0.00182	10718	840	93
P.dawei884	2736282	0.007622	0.00039	5518	1941	84
P.gilbertiana780	2464935	0.020365	0.00118	27709	1358	88
P.goniosperma325	561466	0.016518	0.00092	4873	563	95
P.helleri108	1049454	0.00743	0.00040	4942	1537	87
P.helleri143	2314352	0.022732	0.00226	58810	1637	86
P.helleri509	1803476	0.009332	0.00085	10555	4079	65
P.heptantha328	5136065	0.01079	0.00062	23198	4167	65
P.hexadenia565	4635808	0.020983	0.00200	74053	1791	85
P.hirtiflora714	4942930	0.018929	0.00235	157265	1321	89
P.hyacinthiflora285	1483172	0.008081	0.00039	5076	1406	88

P.ichthyura099	987182	0.028889	0.00145	13760	823	93
P.ilamo407a	3301318	0.025093	0.00107	28421	4970	58
P.ilamo407b	3686986	0.02624	0.00107	28321	4787	59
P.ilamo409	5170926	0.008452	0.00064	12276	5237	56
P.indecora282	224346	0.004401	0.00056	1816	727	94
P.indecora562	3564791	0.008581	0.00016	4924	696	94
P.jeannettae469	125168	0.013384	0.00137	2030	593	95
P.jeannettae720	2463798	0.009812	0.00045	5478	1841	84
P.jorullensis.sal660	3391959	0.012109	0.00125	71215	1296	89
P.jorullensis.sal661	2232890	0.010497	0.00048	14710	1679	86
P.jorullensis.sal663	1318043	0.009413	0.00032	4028	1302	89
P.jorullensis.sal891	842794	0.010917	0.00078	5192	1821	85
P.jorullensis781	1524335	0.030853	0.00151	23436	1327	89
P.kalbreyeri283	40308	0.007119	0.00160	695	134	99
P.kalbreyeri553	4002379	0.019392	0.00132	51911	1524	87
P.kalbreyeri846	10353869	0.018982	0.00246	278255	1659	86
P.ketura330	3253076	0.007107	0.00120	10539	4665	60
P.ketura710	1389029	0.007367	0.00091	28986	1143	90
P.lancearia114	1298904	0.013969	0.00038	6517	1472	88
P.lancearia115	1244970	0.036189	0.00285	20908	805	93
P.lancearia251	1128892	0.037591	0.00423	21689	572	95
P.lancearia399	7677506	0.014959	0.00048	21549	5157	56
P.leptoclada442	1826806	0.010789	0.00026	3554	1285	89
P.leptoclada665	3646426	0.008633	0.00066	27480	749	94
P.leptoclada666	2313457	0.006975	0.00050	18035	690	94
P.lutea319	488765	0.019525	0.00155	3503	604	95
P.lutea320	1023494	0.016559	0.00064	3326	566	95
P.lutea322	923164	0.016678	0.00074	3166	534	95
P.lyra119	1456931	0.005864	0.00029	3557	1359	88
P.magdalenae669	892039	0.007731	0.00052	4147	1512	87
P.micrantha683	1240290	0.013199	0.00040	6505	1343	89
P.micropetala385	1294046	0.017402	0.00090	13832	1495	87
P.micropetala512	26485	0.018926	0.00343	827	293	98
P.micropetala721	3093920	0.011977	0.00042	6012	1930	84
P.misera070	793526	0.004428	0.00045	5399	1256	89
P.misera135	41281	0.017621	0.00193	821	274	98
P.misera257	1963703	0.012544	0.00042	4973	1650	86
P.misera491	2435223	0.008522	0.00053	17184	3510	70
P.misera501	2811849	0.013257	0.00096	29097	4354	63
P.misera503	2470979	0.01677	0.00169	49822	3178	73
P.misera504	7081556	0.017826	0.00072	59416	5028	57
P.mollis455	3752720	0.007584	0.00051	13099	4235	64
P.mollis788	1060902	0.022411	0.00168	22047	1278	89

P.murucuja592	4931599	0.008289	0.00054	12780	5754	51
P.murucuja617	8578727	0.007749	0.00028	11979	5293	55
P.murucuja618	5556367	0.007899	0.00039	12301	5252	55
P.murucuja619	9723257	0.007147	0.00026	12221	5514	53
P.nana716	1878352	0.024758	0.00352	66293	580	95
P.nubicola674	2386565	0.012775	0.00069	11226	1720	85
P.nubicola676	1458688	0.013235	0.00067	5333	1494	87
P.oblongata587	2416667	0.007213	0.00056	9478	4423	62
P.oblongata611	4778762	0.008968	0.00047	15475	5756	51
P.occidentalis261	1364889	0.033153	0.00425	30991	638	95
P.occidentalis337	3008181	0.017728	0.00139	33231	1693	86
P.occidentalis470	27524	0.015383	0.00248	837	221	98
P.occidentalis472a	7098521	0.03069	0.00256	127945	4625	61
P.occidentalis472b	5189115	0.029996	0.00258	94177	4452	62
P.orbiculata616	12036020	0.005823	0.00024	11276	5331	55
P.panamensis698	1729630	0.007598	0.00027	4443	1491	87
P.panamensis787	741800	0.007386	0.00068	4298	1148	90
P.panamensis912	720548	0.015231	0.00170	20501	759	94
P.pardifolia126	1089819	0.008763	0.00073	13524	1128	90
P.pardifolia443	3324774	0.008706	0.00088	12085	4953	58
P.pascoensis189	2701190	0.029549	0.00400	73604	1327	89
P.pascoensis190	404759	0.006223	0.00148	11439	791	93
P.penduliflora580	4131151	0.00849	0.00051	11554	5166	56
P.penduliflora589	3861729	0.007758	0.00071	11584	4983	58
P.penduliflora599	2750507	0.007662	0.00088	10866	5060	57
P.pilossisima044	1197428	0.006906	0.00031	7312	1354	89
P.pilpintu270	762110	0.011323	0.00096	19036	1071	91
P.poeppigii266	1138161	0.007921	0.00130	8022	3346	72
P.punctata056	789985	0.016507	0.00169	16185	1350	89
P.punctata057	263880	0.007808	0.00185	7078	596	95
P.punctata192	244071	0.017367	0.00220	6423	368	97
P.punctata421	178942	0.01279	0.00148	4929	1267	89
P.quadriflora369	58476	0.01617	0.00204	1633	343	97
P.rotundifolia700	4652669	0.016573	0.00174	108816	713	94
P.rotundifolia701	3125884	0.02219	0.00238	67363	1207	90
P.rotundifolia908	1271025	0.013127	0.00103	40629	926	92
P.sandrae127	414628	0.015443	0.00105	6462	776	93
P.saxicola603	7843536	0.008039	0.00042	15340	5307	55
P.sexflora323	772357	0.019887	0.00250	16904	542	95
P.sexflora324	629052	0.023557	0.00244	15624	609	95
P.smilacifolia444	1802626	0.00909	0.00136	10171	4289	64
P.smilacifolia464	1606444	0.00717	0.00070	8879	3810	68
P.sp595	3026632	0.007029	0.00051	10396	4562	61

P.sp612	1804484	0.00746	0.00068	9433	4343	63
P.spnov332	754691	0.032677	0.00258	13884	518	96
P.spnov388	1311134	0.027796	0.00266	28629	2519	79
P.spnov404	506848	0.016534	0.00113	9227	1953	83
P.standleyi272	4290681	0.007347	0.00067	10189	4147	65
P.standleyi273	5446509	0.006479	0.00042	10759	4376	63
P.standleyi395	377231	0.015347	0.00121	6358	2321	80
P.standleyi426	546365	0.011699	0.00061	4278	1780	85
P.standleyi431	7495789	0.008077	0.00033	14383	5274	55
P.standleyi432	251407	0.01061	0.00095	3233	1359	88
P.stenosepala631	1322583	0.016956	0.00166	38521	1128	90
P.subfertilis263	2642921	0.007569	0.00080	10156	4326	63
P.subfertilis449	3257742	0.007594	0.00076	12071	4703	60
P.talamanquensis412	606568	0.01754	0.00121	7302	2762	77
P.tatei068	489697	0.008814	0.00075	6929	932	92
P.tatei166	1432906	0.035043	0.00287	26744	1289	89
P.telesiphe173a	1726705	0.017535	0.00133	38904	1916	84
P.telesiphe173b	1975442	0.020072	0.00157	40694	1171	90
P.telesiphe718	2080264	0.010824	0.00029	4204	1424	88
P.tribolophylla708	57277	0.008648	0.00193	1798	365	97
P.tribolophylla709	2683114	0.018161	0.00198	59974	999	92
P.tribolophylla866	2087225	0.02464	0.00219	51028	1500	87
P.tricuspis150	1923247	0.013393	0.00114	28201	1359	88
P.tricuspis506	6394363	0.009585	0.00040	12725	4995	58
P.tricuspis515	128278	0.01543	0.00158	3407	1314	89
P.tricuspis516	990964	0.02443	0.00247	33209	2074	82
P.tricuspis625a	2465437	0.008437	0.00133	10516	4754	60
P.tricuspis625b	2465437	0.008437	0.00133	10516	4753	60
P.trifasciata105	612528	0.019986	0.00133	11880	883	93
P.trifasciata536	1081809	0.010029	0.00046	3932	1209	90
P.trifasciata537	3261952	0.012347	0.00031	5771	1906	84
P.trinervia078	1881100	0.027298	0.00406	57440	883	93
P.tuberosa437	6605817	0.00802	0.00028	14696	5104	57
P.tuberosa445	788365	0.010321	0.00042	4196	1504	87
P.tuberosa609	5144325	0.01123	0.00054	14589	5418	54
P.tulae581	7034056	0.008352	0.00051	12780	5606	52
P.tulae583	4177583	0.009417	0.00072	11590	5395	54
P.tulae590	2753510	0.006966	0.00077	10942	5197	56
P.tulae614	4442954	0.008184	0.00076	11325	4988	58
P.urnifolia067	977195	0.027768	0.00168	16359	1530	87
P.vespertilio128	1186350	0.031071	0.00492	32430	593	95
P.vespertilio152	690749	0.005641	0.00064	10906	1203	90
P.vespertilio522	4131151	0.00849	0.00051	11554	5178	56

P.vespertilio549	2015145	0.012076	0.00044	5857	1719	85
P.vespertilio598	2972361	0.011468	0.00103	11741	4520	62
P.viridescens039	658704	0.004632	0.00042	4532	1053	91
P.viridescens040	1529219	0.006689	0.00101	15109	1134	90
P.viridescens125	1337031	0.010179	0.00074	19226	1200	90
P.yucatanensis478	103802	0.015037	0.00130	3185	1333	89
P.yucatanensis591	6437224	0.007812	0.00037	11913	4688	60
P.yucatanensis722	3083658	0.009751	0.00027	4623	1557	87
P.yucatanensis92	3153530	0.010804	0.00038	5461	1813	85

Supplementary Table S3Transfer Bootstrap results log, instability index per taxon.

Taxon	Instability Index
P.aff.ichyura405	0.164675
P.aff.lancearia415a	0.004899
P.aff.lancearia415b	0.004899
P.aff.lancearia839	0.004899
P.aff.mexicana310	0
P.aff.mexicana311	0
P.aff.micropetala510a	0
P.aff.micropetala510b	0
P.aff.pohli460	1.071987
P.aff.tribolophylla199	0.837312
P.affinis304	0.140787
P.affinis363	0.216536
P.alnifolia424	0
P.alnifolia820	0
P.alnifolia832	0
P.amalocarpa604	0
P.amalocarpa703	0
P.andersonii630	0.285973
P.andreana049	0
P.andreana102	0
P.andreana256	0.42429
P.anfracta280	1.566606
P.anfracta286	1.566606
P.apetala632	0
P.apetala633	0
P.apetala887	0
P.apetala914	0
P.bicornis141	2.094525
P.bicornis359	2.094525
P.bicornis695	2.094525
P.bicornis696	2.094525
P.biflora052	0.109556
P.biflora244	0.044822
P.biflora288	0.109556
P.biflora423	0.044822
P.biflora613	0.109556
P.boenderi594	0
P.boenderi597	0.155638
P.bogotensis435	0.303864
P.bogotensis439	0.303864

P.bogotensis441	0.303864
P.bucaramangensis641	0
P.caduca886	0.318748
P.calcicola582	0
P.calcicola600	0
P.calcicola610	0
P.cana558	0.318748
P.cana643	0.318748
P.cana885	0.730853
P.candollei646	3.046061
P.candollei783	0
P.carnosisepala121	0
P.cf.candollei782	3.046061
P.cf.chelidonea366	0.346267
P.cf.cuneata074	0
P.cf.cuspidifolia872	0
P.cf.tatei164	0
P.cf.telesiphe88	0.00122
P.cfcuneata901	0
P.chelidonea811	0
P.chelidonea812	0
P.chelidonea857	0
P.chrysosepala106	0.339789
P.chrysosepala107	0.339789
P.colinvauxii878	0.424113
P.condorita281	0.004899
P.cubensis585	0
P.cubensis586	0
P.cubensis601	0
P.cupraea584	0.051149
P.cuspidifolia122	0.59953
P.dawei884	1.313959
P.gilbertiana780	0.008632
P.goniosperma325	0.721006
P.helleri108	0.004899
P.helleri143	0.004899
P.helleri509	0.004899
P.heptantha328	0.941608
P.hexadenia565	0.359643
P.hirtiflora714	0
P.hyacinthiflora285	0.40736
P.ichthyura099	0
P.ilamo407a	0

P.ilamo407b	0
P.ilamo409	0
P.indecora282	0.477804
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P.jeannettae469	0.141051
P.jeannettae720	0
P.jorullensis.sal660	0
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P.jorullensis781	0.003721
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P.occidentalis470	5.545191
P.occidentalis472a	0.3032
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P.standleyi431	0.849125
P.standleyi432	0.849125
P.stenosepala631	0.285973
P.subfertilis263	0.061933
P.subfertilis449	0.061933
P.talamanquensis412	0.894689
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P.vespertilio128	1.566606
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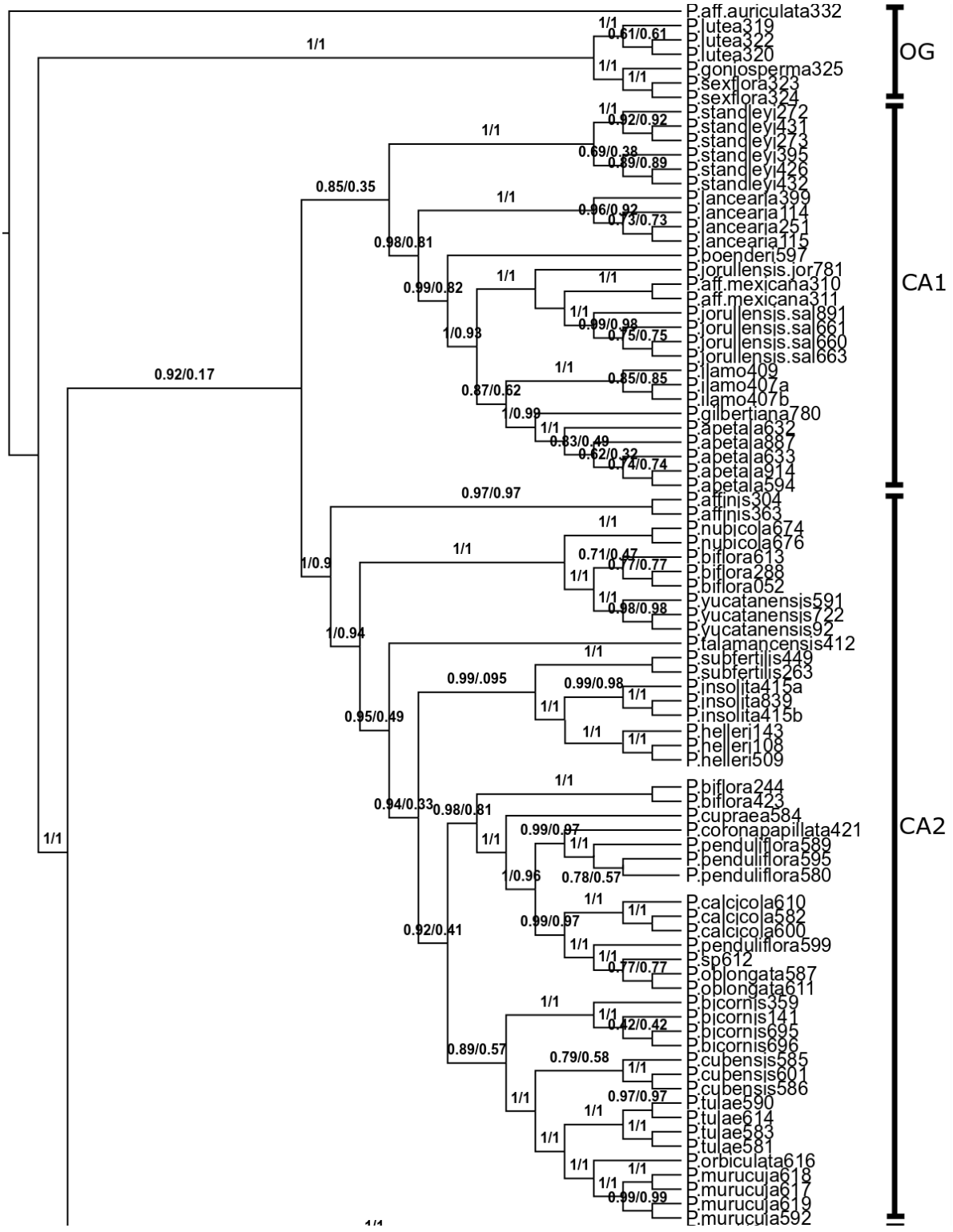
P.yucatanensis478	2.55321
P.yucatanensis591	0.109556
P.yucatanensis722	0.109556
P.yucatanensis92	0.109556

(Next three pages) Supplementary Figure S1. Non-collapsed cladogram. Support values format: TBE/FBP. OG: outgroup, CA: Central America clades, SA: South America clades.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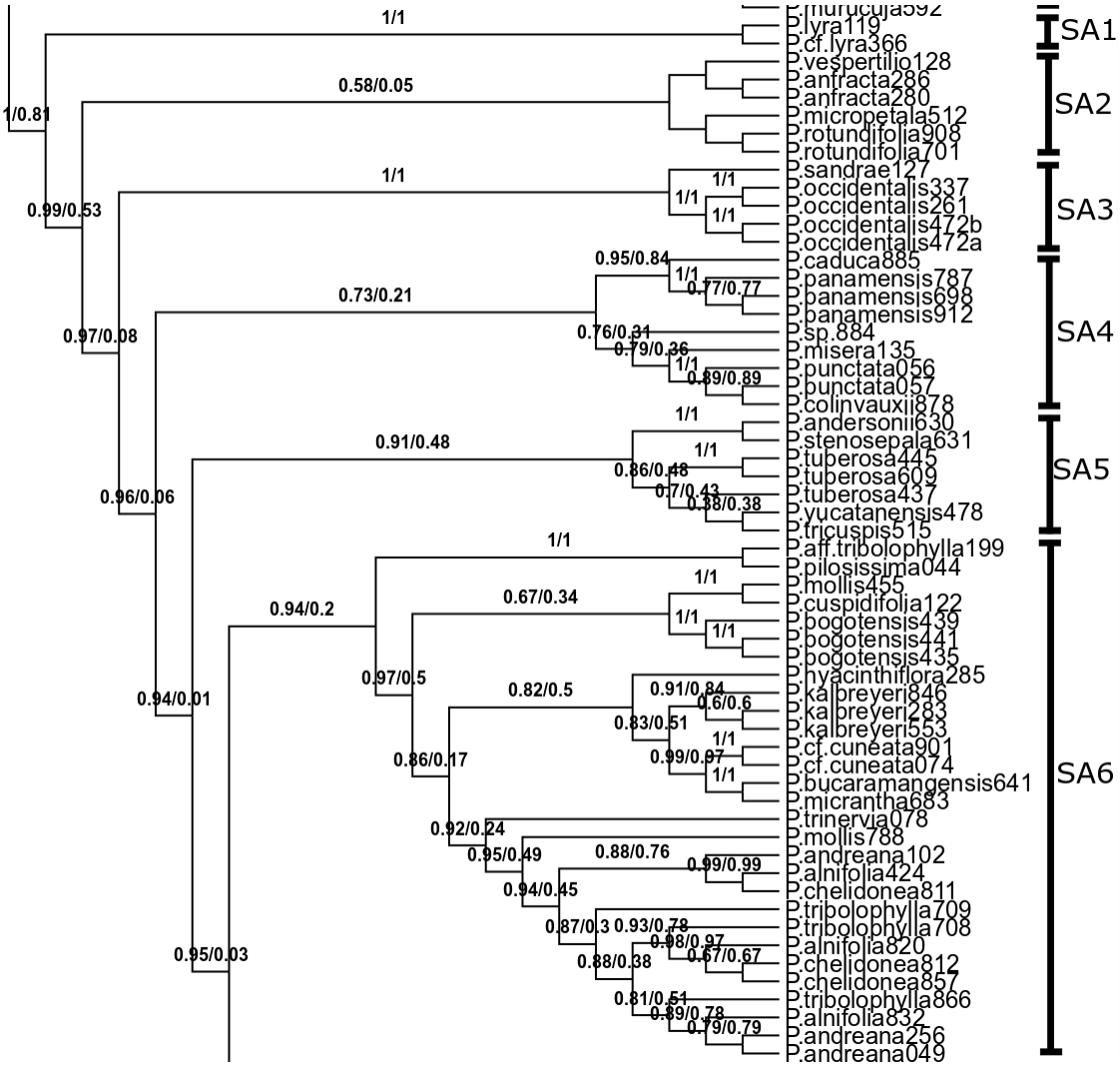
CA2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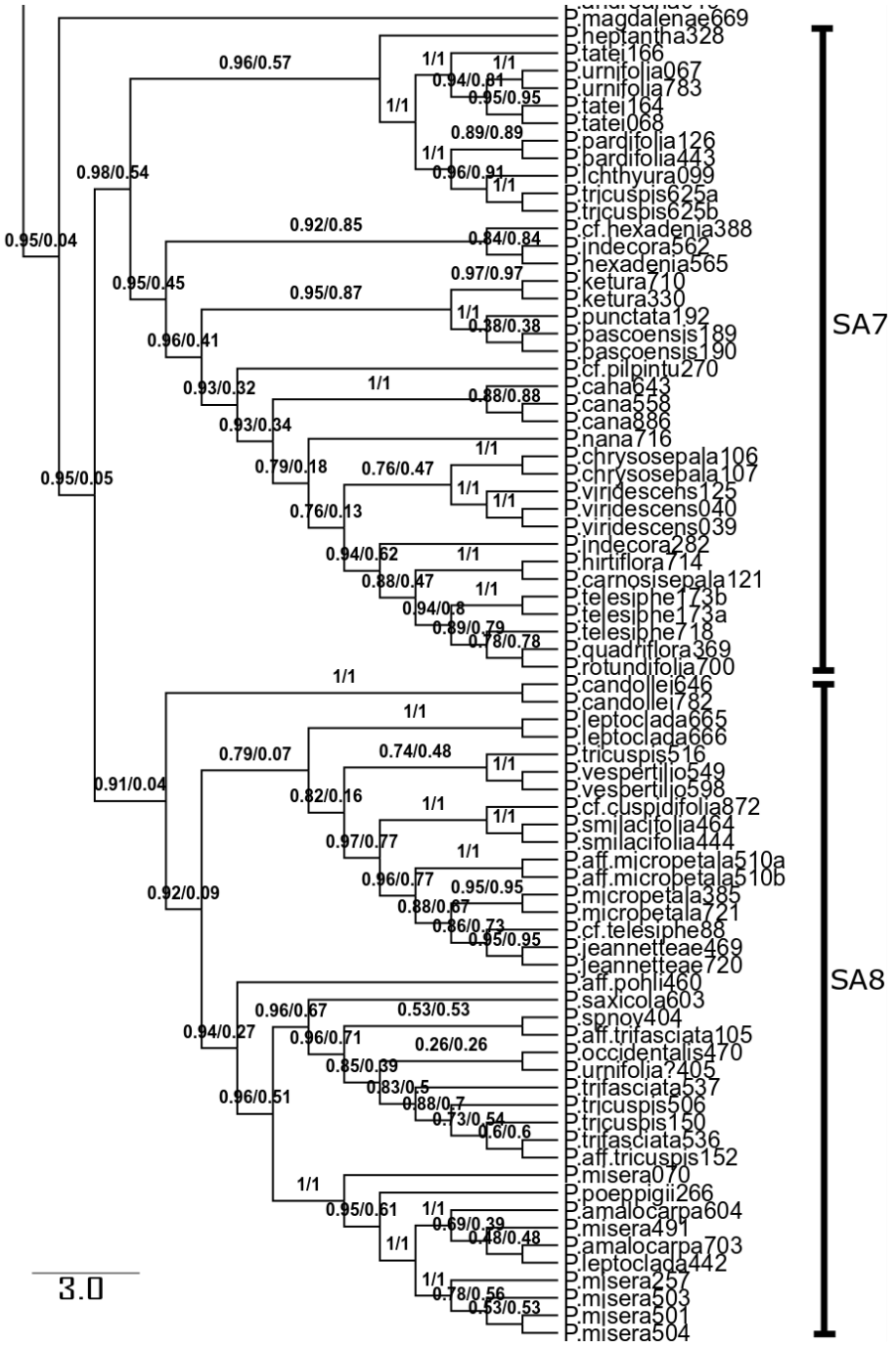
SA1
SA2
SA3
SA4
SA5
SA6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P.leptoclada442
P.misera257
P.misera503
P.misera501
P.misera504
0.95/0.04
0.98/0.54
0.96/0.57
0.95/0.05
0.95/0.45
0.92/0.85
0.95/0.87
0.96/0.41
0.93/0.32
0.93/0.34
0.79/0.18
0.76/0.47
0.76/0.13
0.94/0.62
0.88/0.47
0.94/0.8
0.89/0.79
0.78/0.78
0.91/0.04
0.79/0.07
0.74/0.48
0.82/0.16
0.97/0.77
0.96/0.77
0.92/0.09
0.88/0.67
0.86/0.73
0.94/0.27
0.96/0.67
0.53/0.53
0.96/0.71
0.26/0.26
0.85/0.39
0.83/0.5
0.88/0.7
0.73/0.54
0.6/0.6
0.96/0.51
0.95/0.61
0.69/0.39
0.78/0.56
0.53/0.53
1/1
0.94/0.84
0.95/0.95
0.89/0.89
0.96/0.91
0.84/0.84
0.97/0.97
0.88/0.38
0.88/0.88
0.95/0.95
3.0
SA7
SA8

Supplementary Table S4. RASP model test results.

Model	LnL	numparams	d	e	j	AICc	AICc_wt
DEC	-284.2	2	0.02	0.032	0	572.5	1.80E-21
DEC+J	-235.4	3	0.0034	1.00E-12	0.027	477.1	0.98
DIVALIKE	-279.8	2	0.023	0.024	0	563.7	1.50E-19
DIVALIKE+J	-239.2	3	0.0042	1.00E-12	0.026	484.6	0.023
BAYAREALIKE	-336.7	2	0.032	0.22	0	677.6	2.80E-44
BAYAREALIKE+J	-244.8	3	0.0024	0.0071	0.027	495.8	8.30E-05

	alt	null	LnLalt	LnLnull	DFalt	DFnull	DF	Dstatistic	pval	test	tail	AIC1	AIC2	AIC wt1	AIC wt2	AICweight Ratio model1	AICweight ratio model2
1	DEC+J	DEC	-235.4	-284.2	3	2	1	97.55	5.30E-23	chi-squared	one-tailed	476.9	572.4	1	1.80E-21	5.60E+20	1.80E-21
2	DIVALIKE+J	DIVALIKE	-239.2	-279.8	3	2	1	81.23	2.00E-19	chi-squared	one-tailed	484.4	563.6	1	6.30E-18	1.60E+17	6.30E-18
3	BAYAREALIKE+J	BAYAREALIKE	-244.8	-336.7	3	2	1	183.9	6.90E-42	chi-squared	one-tailed	495.6	677.5	1	3.20E-40	3.14E+39	3.20E-40

Supplementary Table S5. RASP results information.

Dispersal Table:

Area	from	to	within
A	1	3	3
B	8	4	15
C	4	3	7
D	0	1	0
E	3	3	1
F	0	4	0
G	18	2	34
H	1	6	1
I	3.5	12	9
J	7.5	4	6
K	0	4	0

Chapter II: Disentangling the *Passiflora alnifolia* species complex: a phylogenomic and population genomic approach

Abstract

The genus *Passiflora* is a fascinating group of mostly tropical vines that encompasses a broad range of diversity in both floral and leaf morphological features. In the tropical Andes of Colombia and Ecuador, several *Passiflora* species, including *P. alnifolia, P. chelidonea, P. tribolophylla* and *P. andreana,* share highly similar morphology and distribution, have diffuse species limits, and are currently treated as a species complex. Our goal was to determine patterns of genetic structure in the group, clarify how many lineages make up the "alnifolia complex" and, to understand how these lineages correspond to the taxonomy of the group. We sampled leaf tissue from 80 herbarium specimens at the Missouri Botanical Garden, including multiple accessions per species. We used a high-throughput DNA sequencing technique called 2b-RAD to sequence a large number of DNA characters from throughout the genome of each accession. The resulting data was processed using iPyrad and resulted in a data set of 229,040–230,148 characters and 1888–30,883 loci. We reconstructed the phylogeny using RAxML and Tetrad, analyzed genetic structure using STRUCTURE, and PCA, and tested for geneflow using Treemix. Finally, we analyzed these results in the context of both geography and morphology. We found that samples identified as *P. alnifolia, P. chelidonea, P. tribolophylla* and *P. andreana* did not form monophyletic groups, such that the current delimitation of species in the alnifolia complex does not correspond to phylogenetic relationships or patterns of genetic structure. The results of STRUCTURE and phylogenetic analyses identified three main clades inside the alnifolia complex: 1)

one group apparently restricted to middle elevation (1000-2000 m) in the eastern slope of the Ecuadorian Andes, 2) one group found all across the Norther Andes, except the Colombian eastern cordillera and, 3) one group apparently restricted to the west side of the Ecuadorian Andes. Additionally, we confirmed and discovered relationships between close related species to the alnifolia complex: *P. kalbreyeri* and *P. rotundifolia* seem to be the same species as well as *P. micrantha* and *P. bucaramangensis.* Finally, we found that the morphological characters traditionally used to identify the alnifolia complex and close relatives do not have any phylogenetic signal. We propose a new delimitation for *P. alnifolia, P. chelidonea, P. tribolophylla* and *P. andreana* based on geography and genetic analysis; further sampling inside this group is necessary to identify whether some species are of hybrid origin or if this was an artifact of the sampling size.

Keywords: North Andes, species complex, 2b-RAD, Decaloba

1. Introduction

In most biodiversity hotspots, highly diverse plant clades that arose from rapid evolutionary radiations are common. Although a range of processes such as climatological fluctuations and environmental heterogeneity may have given rise to these rapid radiations, the end result is that many species have evolved in a short amount of time. In many of these groups, since the putative species evolved recently, they may not yet have accumulated strong morphological differences to differentiate them, resulting in taxonomically problematic groups (e.g. *Psychotria*, *Burmeistera,* Cucurbitaceae) (Jeffrey, 1980; Taylor, 1994; Lagomarsino et al. 2015). In these groups, detailed genetic, phylogenetic, and morphological studies may be necessary to clarify species boundaries,

which is important for conservation and for all disciplines that use species names as basic units for their studies, including biodiversity-related research.

Due in part to high environmental and climatological heterogeneity associated with mountain uplift, the Andean region of South America has been the site of many rapid evolutionary radiations, resulting in many highly diverse and taxonomically challenging plant groups (e.g. Contreras-Ortiz et al., 2018; Vargas et al. 2017). One example of a highly diverse, plant group (Escobar, 1986; Morales et al., 2016) that has its center of diversity in the Andes is the genus *Passiflora.* With more than 600 species, it is among the 20 most species-rich genera of vascular plants in the New World (Frodin, 2004; MacDougal & Feuillet, 2015-2019), and contains many taxonomically challenging groups and putative species complexes, particularly in the subgenera *Tacsonia* and *Decaloba*.

One group that exemplifies the taxonomic complexity of Andean species of *Passiflora* is the "alnifolia group," a group of five closely related, morphologically similar species that have overlapping geographical distributions in the North Andes. The alnifolia group includes *P. alnifolia* (Killip, 1938; Holm-Nielsen et al., 1988) together with four close relatives, *P. chelidonea, P. andreana*, *P. tribolophylla* and *P. mollis*. The species in the alnifolia group have been traditionally differentiated based on leaf shape, and, number of lobes; flower size, color and anatomy; and flower corona filament shape and length (Killip, 1938; Holm-Nielsen et al., 1988). *P. alnifolia* Kunth (Fig. 1a) (Humboldt et al., 1817) was originally collected in Colombia by Quindío mountains (1500-3000 m) and is distinguished by its whitish flowers and "outer corona filaments abruptly and strongly dilated in upper third, then tapering to a filiform apex" (Killip,

1938) or slightly smaller flower elements (androgynophore 0.7-1.2 cm long; outer corona elements 0.4-0.8 cm). It differs from *P. andreana* Mast. (1883) in that the latter has maroon flowers with an androgynophore 1.6-1.7 cm long and an outer corona elements of 1.1 cm lenght (Fig. 1d) (Holm-Nielsen et al., 1988). *P. chelidonea* Mast. (1879) (Fig. 1b) is distinguished by its "corona filaments about 1 cm long" (Killip, 1938) or "slightly dilated in upper ½, not falcate" (Holm-Nielsen et al., 1988). This species is very similar to *P. tribolophylla* Harms (1922) and the only difference reported by Killip (1983) was that the corona filaments are 0.8 cm long in *P. tribolophylla*. *P. mollis* Kunth (1817) is the most clearly differentiated species in this group, distinguished by its dense and soft indument all across the plants (Killip, 1938), compared to the rest of species that have scarce indument. Overall, the species in the alnifolia group are morphologically very similar and have been differentiated based on very few minute morphological characteristics. Furthermore, many specimens show intermediate morphologies that complicate their identification (Table 1). It is therefore unclear whether the species as currently delimited reflect discrete groups, or instead whether taxonomic changes may be necessary to adequately encompass the natural patterns of variation within the group.

The phylogeny of the alnifolia group was recently investigated as part of phylogenetic and biogeographic study of the larger group section *Decaloba*, a highly diverse group (~120 species). This study showed that section *Decaloba* likely originated in Central America and then dispersed to and subsequently diversified in the Andean region (Acha et al., in prep.). Within the Andean clade of section *Decaloba*, the alnifolia group showed mixed support for its monophyly and was nested within a clade containing an additional of 10 species (clade SA6 in Acha et al. Fig.2B, in prep), most of which

share many morphological features and occupy similar habitats in the northern Andes with the alnifolia group. The previous study, which included 2-4 accessions of each species in the alnifolia group, also showed that most species in the alnifolia group were not monophyletic; however, only a small subset of the geographic and morphological variation within the group was sampled. Additional sampling is necessary to understand how patterns of genetic variation correspond to patterns of geographic and morphological variation within the group and to assess the validity of species.

In this study, we employed a population genomics and phylogenomic approach (e.g. Alcaide et al., 2014; Herrera & Shank, 2016) to help solve the taxonomic conundrum of the *Passiflora alnifolia* group. Population genomics techniques have previously been shown to help untangle species complexes (e.g. Bell and Gonzalez, 2018; Contreras-Ortiz et al., 2018; Dupuis et al., 2017; Wagner et al., 2013), especially when the morphology and geographic distribution of species overlap and when intraspecific gene flow may be ongoing. Data resulting from high-throughput DNA sequencing can also be analyzed to build a phylogenetic framework, thereby providing an evolutionary context. Here, we built on the previous phylogenetic framework developed by Acha et al, including accessions of all 15 species found in the SA6 clade, but sampling more intensively within the alnifolia group to encompass the range of morphological and geographical variation in each species. DNA samples were derived from herbarium specimens, which we identified based on the current taxonomy, and we generated DNA sequence data using 2bRAD-seq approach, a high-throughput DNA sequencing approach that provides a genome-wide view of genetic variation. Our goals were to answer the following research questions: 1) What are the phylogenetic relationships and the patterns

of genetic structure among species in the alnifolia clade? and 2) Do evolutionary relationships and patterns of genetic structure correspond to the described species in the alnifolia group? Our results have important implications for the taxonomy of this diverse and highly variable group.

2. Methods

2.1 Sample selection, DNA extraction and 2b-RAD seq library preparation

Our analysis is based on the phylogenetic framework developed by Acha et al. (in prep.), which showed that 15 species were placed in the larger alnifolia clade. Although the two topologies resulting from RAxML (clade SA6, Figure 2) and BEAST (node 168, Figure 3) in Acha et al (in prep.) differed slightly, mainly in whether *P. rotundifolia* and *P.pilosissima* were placed in the alnifolia clade, in the present study we included all accessions placed in the "alnifolia" clade in either of these topologies, totaling 30 accessions. We then added samples from the most problematic species (*P.alnifolia*, *P.chelidonea, P.andreana* and *P. tribolophylla),* by sampling leaf tissue from herbarium specimens. Most of the worldwide collections of *Passiflora* section *Decaloba* are held in the Missouri Botanical Garden herbarium (MO), which has been a center for taxonomic research in section *Decaloba*. To identify appropriate specimens for inclusion in the study, we initially used the current determinations for all the herbarium collections in MO as a reference and studied the herbarium specimens to correct any evident misidentifications. Specimens were classified to species according to the taxonomic descriptions (Table 1). We sampled leaf tissue from 80 herbarium specimens that were not included in the Acha et al. (in prep.) study and we used the protocols described in Acha et al. (in prep.) for the DNA extraction and 2b-RAD library preparation (Wang et

al. 2012). Briefly, we extracted the DNA using the CTAB DNA extraction protocol for plants (Doyle and Doyle, 1987). Then, we quantified the DNA concentrations in each sample using a Qubit™ fluorometer (ThermoFisher) and we cleaned the samples using a GENECLEAN® turbo kit (MP Biomedicals). We followed the 2b-RAD seq protocol using the restriction enzyme BcgI (New England Biolabs) as described by Aglyamova and Matz (2014; available at: https://github.com/z0on/2bRAD_denovo), with some modifications. We sequenced the final library for 1x50 cycles on an Illumina HiSeq 4000 sequencer at Duke University (2018).

2.2 Analysis of DNA sequence data

2.2.1 Sequencing quality control, assembly of loci, and SNP calling

For data analysis, we pooled the 30 sequences previously generated in the alnifolia clade by Acha et al. (in prep.) and analyzed them concurrently with new sequences generated in this study. We used the same tools and parameters as described in Acha et al. (in prep.) for demultiplexing (2bRAD_denovo script by M. Matz) and quality control (FastQC and FastX toolkit) of our 2b-RAD seq data. We used iPyrad v0.7.28 (Eaton and Overcast, 2016) to assemble loci and conduct SNP calling. Because the nature of the different analysis methods used, we generated two main assemblies: 1) The "complete" dataset was generated only for phylogenomic analyses and contained all samples, including two outgroups. Loci were retained with a maximum of 20 SNPs per locus and a threshold of 90% missing information per sample. The resulting 36-bp loci, including invariant sites, were concatenated for phylogenomic analysis. 2) The "reduced" dataset excluded the outgroups and the samples that had more than 50% missing information in the assembly. Additionally, we retained loci with a maximum of 15

maximum SNPs allowed per locus, allowing the recovery of more loci in potential messy data (Eaton and Overcast, 2016). For the "reduced" assembly, we then generated two data sets, one for phylogenomic analysis that consisted of the full 36-bp loci that were concatenated (which included invariant sites), and another for coalescent and population genomic analysis that retained only the SNPs. All other parameters remained the same as described previously in Acha et al. in prep. (Supplementary table S1).

2.2.2 Phylogenetic analyses

We conducted phylogenetic analysis of the "complete" and "reduced" concatenated datasets using RAxML 8.2.10 (Stamatakis, 2014) using the GTRCAT model with 1000 rapid bootstraps (FBP) and transfer bootstrap expectation (TBE) calculations (Lemoine et al. 2018). We considered high support values to be those >85% for TBE and >75% for FBP. As an alternative to the RAxML analysis. we also used tetrad, a species tree inference method. Tetrad is based on the SVD quartets method (Chifman & Kubatko 2014, 2015) and is implemented through the iPyrad analysis toolkit. We ran tetrad with 1000 bootstraps and sampling all the quartets. We compared tetrad to the "reduced" RAxML tree using the online tool https://phylo.io/ (Robinson et al., 2016), obtaining three distances values: Robinson-Foulds (Robinson and Foulds 1981), subtree pruning and regrafting (SPR, Penny and Hendy 1985) and the Euclidean distance.

2.2.3 Population structure

We conducted an analysis of population structure using the Bayesian program STRUCTURE as implemented by iPyrad in Jupyter Notebooks (Pritchard et al., 2000). We used the "reduced" dataset that contained only SNPs for this analysis. We ran one million generations with 10% burnin for K-values from 1-16, with 20 replicates at each

value of K (Jakobsson and Rosenber, 2007). To select the optimal value of K, we used Structure Harvester to plot the –ln likelihood values at each K and calculate ΔK (Evanno, et al., 2005), excluding replicates with high variance that did not converge. In addition to generating the STRUCTURE bar plots, we mapped the best K-value replicate results in a map using QGIS to show any geographic genetic structure pattern (QGIS Development Team, 2019).

We also analyzed patterns of genetic structure in the "reduced" dataset using Principal Component Analysis (PCA) to complement our STRUCTURE analysis. We implemented PCA through iPyrad in Jupyter notebooks in a series of runs with different parameters. First, we did a series of preliminary runs to detect any outliers that could be skewing the results and removed them one by one, rerunning the PCA after each sample removal. Once we removed all the outliers, we proceeded to plot using the results in R, color coding the samples based on genetic cluster (R Core Team, 2019).

2.2.4 Gene flow

We analyzed the data to understand gene flow across the phylogenetic tree using Treemix (Pickrell & Pritchard, 2012). We applied this analysis to our dataset with the expectation that it would detect events of hybridization between species or populations (Pickrell et al., 2012). First, we used the best K-value from our STRUCTURE results to define population hypotheses and possible migration events. Then, we used iPyrad to test for 0-13 events of migration with 500 bootstraps, size correction and optimization of the migration edges. We also analyzed our dataset without population assignation with 0-10 events of migration. We chose the best migration event number based on the migration

weight and overall standard error and plotted the trees and migration events as well as the residual matrix heatmaps using R.

2.3 Morphological analysis

Finally, to understand how morphological features correspond to the patterns revealed in the phylogenomic and population genomic analysis, we measured morphological characteristics that have been reported as diagnostic for the different species (Killip, 1938; Holm-Nielsen et al. 1988) from all specimens successfully sequenced in the study. We measured: 1) leaf length/width ratio, considering leaf length as midvein length based on Leaf Architecture Working Group manual (1999). We grouped this trait in three different categories: 1-1.49 (leaves with similar leaf length and width), 1.5-2 (leaves longer than wide) and more than 2 (leaves extremely longer than wide). 2) Lobe size pattern, taking into account the relative length of the lateral and central lobes and scoring as: the same length (including truncate leaf apex), central lobe absent, central lobe shorter than the lateral lobes, central lobe longer than the laterals or lateral lobes absent. 3) Lateral lobe depth, assigning a value depending if the lateral lobe extension length was greater or less than ¼ of midvein length. 4) Gland position in relation to the overall position of glands across the leaf. 5) Gland position in relation to the main secondary veins. 6) Indument density. 7) Peduncule plus pedicel length. 8) Flower diameter and 9) Fruit length. Characters 4 and 5 were not reported in the past literature, but were included in this study based on previous herbarium observations.

3. Results

3.1 Locus assembly and SNP calling

Of the original 80 samples that met our minimum DNA concentration for library preparation, only 28 (appendix) produced positive results in the amplification test. The "complete" dataset therefore included 58 samples, with 28 new accessions and 30 accessions previously generated as described in Acha et al. (in prep.). This assembly included two outgroups, *P*.aff. *dawei* and *P. magdalenae,* and 56 ingroups representing 15 species (*P. alnifolia, P. andreana, P. bogotensis, P. chelidonea, P. tribolophylla* (and one *P*. aff. *tribolophylla), P. rotundifolia, P. kalbreyeri, P. pilossisima, P. hyacinthiflora, P. mollis, P. hyacinthiflora, P*. cf. *cuneata, P. micrantha, P. bucaramangensis, P. trinervia*) (appendix). The raw number of 36 bp loci that passed the initial sequencing quality control procedures was 230,148 and after applying the iPyrad assembly parameters, we obtained 30,883 36-bp loci, for a final concatenated length of 1,111,788 bp. The final dataset included a large percentage of missing data, with most of the samples having 80-90 % of missing information.

For the "reduced" dataset we excluded three samples with high percentage of missing dara: P.alnifolia823, P.kalbreyeri283 and P.tribolophylla708, resulting in a total of 54 samples. One outgroup (sample P.aff.dawei884) was excluded from this dataset for the analyses of population structure (STRUCTURE and PCA). The raw number of 36 bp loci from the "reduced" dataset that passed the initial sequencing quality control procedures was 229,040 and the final number after applying the iPyrad assembly parameters was 1,888. When concatenated, this data set was 67,968bp in length and the final SNP data set included 5,366 SNPs. This dataset included a low percentage of missing data, with most of the samples having between 8-22 % missing data.

3.2 Phylogenetic analyses

We conducted phylogenetic analysis using RAxML based on our two datasets, with the “reduced” data set having fewer loci and less missing data than the “complete” data set. The topologies resulting from these two data sets were largely similar, but the “reduced” dataset (Figure 2) showed overall greater support in most nodes and particularly along the backbone of the phylogeny than the “complete” dataset (supplementary material 1).

The phylogenetic reconstruction based on the “reduced” dataset in RAxML (Figure 2) placed six clades as successive sisters to the alnifolia group: 1) a weakly supported clade of composed of P.insolita415a placed as sister to *P. pilosissima* + P.aff.tribolophylla199, 2) a strongly supported clade (100% TBE) composed of two accessions of *P.* cf. *cuneata* placed as sister to a clade composed of the samples P.micrantha683 and P.bucaramangensis641, 3) a strongly supported clade (100% TBE) composed of two accessions of *P. rotundiflora* nested within two accessions of *P. kalbreyeri*, 4) one accession of *P. hyacinthiflora*, 5) a weakly supported clade (69%TBE) composed of two strongly supported (100%TBE) subclades, one containing samples P.mollis455 plus P.cuspidifolia122, and the other containing three accessions of *P. bogotensis,* and 6) one accession of *P. trinervia*. The main node of the clade containing most accessions of the alnifolia group was strongly supported (100%TBE). Within the “core alnifolia” clade, a weakly supported clade comprised of two accessions of *P. alniflolia* and one accession each of *P. mollis*, *P. chelidonia,* and *P. andreana* was placed as sister to the remainder of the group. Next, samples P.alnifolia197, P.tribolophyllia709, and P.tribolophylla866 were placed as strongly supported successive sister to two larger clades, one containing 9 accessions of *P. alnifolia* and five accessions of *P. chelidonea*,

and the other containing three accessions of *P. chelidonea*, one accession of *P. andreana*, and nine accessions of *P. alnifolia*. Although some of the subclades within the core alnifolia group were strongly supported, none of the five species were recovered as monophyletic. Indeed, of all the species with multiple accessions, we recovered only two monophyletic species: *P. bogotensis* (100% support) and *P*.cf. *cuneata* (100%). Although relationships were largely similar in the "complete" data set (supplementary material 1), the topology differed in placing *P. kalbreyeri* and *P. rotundifolia* as nested within the core alnifolia group, and nodes along the backbone, particularly those leading to the clade in which *P. kalbreyeri* and *P. rotundifolia* were placed, overall showed much poorer support.

The tetrad tree showed a wide range of support values across the phylogeny (12-100%), but generally showed lower support for some root nodes (53% bootstrap support) and most of the backbone nodes (Figure 3) than the "reduced" RAxML tree. The main clades that received strong support in the "reduced" RAxML tree were also recovered in the tetrad tree. Branches that received weak support in the "reduced" RAxML generally collapsed or showed differing relationships in the tetrad tree. *P. bogotensis* and *P*. cf. *cuneata* were the only species shown to be monophyletic. The comparison between the "reduced" RAxML tree and tetrad showed a moderate difference between the two trees (Supplementary material 2, Robinson-Foulds distance: 44/0.43, Euclidean distance: 119.892 and SPR: 15)

3.3 Population structure

Before assessing the optimal value of K for the STRUCTURE analysis, we removed 1-6 (19 in the case of K=1) replicate runs at each value of K that had high

variance and did not converge, totaling 46 of the total 320 replicates. Inspection of the –ln likelihood values and the Evanno approach showed that the optimal value of K was K=13 (Supplementary material 3, Figure 2 and 3). Most individuals showed admixture proportions that were assigned predominantly to a single cluster, but some showed admixture across several groups, and several species shared highly similar, yet complex admixture proportions (Figure 2 and 3). In most cases, accessions placed into the same structure cluster or the groups of accessions sharing similar admixture patterns are consistent with the strongly supported main clades identified in the phylogenetic analyses. These groups included: 1) *P.* aff. *lancearia* from Guatemala, which was grouped into its own unique cluster (cluster 11 in Fig. 4; yellow), 2) *P. pilossisima* and *P.aff.tribolophylla* shared highly similar admixture proportions and were predominantly assigned to their own unique cluster (cluster 1; sea foam green), 3) two accessions of *P.* cf. *cuneata* were assigned to their own unique cluster (cluster 7; pink), 4) the two accessions of *P. rotundifolia* and two of *P. kalbreyeri* from the Lesser Antilles and Venezuela were grouped into one cluster (cluster 2; medium green), 5) the three accessions of *P. bogotensis* were predominantly assigned to one cluster (cluster 9; lime green), and 6) P.mollis455 and *P. cuspidifolia* were predominantly assigned to a single cluster (cluster 6; dark blue), with *P. cuspidifolia* also showing some membership in the *P. bogotensis* cluster 1. *P. micrantha* and *P. bucaramagensis* were placed predominantly in a single cluster (cluster 5; periwinkle), while *P. hyacinthiflora* formed a unique, highly admixed accession that showed the strongest membership in cluster 5 (periwinkle). Similarly, *P. trinervia* showed strong admixture, with roughly equal assignment to three separate clusters.

Within main core alnifolia clade as identified in the phylogenetic analysis, we found several unique groups: 1) Samples P.mollis788 and P.alniflolia860 showed highly similar patterns and were predominantly grouped into one cluster (cluster 13; beige), but also showed admixture in many other clusters, 2*)* Samples P.andreana102, P.chelidonea811 and P.alnifolia424 were predominantly assigned to a single cluster (cluster 12; mustard yellow), 3) one of the two larger clades of *P. alnifolia* and *P. chelidonea* identified in the phylogeny was assigned to a cluster (cluster 3; salmon), and 4) another main clade composed primarily of *P. alnifolia* accessions was grouped into one cluster (cluster 8; magenta). The remaining accessions in the core alnifolia clade (e.g., samples P.chelidonea197, P.chelidonea 856, P.chelidonea801, P.chelidonea765, P.tribolophylla709, P.tribolophylla866 and P.alnifolia796) were shown to be highly admixed, predominantly among clusters 3, 8, 12, and 13 (i.e., the magenta, salmon, mustard yellow and beige clusters). Overall, we found very little geographical patterns to the genetic structuring in the alnifolia group except in two cases: cluster 12 (mustard yellow) appears to be exclusively from the east slope of the north Ecuadorian Andes and cluster 3 (salmon) are found on the west slope of the same region (Figure 4).

For the PCA analysis, we progressively removed the most divergent 8 samples (samples P.insolita415a, P.aff.tribolophylla199, P.pilosissima044, P.mollis455, P.cf.cuneata074, P.cfcuneata901, P.bucaramangensis641 and P.micrantha683) until we recovered an ordination graph with less highly divergent outliers (supplementary material 4a). The proportion of variance represented by the first four (PC1-PC4) principal components was 8.3%, 6.8%, 6.6% and 6%, respectively. Our PCA results showed the data distributed across three vertices, one composed by all *P. bogotensis* samples (cluster

9), the second by all *P. kalbreyeri* and *P. rotundifolia* samples (cluster 10) and the last one by most of the *P. alnifolia, P. chelidonea* samples (cluster 3). Additionally, the samples that matched to the cluster 12 are separated from the rest of samples of similar species (supplementary material 4a).

We ran a separate PCA analysis including the samples from the core alnifolia clade (supplementary material 4b). We progressively removed 6 samples that appeared to be skewing the results: P.alnifolia424, P.andreana102, P.chelidonea811, P.alnifolia860, P.mollis788 and P.chelidonea197. The proportion of variance represented by the first four (PC1-PC4) principal components was 13.4%, 10%, 9% and 8.6%. We ended with 29 samples that corresponded to a mix of *P. alnifolia, P. chelidonea* and *P. andreana* and that were clearly grouped following the genetic clusters of STRUCTURE and tetrad. The individuals with high admixture or "hybrids" were placed in the middle of the PC1 axis and in some cases (P.chelidonea801, P.chelidonea 856) showed a clear separation in PC2.

3.4 gene flow

Our series of treemix analyses showed that 11 events of migration had the lowest standard error (+/-0.9) when we assigned population identity to the samples based on our STRUCTURE clusters (Supplementary material 5a-c). Furthermore, we found support (migration weight higher than 0.25) for five migration events: *P. trinervia*→*P.* cf. c*uneata*, P.mollis455→*P. trinervia*, (P.cuspidifolia122, P.mollis455) → (*P. hyacinthiflora, P. bogotensis*), (*P. hyacinthiflora*, *P. bogotensis*) → *P. rotundifolia* and *P. alnifolia*→ (*P. pilossisima*, *P*. aff. *tribolophylla*). When we ran Treemix without any population assignation, we recovered support for 10 migration events (standard error +/-3.1, Supplementary material 5d), five having a migration weight higher than 0.25:

P.alnifolia113→ P.andreana049, P.alnifolia864→ P.alnifolia841, P.alnifolia796→ P.alnifolia864, P.aff.dawei884→ P.insolita and P.aff.dawei884→ P.kalbreyeri553 (Supplementary material 5e).

3.5 morphological characters

We obtained morphological measurements from 35 of the samples identified as one of the main five species in the "alnifolia clade" (Figure 3). 29 of these samples had some reproductive structure that could be measured. We did not recover any strong morphological pattern across any of the clades and genetic clusters, as most of the subclades showed strong variation of morphology (characters 1,2,6 and 7). Reproductive characters (7,8 and 9) contained data gaps in each of the subclades and also lacked any clear pattern of variation. The only characters with apparent phylogenetic signal were related to gland position on the leaf (characters 4 and 5): most of cluster 3 individuals seemed to have glands all across the leaf and positioned inside and outside the main secondary veins, whereas most of cluster 8 samples had glands all across the leaf with a gap in the middle, only inside the main secondary veins.

4. Discussion

Our goals were to answer the following research questions: 1) What are the phylogenetic relationships and the patterns of genetic structure among species in the alnifolia clade? 2) Do evolutionary relationships and patterns of genetic structure correspond to the described species in the alnifolia group? Below, we explore each of these questions and discuss the possible taxonomic implications for this work.

4.1 Evolutionary relationships and patterns of genetic structure

The first goal was to reconstruct the phylogeny and analyze patterns of genetic structure in the alnifolia clade. Although the "complete" RAxML (Supplementary material 1), the "reduced" RAxML (Figure 2) and tetrad (Figure 3) phylogenies were built from different datasets, the main subgroups of species identified by these differing approaches were largely concordant, and most of them were also in accordance with STRUCTURE results. For example, all approaches recovered groups such as: 1) *P. pilosissima* together with sample P.aff.tribolophylla199, 2) P. *kalbreyeri* together with *P. rotundifolia*, 3) the three accessions of *P. bogotensis*, 4) the two accessions of *P.* cf. *cuneata*, 5) and *P. cuspidifolia* together with sample P.mollis455. However, the approaches differed in how they reconstructed the relationships among these groups. The most significant difference between the "complete" RAxML and "reduced" RAxML/tetrad tree was in the placement of the rotundifolia-kalbreyeri clade. The reduced RAxML/tetrad trees both showed this clade as an early divergent group, whereas the "complete" RAxML tree recovered this clade as one of the most early diverging. Because the placement of rotundifolia-kalbreyeri clade within the core alnifolia clade in the "complete" RAxML phylogeny dramatically lowered the support values for most of the tree, and because it differs from the relationships found in all other analyses, this likely represents an erroneous placement, possibly due to the high proportion of missing data in this dataset. However, even the reduced RAxML and tetrad trees differed in many of the relationships among clades, which may be the result of this group representing a rapid radiation, whereby the divergences among these taxa occurred so quickly that there was not sufficient time for mutations to accumulate and track them.

The conflicting placements of clades may also be in part due to intraspecific hybridization; for example, STRUCTURE results showed that several taxa, including *P. hyacinthiflora*, sample P.cuspidifolia122, and *P. trinervia,* could be of hybrid origin. However, these species are very distinguishable morphologically and it is possible that STRUCTURE analysis recovered them as hybrids because we included only one individual of each species. We were unable to attain additional samples for these species because they are extremely rare and are poorly represented in herbarium collections. Additional field sampling and DNA sequencing is needed to test whether these very interesting, rare species are of hybrid origin.

Within the core alnifolia group, we found consistent support for a few main groups. All approaches recovered a clade of P. andreana102+P. chelidonia811+ P.alnifolia424 (mustard yellow; structure cluster 12). The samples that form this cluster have the biggest flowers of all of the clade and.are exclusively found on the eastern slope of the Ecuadorean Andes and tend to grow under 2000 m of elevation; this geographic pattern supports the conclusion that may be an independent linage that needs to be treated as a different species. All approaches also recovered a group containing P.mollis788 and P.alnifolia860 (beige; cluster 13), which also could indicate that they are a distinct lineage; additional morphological analysis is needed to see if they are distinct. The distinctiveness of these two groups was also supported by PCA, as these accessions were so divergent that we removed them so that we could visualize the differences among the remainder of the core alnifolia group

The remainder of the core alnifolia group accessions were placed into a large clade in the “reduced” RAxML and tetrad trees. Within this clade, STRUCTURE largely

placed the individuals into two genetic clusters: cluster 3 (salmon) and cluster 8 (magenta). The individuals that showed low admixture in STRUCTURE correspond to two distinct clades recovered in the both "reduced" RAxML and tetrad trees, but STRUCTURE also identified many individuals that appeared to be admixed between these two groups and that were placed at the base of the two large clades in the phylogenies. For example, the two samples identified as *P. tribolophylla* appeared to be admixed and were placed at the base of subclades together with the sample P.chelidonea197. In terms of taxonomy, these results could be interpreted in two ways: either this could represent two species that show extensive interspecific hybridization, or it could represent a single species that shows some intraspecific variation. Geographically, the ranges of these two groups overlap; cluster 3 occurs on the west slope of the Andes, whereas cluster 8 occurs all across both slopes, reaching the Colombian central mountain chain (figure 3).

4.2 Correspondence between described species and the evolutionary relationships and patterns of genetic structure in the alnifolia group

Within the alnifolia clade, even when initially identifying specimens for the study, many of the combinations of characteristics that are supposed to be diagnostic for the species were highly variable and overlapped among specimens, rendering identifications very difficult. Although we used the traditional species descriptions to identify the specimens, none of the species formed a cohesive genetic group. Based on our herbarium measurements, we have found that the morphological features that were used to differentiate the species do not correspond to any of the genetic groups recovered on the study. Leaf shape is highly variable and does not show any evident pattern that matched

the current genetic groups (Figure 3). The indument, which supposedly is used to identify P. mollis, similarly occurs in two separate clades within this group. On the same note, based on the 28 fertile specimens, flower size seems to be highly variable within clades, especially in the "core alnifolia" species. *P. andreana* has been traditionally distinguished from the rest of the "alnifolia" clade species by its big and maroon flowers. In this study we did not find any support for the monophyly or genetic structure of this species as is currently delimited, but we found support for a clade and genetic cluster (cluster 12) with the biggest flowers of all the clade that needs further study. Our results indicate that the traditional species, many of which were likely described based on only a few specimens, likely represent a small subset of the morphological variation present in what should be recognized in one or two highly variable species. Based on this, we propose that apart from the two groups that may represent new species, *P. alnifolia, P. chelidonea, P. andreana,* and *P.tribolophylla* should be treated as a single species.

5. Conclusions

We built a well-sampled phylogenetic reconstruction for the "alnifolia" group in *Passiflora* section *Decaloba.* This analysis was accompanied by analyses of genetic structure and gene flow analyses, which have for the first time clarified the relationships within and among the species in the group. To our knowledge, this study is one of only a few that has applied a population genomic approach to study Andean organisms, especially in the northerb Andes. Although there have been few population genetic studies in the Andean region that focused on animals (Bernal et al., 2008; Masello et al., 2011), pathogens (Cárdenas et al., 2011) and plants (Trénel et al., 2008; Oleas et

al., 2012), the use of high-throughout sequencing techniques is, as yet, infrequent in Andean organisms population genetics studies.

The phylogenomic approach in this study revealed that the full group of 15 species likely represents a rapid radiation. Based on the combination of phylogenetic and population genomic analyses, our study also suggests that several species could potentially be the product of interspecific hybridization and merit additional sampling and analyses. The analysis also recovered the monophyly of the core alnifolia clade, but, none of the accepted species as currently described were recovered in any of our phylogenetic or population genomic analyses. Within the core alnifolia clade, we potentially identified two genetic groups that could potentially represent new species, but our results suggest that all of the currently recognized species (*P. alnifolia, P. chelidonea, P. andreana*, and *P.tribolophylla)* should be subsumed into a single variable species that needs re-circumscription. Additionally, *P. kalbreyeri* and *P. rotundifolia* form a strong clade and a genetic cluster, therefore we recommend to further studies to determine whether to treat these two species as one.

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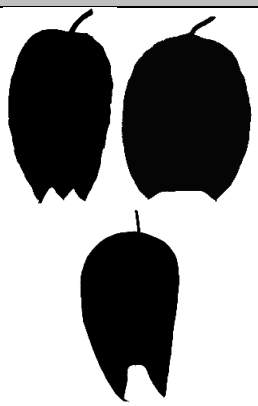
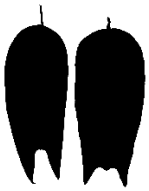
Tables

Table 1. Morphological characters of the main five species of the alnifolia clade. all the information is based on the original descriptions and taxonomic treatments that included these species. Leaf shape and corona filament shape figures were taken from real herbarium collections and life pictures. NA values indicate there are not published sources available for that trait.

Character	*P. alnifolia*	*P. andreana*	*P. chelidonea*	*P. tribolophylla*	*P. mollis*
Leaf shape					
Leaves length (cm)	2.6-10.6	2.8-4.6	5.4-14	3-12	5-10.56
Leaves width (cm)	1.4-8.3	2-3.8	2.7-4.7(8)	1-4	3-7.5
Lobes	2-3	2-3	2-3	2	2-3
Indument	Glabrescent, finely puberulent above	Glabrous above, pubescent bellow	NA	Puberulent bellow	Pubescent or glabrescent above, tomentose bellow
Glands	yes	NA	yes	NA	6-7, between secondary veins, near base
Flower peduncle length (cm)	2-4.6	2.1-3.2	2-3	NA	0.5-1
Flower pedicel length (cm)	NA			1.5-2.5	NA
Flower diameter (cm)	(3.2-)3.8-5.8	5-5.2	2.5-5	2.4	1.1-1.8, 3-3.5
Corona first row filaments shape					
Fruit size (cm)	1.3-2	1-1.2	1.5-2.5	NA	1

Appendix. List of samples included in this study.

Sample name	Collector	Collection Number	Institution	Country
P.alnifolia063	Jorgensen	2484	MO	Ecuador
P.alnifolia112	Ulloa	2182	MO	Ecuador
P.alnifolia113	Jorgensen	2438	MO	Ecuador
P.alnifolia157	Jorgensen	2439	MO	Ecuador
P.alnifolia348	MacDougal	1930	MO	Ecuador
P.alnifolia36B	Ulloa	2210	MO	Ecuador
P.alnifolia41	Croat	96520	MO	Ecuador
P.alnifolia424	Jorgensen	2475	MO	Ecuador
P.alnifolia64	Croat	93100	MO	Ecuador
P.alnifolia775	Smith	1954	MO	Ecuador
P.alnifolia796	Betancur	2578	MO	Colombia
P.alnifolia820	Dodson	10887	MO	Ecuador
P.alnifolia823	Penafiel	8	MO	Ecuador
P.alnifolia828	Croat	96490	MO	Ecuador
P.alnifolia832	Drew	E-265	MO	Ecuador
P.alnifolia841	Dawe	771	MO	Colombia
P.alnifolia849	Penafiel	1197	MO	Ecuador
P.alnifolia850	Jorgensen	2437	MO	Ecuador
P.alnifolia851	Harling	9880	MO	Ecuador
P.alnifolia860	Albert de Escobar	1856	MO	Colombia
P.alnifolia864	Jorgensen	56439	MO	Ecuador
P.andreana049	Jorgensen	2476	MO	Ecuador
P.andreana102	Jorgensen	2478	MO	Ecuador
P.andreana256	Jorgensen	2477	MO	Ecuador
P.bogotensis435	Krosnick	405	MO	Colombia
P.bogotensis439	Krosnick	383	MO	Colombia
P.bogotensis441	Krosnick	503	MO	Colombia
P.cf.cuneata074	Ramos	3588	MO	Colombia
P.cf.cuneata901	Daniel (Hermano)	147	MO	Colombia
P.chelidonea197	Fonnegra	5631	MO	Colombia
P.chelidonea765	Jorgensen	2485	MO	Ecuador
P.chelidonea798	Freire	2841A	MO	Ecuador
P.chelidonea801	Gentry	55126	MO	Colombia
P.chelidonea811	Knapp	6204	MO	Ecuador
P.chelidonea812	Jorgensen	61638	MO	Ecuador
P.chelidonea817	MacDougal	1940	MO	Ecuador
P.chelidonea818	MacDougal	1909	MO	Ecuador
P.chelidonea856	Benavides	9594	MO	Colombia

P.chelidonea857	Ulloa	2213	MO	Ecuador
P.cuspidifolia122	Stein	3686	MO	Colombia
P.hyacinthiflora285	Hernandez	195	MO	Colombia
P. insolita415a	MacDougal	6213	MO	Guatemala
P.kalbreyeri283	Davidse	21150	MO	Venezuela
P.kalbreyeri553	Porter-Utley	415	MO	NA
P.kalbreyeri846	Weitzman	112	MO	Venezuela
P.micrantha683	Fosberg	22018	MO	Colombia
P.mollis455	Gentry	48035	MO	Colombia
P.mollis788	Escobar	420	MO	Colombia
P.rotundifolia701	Stehle	123	MO	Leeward Islands, Guadeloupe
P.rotundifolia908	Stehle	2585	MO	Leeward Islands, Guadeloupe
P.trinervia078	Ramos	3000B	MO	Colombia
P.tribolophylla708	Luteyn	12480	MO	Colombia
P.tribolophylla866	Albert de Escobar	1022	MO	Colombia
P.tribolophylla709	Lehmann	BT859	MO	Colombia
P.bucaramangensis641	Killip	17046	MO	Colombia

Figures

Figure 1. Example of the flower morphology found in the "alnifolia" clade. A) *P. alnifolia*, B) *P. chelidonea* (Photo: A. Hernández), C) *P. trinervia* (Photo: www.passifloratuin.com), D) *P. andreana* (Photo: P.M. Jørgensen), E) *P. hyacinthiflora* (Photo: A. Hernández).

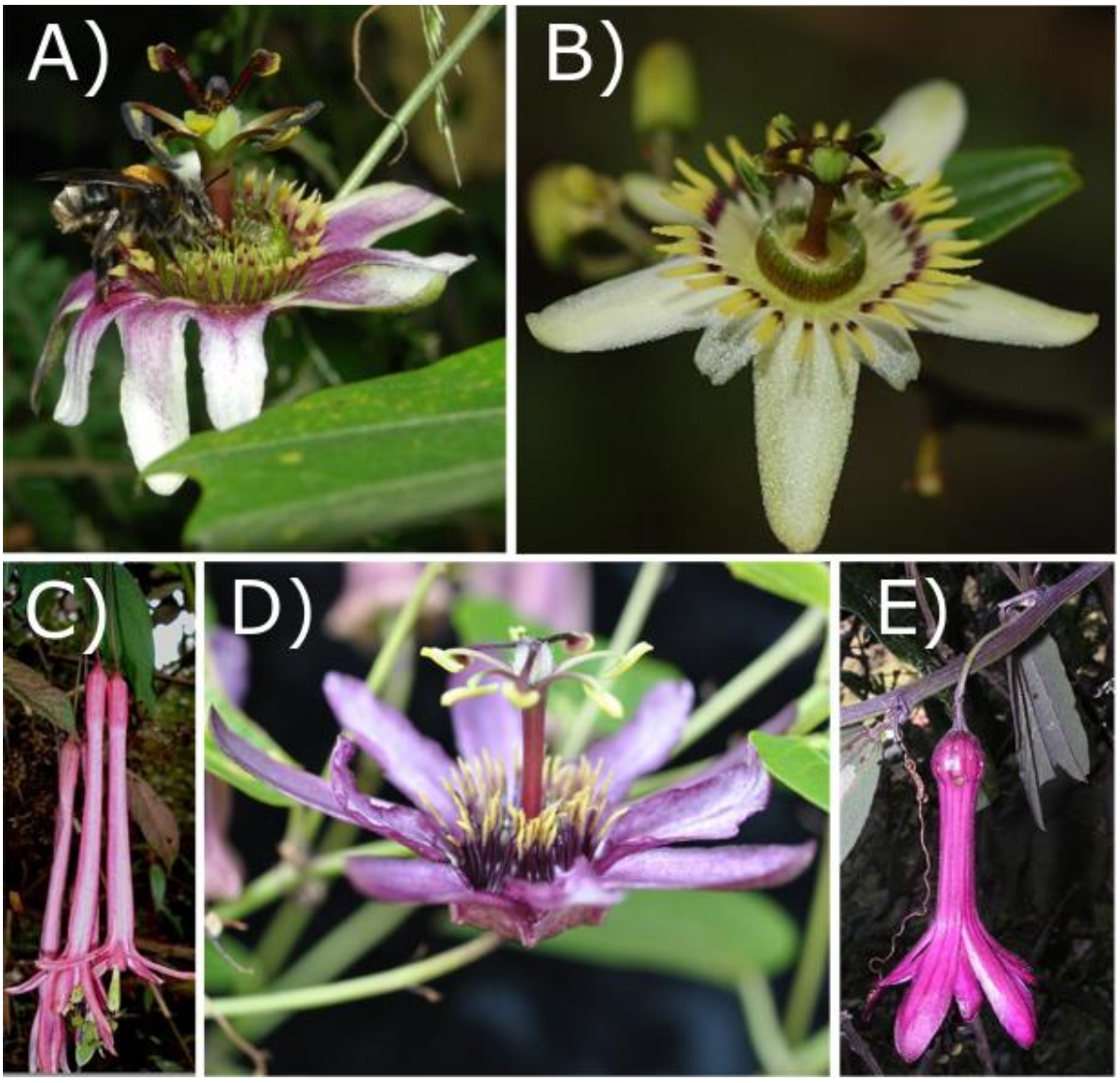


Figure 2. RAxML tree and STRUCTURE bar plot for K=13RAxML tree for the "reduced" dataset with 50% missing information threshold and TBE/FBP values. Branches with only one value have the same value for TBE and FBP.

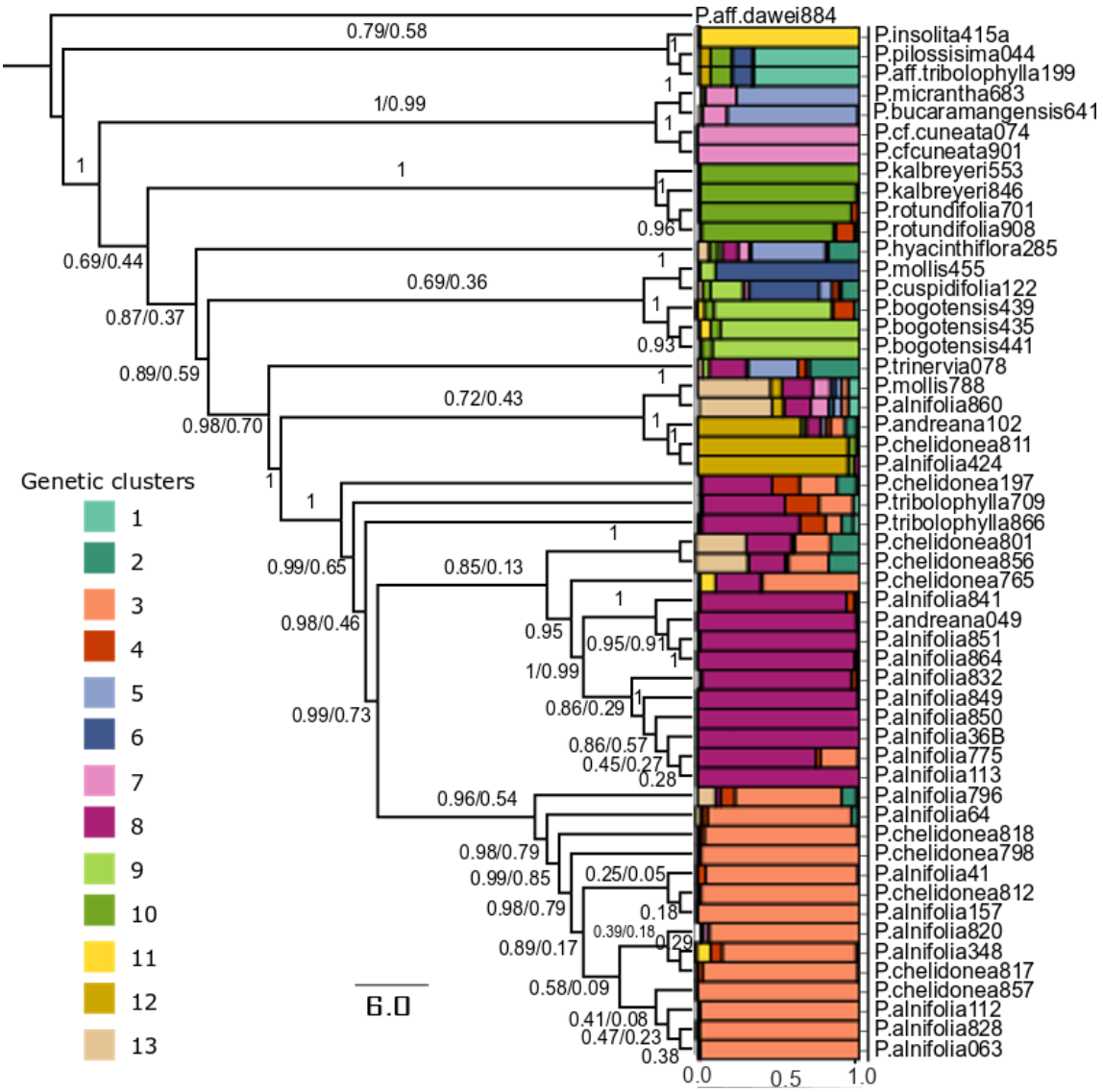


Figure 3 (next page). Tetrad tree and STRUCTURE bar plot for K=13.

P.aff.dawei884 is used as an outgroup and was included only in the tetrad analysis. Branch support values are transfer bootstraps. Nine selected morphological characters are mapped to the right, gray boxes indicate insufficient material and multicolor boxes indicate more than one state present. **1)** Leaf (midvein) length/width ratio: red (1-1.49), yellow (1.5-2), blue (more than 2). **2)** Lobe size pattern: red (lateral lobes and central lobe the same length), yellow (central lobe absent), blue (central lobe shorter than the lateral lobes), green (central lobe longer), black (lateral lobes absent).**3)** Lobes depth: red (lateral lobe extension length greater than ¼ of midvein length), yellow (lateral lobe extension length less than ¼ of midvein length) **4)** Gland position 1: yellow (base of the leave only), green (from the base to 2/3 length of the leaf), black (from the base to the middle of the leaf), red (all along the leaf), blue (on the base and tip of the leaf with a gap in the middle). **5)** Gland position 2: red (inside main secondary veins), yellow (inside and outside secondary veins), blue (different leaves with either of these characteristic). **6)** indument: red (abundant), yellow (medium), blue (scarce). **7)** Peduncule + pedicel length in cm: red (equal or less than 2 cm), yellow (from 1.2 to 3 cm), blue (from 3.1 to 4 cm), green (more than 4.1 cm) **8)** Flower diameter in cm: red (from 2 two 3.9 cm), yellow (less than 2 cm), blue (more than 4 cm). **9)** Fruit length in cm.

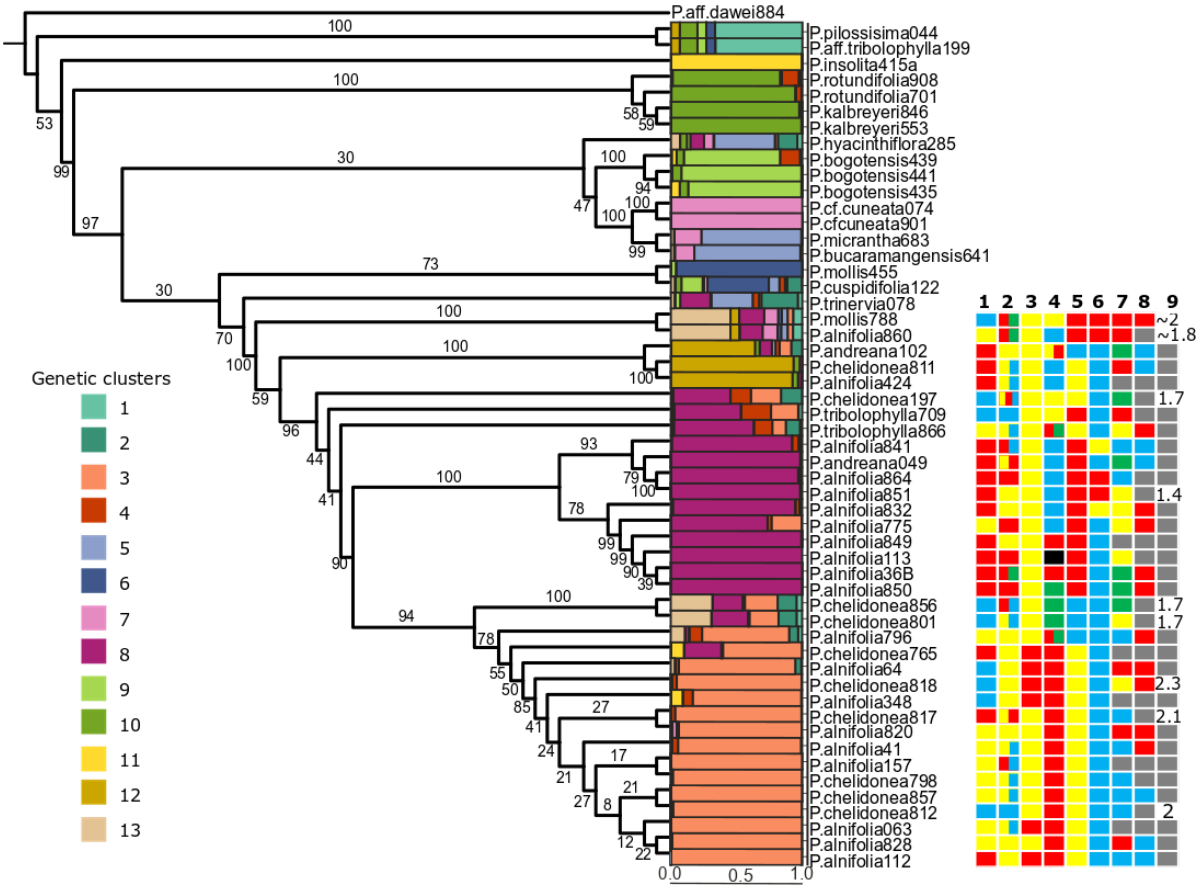
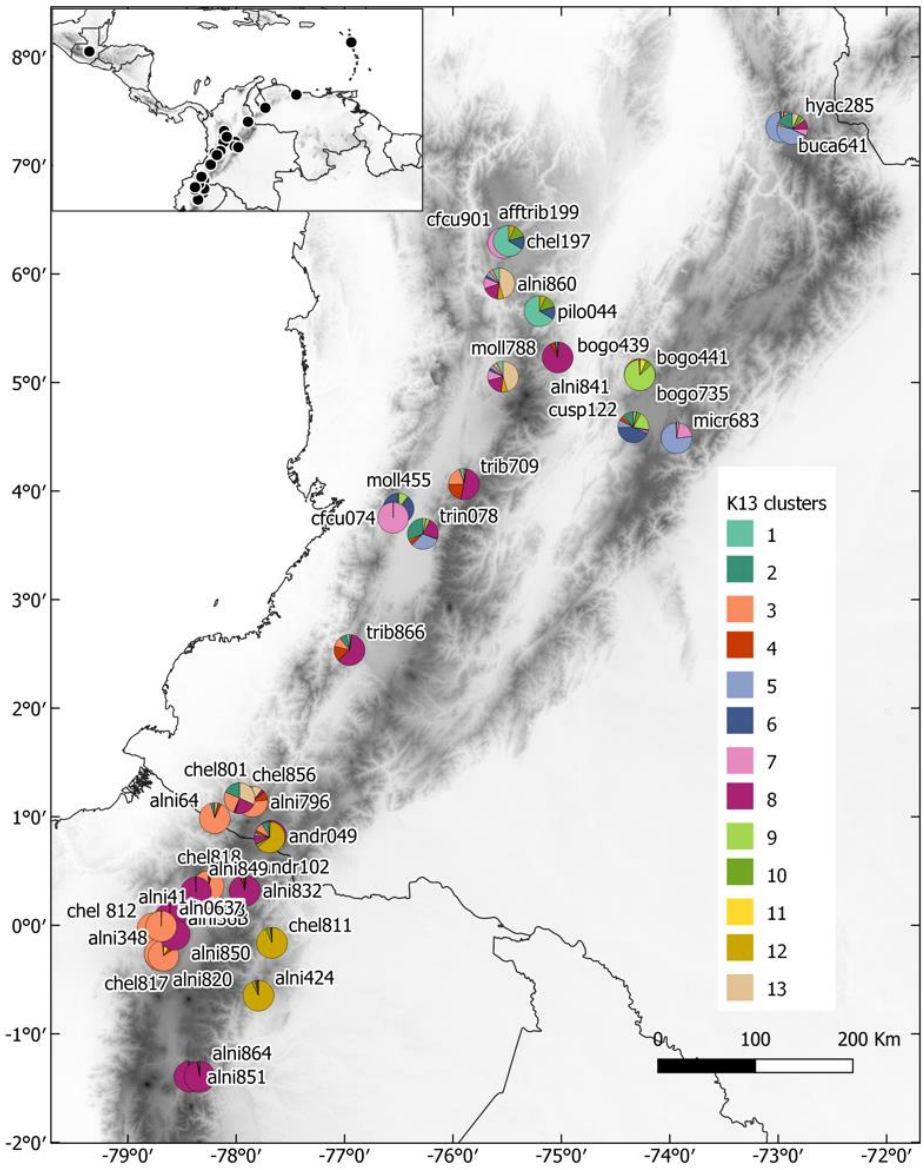
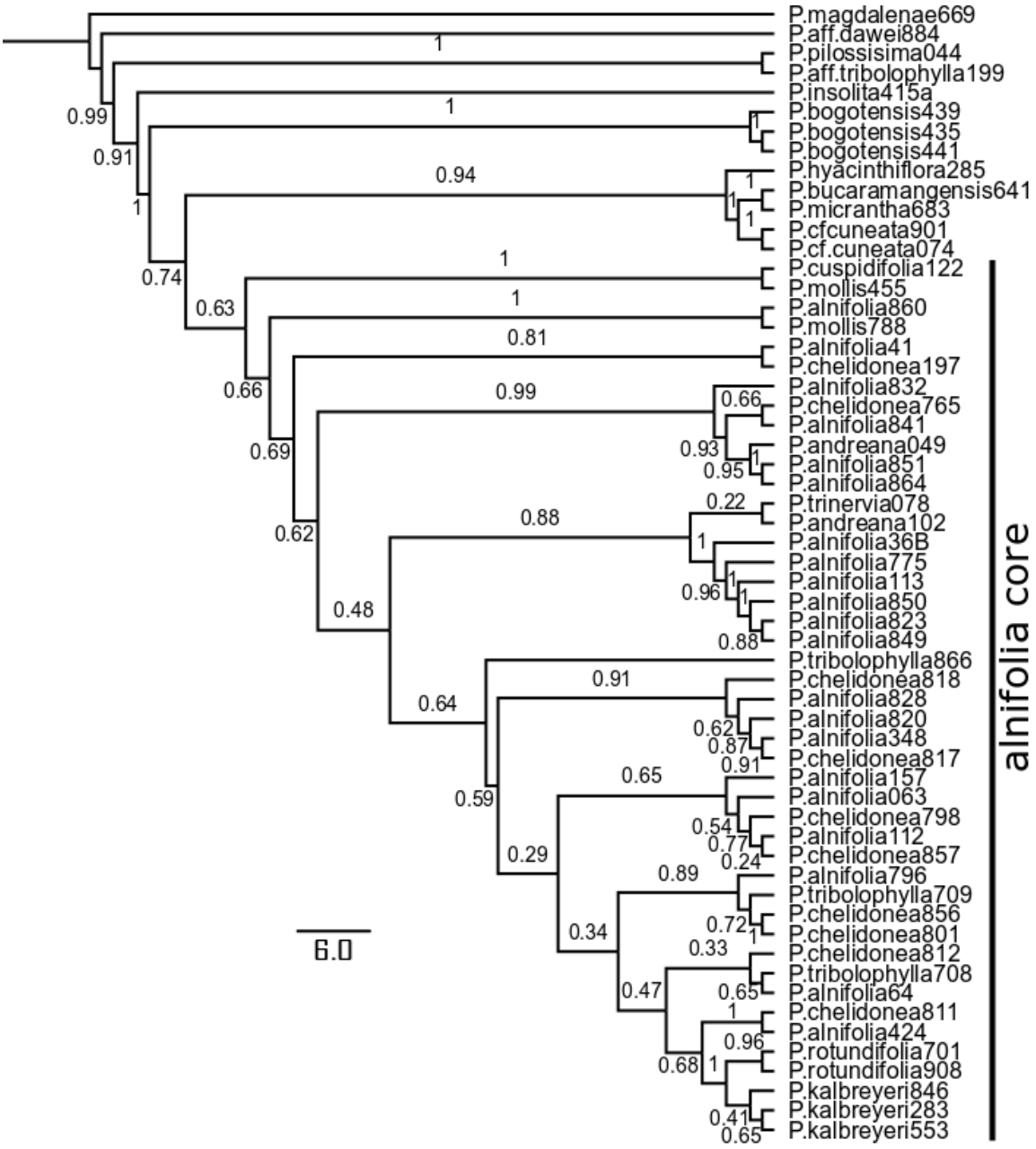

P.aff.dawei884
P.pilossisima044
P.aff.tribolophylla199
P.insolita415a
P.rotundifolia908
P.rotundifolia701
P.kalbreyeri846
P.kalbreyeri553
P.hyacinthiflora285
P.bogotensis439
P.bogotensis441
P.bogotensis435
P.cf.cuneata074
P.cfcuneata901
P.micrantha683
P.bucaramangensis641
P.mollis455
P.cuspidifolia122
P.trinervia078
P.mollis788
P.alnifolia860
P.andreana102
P.chelidonea811
P.alnifolia424
P.chelidonea197
P.tribolophylla709
P.tribolophylla866
P.alnifolia841
P.andreana049
P.alnifolia864
P.alnifolia851
P.alnifolia832
P.alnifolia775
P.alnifolia849
P.alnifolia113
P.alnifolia36B
P.alnifolia850
P.chelidonea856
P.chelidonea801
P.alnifolia796
P.chelidonea765
P.alnifolia64
P.chelidonea818
P.alnifolia348
P.chelidonea817
P.alnifolia820
P.alnifolia41
P.alnifolia157
P.chelidonea798
P.chelidonea857
P.chelidonea812
P.alnifolia063
P.alnifolia828
P.alnifolia112
0.0
0.5
1.0
Genetic clusters
1
2
3
4
5
6
7
8
9
10
11
12
13
1 2 3 4 5 6 7 8 9
~2
~1.8
1.7
1.4
1.7
1.7
2.3
2.1
2

Figure 4. STRUCTURE best likelihood K13 values mapped over an Andes digital elevation mode. The colors match to Figure 2**.** Four accessions are not represented in this map: P.insolita415a from Guatemala, P.rotundifolia701 form the lesser Antilles and P.kalbreyeri846-553 from Venezuela. Inset map: the general distribution of all samples included in this study.

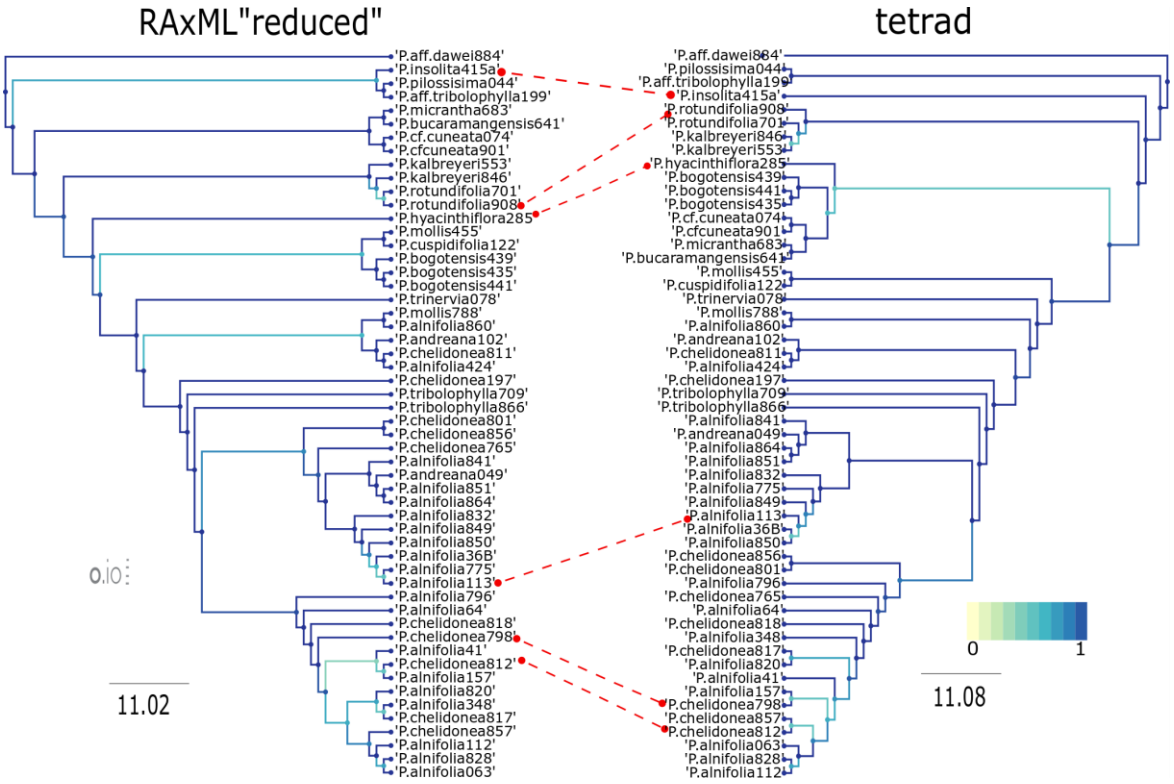


Supplementary material

Supplementary material 1. RAxML tree for the "complete" dataset with 90% missing information threshold and TBE values**.**



Supplementary material 2. Comparison of the "reduced" RAxML and tetrad trees using Phylo.io. The color of the nodes represents a variant of the Jaccard Index used to show the similarity between trees (1 or blue more similar). The red dashed lines mark examples of the most relevant differences found between both trees.

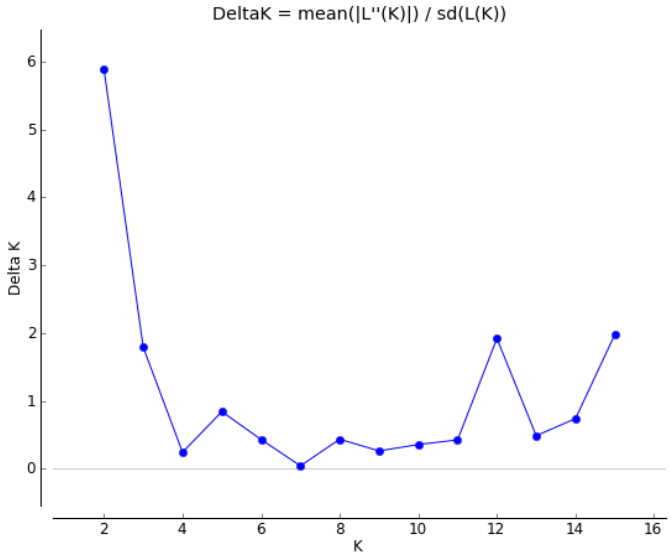


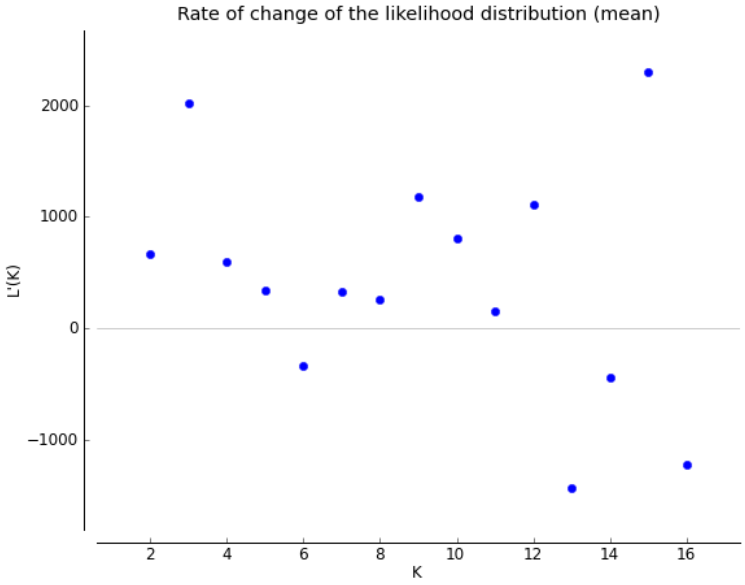
Supplementary material 3.

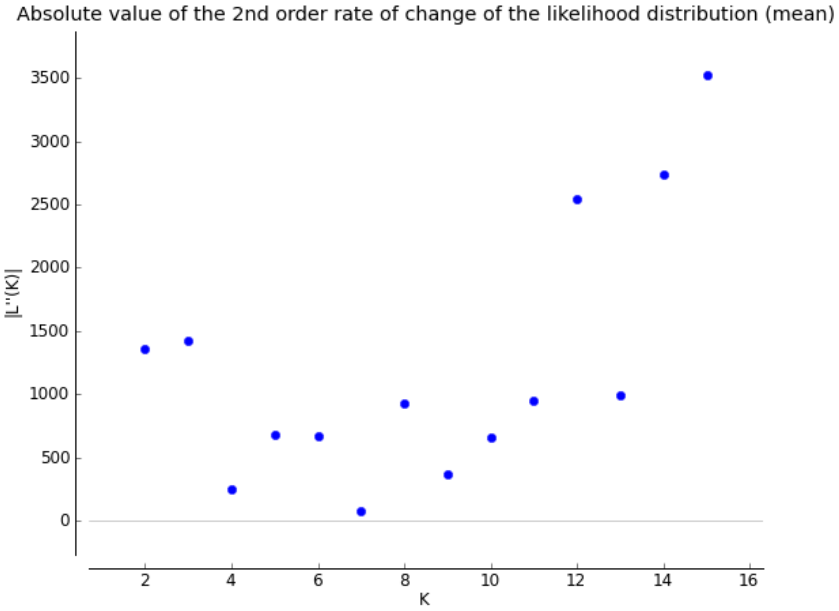
3a) STRUCTURE Evanno table results.

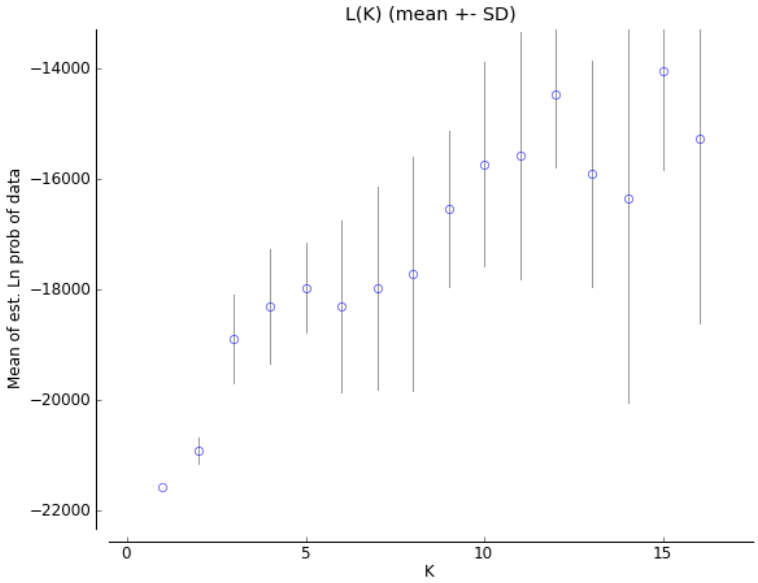
K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	1	-21576.800000	0.000000	—	—	—
2	20	-20914.600000	230.347561	662.200000	1356.540000	5.889101
3	20	-18895.860000	797.564727	2018.740000	1426.032632	1.787984
4	19	-18303.152632	1032.891832	592.707368	249.537090	0.241591
5	17	-17959.982353	807.774941	343.170279	678.723220	0.840238
6	17	-18295.535294	1553.076955	-335.552941	665.794485	0.428694
7	16	-17965.293750	1842.835556	330.241544	76.141127	0.041317
8	15	-17711.193333	2131.258987	254.100417	924.815139	0.433929
9	18	-16532.277778	1411.029248	1178.915556	368.094921	0.260870
10	14	-15721.457143	1848.659907	810.820635	656.770635	0.355269
11	14	-15567.407143	2249.636005	154.050000	953.757143	0.423961
12	15	-14459.600000	1324.555839	1107.807143	2542.399451	1.919435
13	13	-15894.192308	2049.426528	-1434.592308	993.684615	0.484860
14	10	-16335.100000	3713.239409	-440.907692	2740.077692	0.737921
15	10	-14035.930000	1783.727631	2299.170000	3524.400000	1.975862
16	10	-15261.160000	3348.616590	-1225.230000	—	—

3b) Structure harvester results

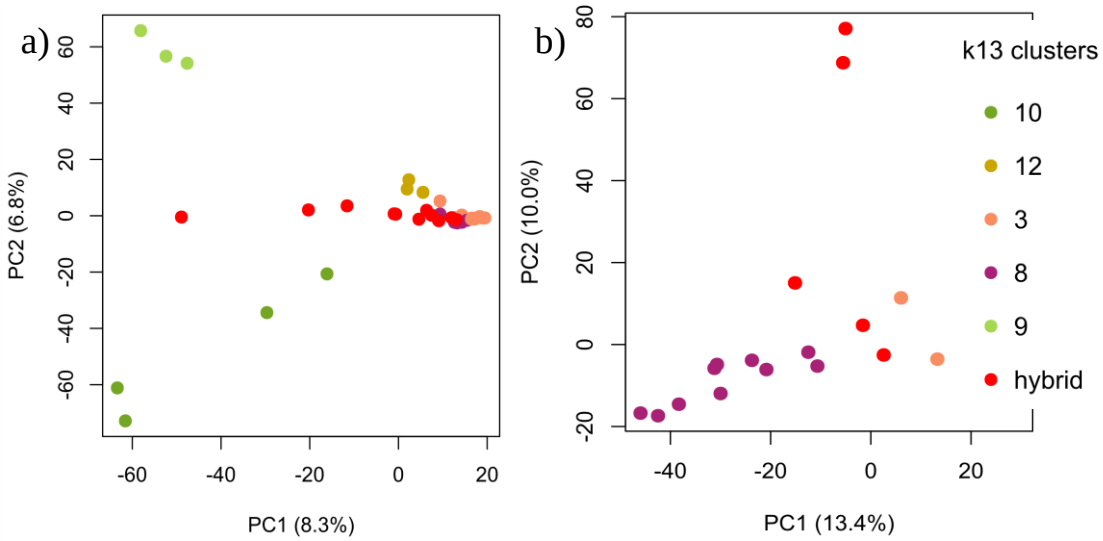





Absolute value of the 2nd order rate of change of the likelihood distribution (mean)
|L''(K)|
3500
3000
2500
2000
1500
1000
500
0
2
4
6
8
10
12
14
16
K


L(K) (mean +- SD)
Mean of est. Ln prob of data
−14000
−16000
−18000
−20000
−22000
0
5
10
15
K

Supplementary material 4. PCA results color coded to K=13. The colors match figures 1 and 2 with the exception that the individuals with high admixture are marked as red dots. a) includes all the samples from the STRUCTURE analysis minus outliers. b) Includes only samples from the "alnifolia core" clade including clusters 3 and 8.

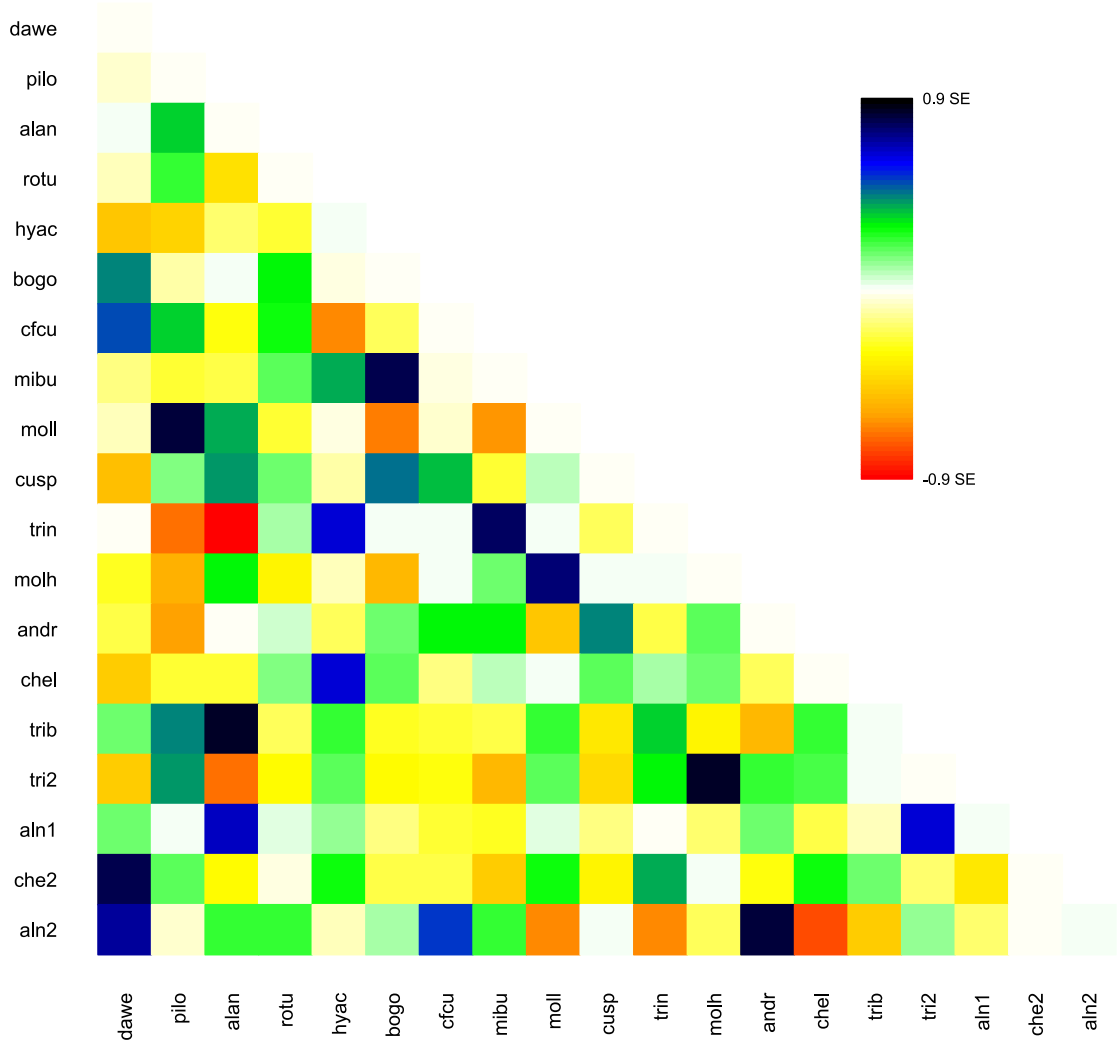


Supplementary material 5. Treemix inputs and results

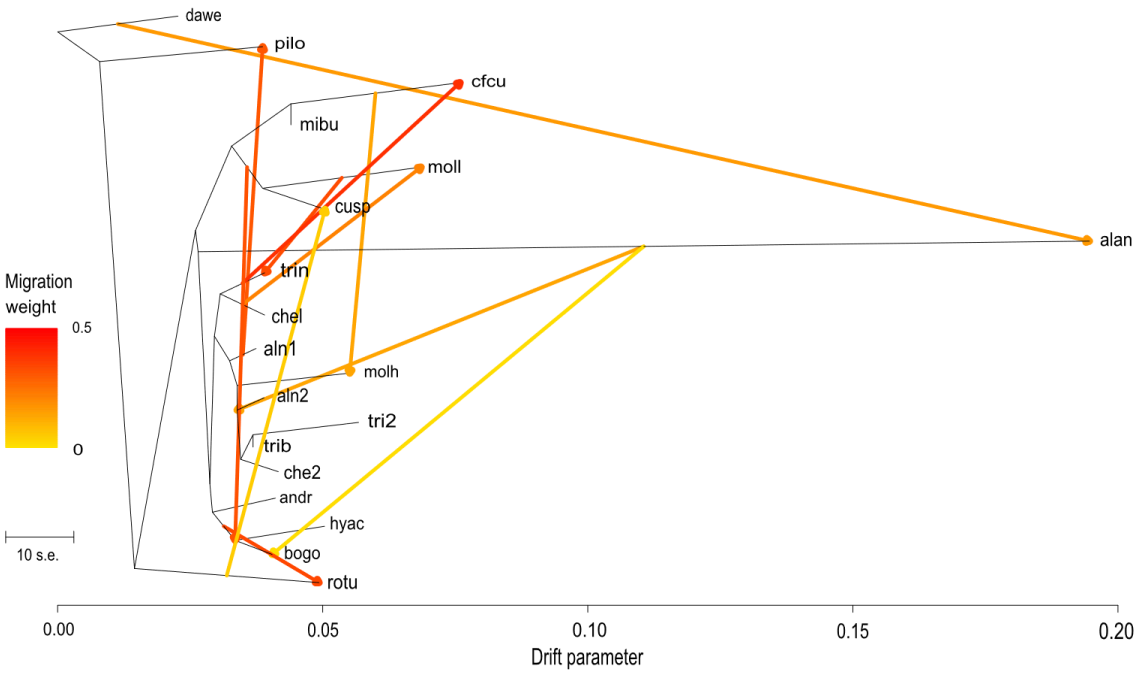
5a) Population groups based on the STRUCTURE results.

Structure group	Samples
dawe	P.dawei884
pilo	P.pilossisima044, P.aff.tribolophylla199
alan	P.insolita415a,
rotu	P.rotundifolia908, P.rotundifolia701, P.kalbreyeri846, P.kalbreyeri553
hyac	P.hyacinthiflora285
bogo	P.bogotensis439, P.bogotensis441, P.bogotensis435
cfcu	P.cf.cuneata074, P.cfcuneata901
mibu	P.micrantha683, P.bucaramangensis641
moll	P.mollis455
cusp	P.cuspidifolia122
trin	P.trinervia078
molh	P.mollis788, P.alnifolia860
andr	P.andreana102, P.chelidonea811, P.alnifolia424
chel	P.chelidonea197
trib	P.tribolophylla709
tri2	P.tribolophylla866
	P.alnifolia841, P.andreana049, P.alnifolia864, P.alnifolia851, P.alnifolia832, P.alnifolia775, P.alnifolia849,
aln1	P.alnifolia113, P.alnifolia36B, P.alnifolia850
che2	P.chelidonea856, P.chelidonea801
	P.alnifolia796, P.chelidonea765, P.alnifolia64, P.chelidonea818, P.alnifolia348, P.chelidonea817, P.alnifolia820,
	P.alnifolia41, P.alnifolia157, P.chelidonea798, P.chelidonea857, P.chelidonea812, P.alnifolia063, P.alnifolia828,
aln2	P.alnifolia112

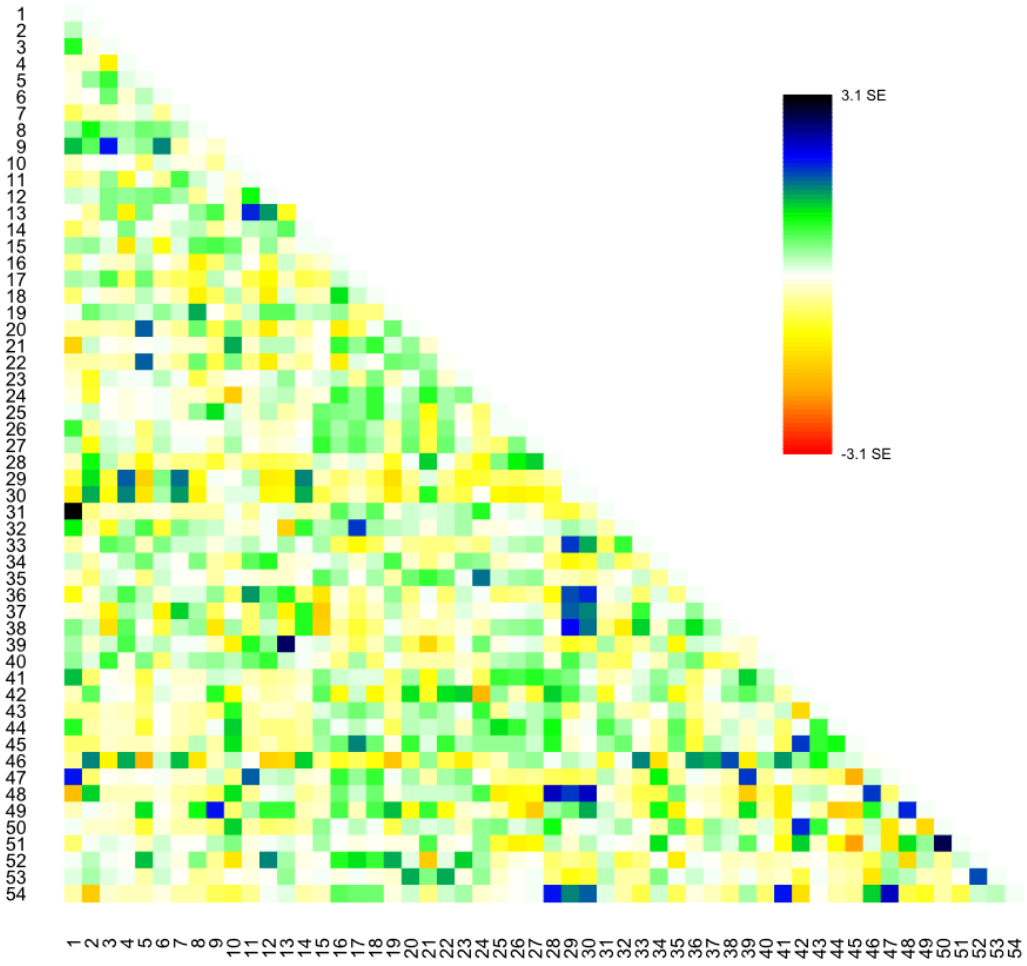
5b) Standard error heatmap for 11 events of migration with population assigned to samples based on STRUCTURE. See 5a) for the complete name list.



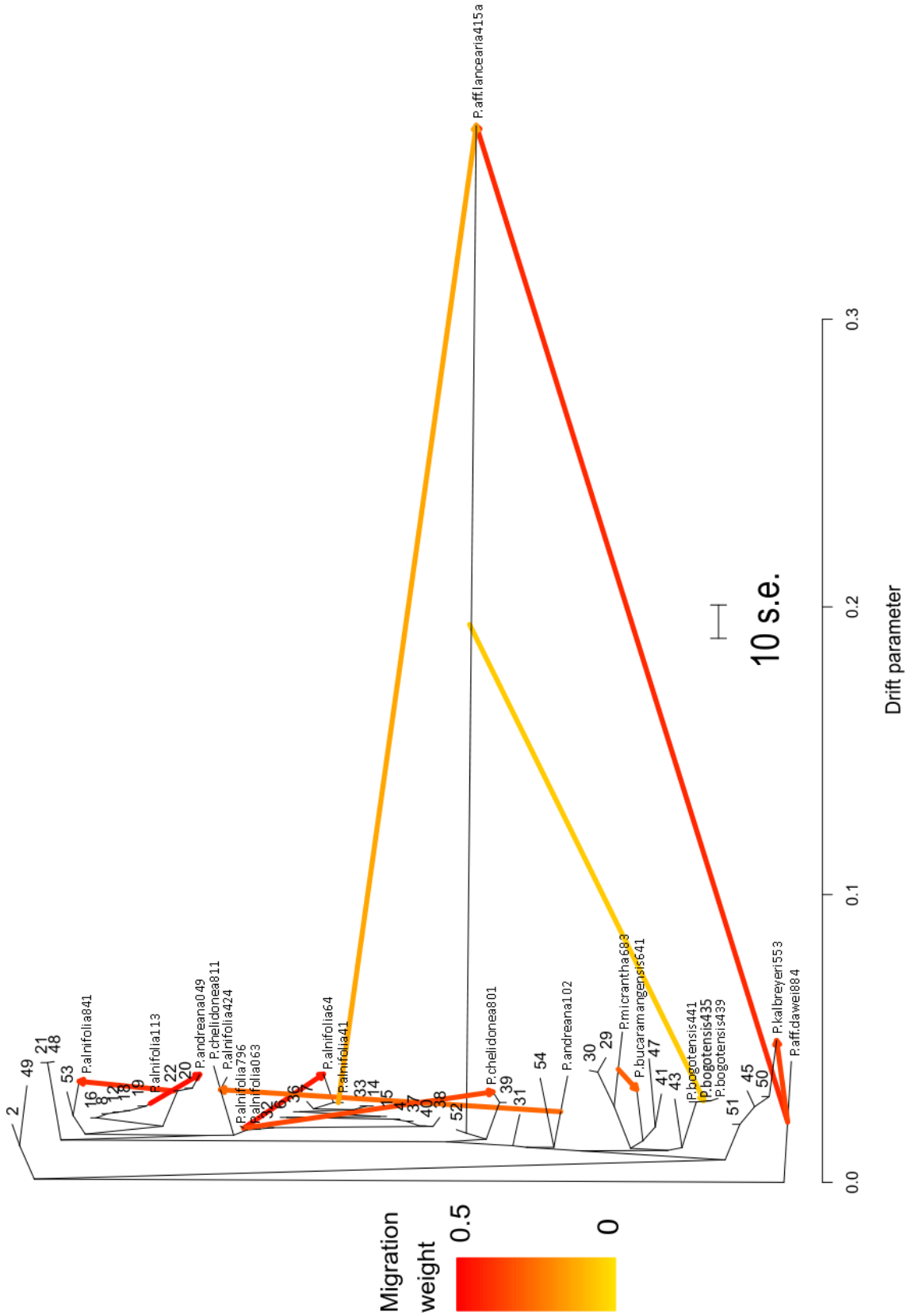
5c) Treemix tree showing 11 events of migration with population assigned to samples based on STRUCTURE. See 5a) for the complete name list.



5d) Standard error heatmap for 10 events of migration with no population assigned to samples. See 5f) for the complete name list.



5e) Treemix tree showing 10 events of migration with no population assigned to samples. See 5f) for the complete name list.


P.aff.lancearia415a
P.alnifolia841
P.alnifolia113
P.andreana049
P.chelidonea811
P.alnifolia424
P.alnifolia796
P.alnifolia063
P.alnifolia64
P.alnifolia41
P.chelidonea801
P.andreana102
P.micrantha683
P.bucaramangensis641
P.bogotensis441
P.bogotensis435
P.bogotensis439
P.kalbreyeri553
P.aff.dawei884
2
49
21
48
53
16
8
18
19
22
20
36
31
33
14
15
47
37
40
38
52
39
31
54
30
29
47
41
43
45
50
51
0.0
0.1
0.2
0.3
Drift parameter
10 s.e.
Migration weight
0.5
0

5f) List of samples names and number corresponding to figure 5d) and 5e).

Number	Sample
1	P.insolita415a
2	P.aff.tribolophylla199
3	P.alnifolia063
4	P.alnifolia112
5	P.alnifolia113
6	P.alnifolia157
7	P.alnifolia348
8	P.alnifolia36B
9	P.alnifolia41
10	P.alnifolia424
11	P.alnifolia64
12	P.alnifolia775
13	P.alnifolia796
14	P.alnifolia820
15	P.alnifolia828
16	P.alnifolia832
17	P.alnifolia841
18	P.alnifolia849
19	P.alnifolia850
20	P.alnifolia851
21	P.alnifolia860
22	P.alnifolia864
23	P.andreana049
24	P.andreana102
25	P.bogotensis435

26 P.bogotensis439

27 P.bogotensis441

28 P.bucaramangensis641

29 P.cf.cuneata074

30 P.cfcuneata901

31 P.chelidonea197

32 P.chelidonea765

33 P.chelidonea798

34 P.chelidonea801

35 P.chelidonea811

36 P.chelidonea812

37 P.chelidonea817

38 P.chelidonea818

39 P.chelidonea856

40 P.chelidonea857

41 P.cuspidifolia122

42 P.aff.dawei884

43 P.hyacinthiflora285

44 P.kalbreyeri553

45 P.kalbreyeri846

46 P.micrantha683

47 P.mollis455

48 P.mollis788

49 P.pilossisima044

50 P.rotundifolia701

51 P.rotundifolia908

52 P.tribolophylla709

53 P.tribolophylla866

54 P.trinervia078

Chapter III: Taxonomic changes and novelties in Andean Passion flowers (*Passiflora* subgenus *Decaloba*, section *Decaloba*)

Abstract

We make taxonomic changes in *Passiflora* section *Decaloba* based on the results of a previous phylogenetic reconstruction and a population genomics study, focusing on non-monophyletic species, as reported previously. Using the original species delimitations, herbarium specimens, and reported morphological variation we propose the following taxonomic changes: 1) We subsume *P. micrantha* into *P. bucaramangensis* and propose two varieties for this species: *P. bucaramangensis* var. *bucaramangensis* and *P. bucaramangensis* var. *micrantha*, 2) we synonymize *P. kalbreyeri* into *P. rotundifolia,* 3) We recognize *P. jorullensis* and *P. salvadorensis* as different species, 4) we discuss *P.biflora* Lam. and support the recognition of two species: *P. biflora* s. str, and *P. brighamii*, 4) we subsume *P. chelidonea, P. tribolophylla*, and *P. bauhiniifolia* into *P. alnifolia,* and 5) we update the description of *P. andreana*.

Keywords: Species complex, North Andes, Central America

1. Introduction

Passiflora L. displays an extraordinary amount of morphological variation. Because of this, it has been divided anywhere from 4 to 22 subgenera (Masters, 1872; Killip, 1938; Feuillet & MacDougal, 2004). The currently accepted subgenera are: *Passiflora*, *Deidamioides* (Harms) Killip, *Astrophea* (DC.) Mast, *Tryphostemmatoides* (Harms) Killip, *Tetrapathea* (DC.) P.S. Green, and *Decaloba* (DC.) Rchb. (Krosnick et al. 2013). All six subgenera have been supported as monophyletic groups based on

phylogenetic analysis using traditional Sanger DNA sequencing of a limited number of nuclear and plastid molecular markers (Krosnick et al., 2013; Buitrago et al., 2018). In addition to the subgeneric division, *Passiflora* subgenera have been subdivided into multiple ranks (supersections, sections, subsection, and series), some of which have morphological, geographical, or molecular support (Feuillet and MacDougal, 2004; Krosnick et al. 2013; Acha, in prep.). *Passiflora* subgenus *Decaloba* is currently divided into seven supersections, of which supersection *Decaloba* (DC.) J.M. MacDougal & Feuillet is estimated to be one of youngest clades (Krosnick et al. 2013; Sader et al., 2019). Supersection *Decaloba* is a large, monophyletic group that contains ca. 130 species, including several putative species complexes and many undescribed new species. Two key morphological synapomorphies of supersection *Decaloba* are the presence of cernuous (drooping) shoot tips and loss of petiolar nectaries. Aside from these characteristics, plants in this clade have a strong tendency toward bilobed or even unlobed leaves. Supersection *Decaloba* is divided in two sections: section *Xerogona* (Raf.) Killip and section *Decaloba* DC. Section *Decaloba* is differentiated from section *Xerogona* by the presence of nectaries on the leaf blade located mostly between the main secondary veins and by having seeds with rugulose ridges (Krosnick et al. 2013).

Section *Decaloba* (~120 species) contains 20% of the species in *Passiflora* and is characterized by some of the most recalcitrant species limits within the genus. The alpha taxonomy in this group has until recently lacked a comprehensive phylogenetic framework for reference. In 2013, Krosnick et al. confirmed the monophyly of section *Decaloba* and suggested potential synapormorphies for section *Decaloba* and some of its constituent subclades; these included leaf shape, leaf gland position, and certain floral

structures. The most complete recent study in this group, Acha et al (in prep.), provided a largely well-resolved phylogeny of the group, identified major clades, and tested the monophyly of many of the species in the group. Although many of the species in section *Decaloba* were shown to be monophyletic, many others were revealed to be non-monophyletic and in need of additional taxonomic research. Furthermore, an additional phylogenetic study was conducted on a taxonomically problematic group in section *Decaloba,* the alnifolia group, which revealed that several recognized species are not cohesive genetically and need recircumscription, and identified a genetically and morphologically distinct putative new species that is in need of further study.

Based on evidence from the most recent phylogenetic reconstruction for the group, we identified taxa that were potentially in need of taxonomic changes and conducted an exhaustive review of the relevant herbarium specimens, the original descriptions, and the taxonomic bibliography available for the species. In this paper, we make several taxonomic changes within section *Decaloba;* we propose several changes of rank, the synonymizing of several species, and lastly, we identify and discuss a probable new species.

2. Methodology

We examined all the herbarium specimens available at the Missouri Botanical Garden herbarium (MO). This institution has the largest current collection of Section *Decaloba* species, including loans from other herbaria. We measured the herbarium specimens according to the parameters described in Boza et al. (2018), with the exception that we measured lateral lobe width in a perpendicular angle to the secondary vein and lateral lobe length from the base of this line to the apex. Additionally, we used all the

information available in the MO Tropicos database to obtain nomenclatural information and distributional data that was later used to map the species distributions using QGIS. Other resources that we used were the JSTOR Global Plants database, where we obtained images of the type specimens, the *Passiflora* working group photo database, and the original species descriptions.

3. Results

3.1 *Passiflora bucaramangensis* and *Passiflora micrantha* change of rank

Passiflora bucaramangensis Killip, J. Wash. Acad. Sci. 20: 376. 1930. Type: Colombia: Santander, near La Baja, N of Bucaramanga, 2400 m, 8 Jan 1927. *Killip & Smith 16787* (Holotype: US; Isotypes: A, F!, GH, K!, MO!, NY!, PH, S, US)

Vines, glabrous or glabrescent, with simple trichomes. Stipules 1.5–2 X 0.25–0.5 mm, narrowly triangular, apex acuminate; petioles 0.5-2.5 cm X 0.1–0.6 mm; leaf laminas 1.2–3.4 X 2.8–7 cm, rounded or slightly cordate at the base, glabrous, 3-lobed, lateral lobes 0.5–2.8 X 0.7–1.8 cm, with apex obtuse, rounded, or mucronulate; midvein 0.9–2 cm long, central lobe 0.2–0.7 X 0.7–2.2 cm, shorter than lateral lobes, with apex obtuse, rounded, retuse, or mucronulate, or sometimes almost absent; angle between the lateral veins 76°–115°; ocellate extrafloral nectary glands 6–10, placed in some leaves only between the main secondary veins and in others also outside. Peduncles 0.9– cm X 0.3–0.5, solitary or in pairs; bracts with papillose teeth in the apex, floral stipe 1.3–2 mm. Flowers white sometimes with purple parts, 1.2–2 cm diameter ; sepals 5–8 X 1.5–3 mm, oblong-triangular, apex obtuse; petals 3-6 X 2.5-6 mm, ovate, apex rounded-obtuse to praemorse; corona filaments in 2 series; filaments of outer series 30–31, 2–3.5 mm long, white with a purple band in the middle or purple, yellow tips erect, narrowly linear-

clavate; filaments of inner series 1–2.5 mm long, filiform or clavate; operculum plicate to denticulate; androgynophore 2–4 mm; anthers 1-2.5 X 0.7–1.5 mm; ovary ~1.4 mm long, ellipsoid, densely pubescent; styles1.5–2.5 mm; Fruits 0.8–1 cm diameter, globose, dark purple when mature; seeds ~3 mm long, testa transversely grooved, with 4-5 sulci, the ridges rugulose.

Distribution (figure 2). *Passiflora bucaramangensis* is found in the northern part of the Colombian oriental cordillera, between 1600-2600 m.

Key to the varieties of *Passiflora bucaramangensis*

1a. Leaves with shorter lateral lobes (0.5–1.1 cm) and a wider angle between secondary veins (91°–115°)…… *Passiflora bucaramangensis* var. *bucaramangensis* (Fig.1C)

1b. Leaves with longer lateral lobes (1.1–2.8 cm) and a narrower angle between secondary veins (76°–91° ……*Passiflora bucaramangensis* var. *micrantha* (Fig.1D)

Passiflora bucaramangensis var. **micrantha (Killip) Achá, comb. nov.** *Passiflora micrantha* Killip, Publ. Field Mus. Nat. Hist., Bot. Ser. 19: 196. 1938. Type: Colombia: Boyacá, près de Bogotá, Chinquinquirá [Chiquinquirá, 05°37’08”N 073°49’12”W], July 1909, *Frère Apollinaire [Brother Apollinaire Marie] s.n.* (Holotype: G!, photo: NY; Isotypes: US!, BOG [transferred to COL?])

Discussion

We decided to subsume *Passiflora micrantha* Killip into *Passiflora bucaramangensis* Killip due to their extremely close genetic relationship and the limited morphological differences found between them. These taxa were sister groups in a recent

phylogeny (Acha, in prep.) and they form part of a single genetic cluster. The apparent leaf shape differences are only significant when one compares the leaf lateral lobe length and the angle between veins in specimens that exhibit the extremes in the range of variation. When all specimens were examined, we found continuous variation in the values of these traits and no apparent gap in the morphology. We suspect the leaf morphology could be a very plastic trait in section *Decaloba*, as it has been shown to demostrate a large range of variation in closely related species (the "alnifolia group" in Acha in prep.). Still, we consider these differences relevant enough to recognize two varieties for *P. bucaramangensis*.

Specimens examined

Passiflora bucaramangensis* var. *micrantha

COLOMBIA**. Boyacá:** près de Bogotá, Chiquinquirá [05°37'08"N 073°49'12"W], July 1909 *Felix [Tomeón-Felix] s.n.* (E, US, P). **Cundinamarca:** Coachí, 04°31'44"N 073°54'24"W, 1600 m, *Frère Apollinaire s.n.* (BOG, NY COL*)*; hills south of Ubaque, 04°29'N 073°56'W, 2150 m, *F. R. Fosberg 22018* (NY).

Passiflora bucaramangensis* var. *bucaramangensis

COLOMBIA. **Santander**: vicinity of California, 07°21'N 072°58'W, 2000 m, *E. P. Killip 18842* (BM, GH, NY, US); Municipio de Onzanga, vereda Chaguaco, finca Bellavista, 2000 m, *C. Orozco 1883* (COL); California, 07°21'N 072°58'W, 2200 m, *E. P. Killip 17046* (GH, NY, US); Tona, 07°08'N 072°59'W, 1800-1900 m, *R. Bernal 3545* (COL).

3.2 *Passiflora rotundifolia* and *Passiflora kalbreyeri* synonymy

Passiflora rotundifolia L. Sp. Pl. 957. 1753. *Decaloba rotundifolia* (L.) M. Roem., Fam. Nat, Syn. 2: 159. 1846. (Lectotype: [icon] the engraved illustration, fig. 2, on the right side of the frontispiece title page of Barrelier, Pl. Galliam Hisp. Ital. Obs. Icon. Æneis Exhib. 1714;

Epitype: West Indies: Martinique [without further locality], Nov 1867, L. Hahn 177 (K [herb. Hooker]; Isoepitypes: BM [barcode BM000915306], P [barcode P00562590])

Passiflora kalbreyeri Mast., Journal of Botany, British and Foreign 21: 36. 1883. Type: Nov. Granata [Colombia: Norte de Santander], Prov. Ocana [Ocaña], 6000 ft., 17 Nov 1869, *Kalbreyer 1253* (Holotype: K!; Isotype: B [destroyed], photo: F, MO).

Vines, ferruginous to yellowish tomentose; stems-angular. Stipules 2–3(8)X 1–1.5 mm, narrowly triangular (rarely lingulate), apex acuminate (or rounded); petioles 1–3 cm X 0.8–1.8 mm; leaf suborbicular, ovate or obcordate laminas 2.6–12 X 2.3–9.5 cm, rounded or slightly attenuate at the base, ferruginous tomentose, (2-)3-lobed, lateral lobes 0.2–2.8 X 1–2.3 cm, with a obtuse, rounded, or mucronulate apex; midvein 2.3–10.5 cm long, central lobe 0.2–0.9 X 0.9–4.3 cm, with a obtuse, rounded or mucronulate apex or sometimes almost absent, central lobe apex retuse or mucronulate; angle between the lateral veins 36°–49°; 4–11 glands placed usually between the main secondary veins, never reaching the leaf apex but with variable position along the leaf; peduncles 1–4 cm X 0.5 mm, solitary or in pairs; floral bracts 2–8 X 2–3 mm, acute or 3–4-toothed toward apex. floral stipe 3 mm long. Flowers white, sometimes pink or purple parts, 2.8–4 cm diameter; sepals 11–16 X 4–6 mm, oblong-triangular, apex obtuse, white or white-pinkish; petals 5–7 X 2–4mm, obelliptic, apex rounded, white or white-pinkish; corona filaments in 2 series, filaments of outer series 26–37, 4–5.5 mm, erect, linear to cultrate,

white with yellow tips and a purple band in the middle; filaments of inner series 3–6 mm, filiform; operculum 2–2.5 mm, plicate; androgynophore 4–6 X 1 mm; ovary ellipsoid, densely pubescent; styles 4.5 mm. Fruits 1.5 cm in diameter, globose; seeds broadly ovoid, testa transversely grooved, with 5-6 sulci with rugulose ridges.

Distribution (Fig. 3). This species is distributed from the Lesser Antilles to the Venezuelan Coastal range and Cordillera of Mérida, reaching the northern region of the Colombian Oriental cordillera.

Discussion

Passiflora rotundifolia was known as a species confined to the Lesser Antilles (Killip 1938), whereas *P. kalbreyeri* was thought to be exclusively from the northern part of Venezuela and adjacent Colombia. In our latest study (Acha in prep., Figs. 2 and 3) we discovered that these species formed a well-supported clade and that depending of the analysis, either *P. rotundifolia* or *P. kalbreyeri* were non-monophyletic, as they contained within their clades the samples of the other species. In addition to the results of phylogenetic reconstruction, population genomics analyses also revealed that *P. kalbreyeri* and *P. rotundifolia* form a single genetic cluster. These patterns are very similar to those found in other species with similar distribution, such as *Passiflora rubra* (Boza et al., 2018) and *Bernardia corensis* (Cervantes et al., 2016).

Based on the specimens examined, we found subtle differences between the mainland and insular samples, nevertheless we do not consider these to be enough to recognize them as two different species. The mainland samples tend to have bigger stipules (2–2.5 (8) mm) than the insular samples (1.4–2 mm). The insular specimens tend to have narrower lateral leaf lobes (1–1.2 cm vs. 1.6–2.3 cm) and longer central lobes (7–

9 mm vs. 2–5 mm) than the mainland specimens. Finally, the insular samples tend to have bigger flowers (3–3.8 cm diameter) than the mainland samples (2.5-3 cm diameter).

Specimens examined

COLOMBIA. **Norte de Santander:** between Pamplona y La Isla, 07°23'N 072°38'W, *E. P. Killip 19801* (A, GH, MO, NY, US); 2420 *J. Escobar1094.* LEEWARD ISLANDS. **Guadaloupe:** 16°15'N 061°35'W, 1000 m, *H. Stehle 123* (NY); 16°01'N 061°41'W, 620 m, *H. Stehle 2585* (P, US); Bains Jaunes, 16°02'00"N 061°40'23"W, 920 m, *H. Stehle 697* (US); 16°01'N 061°41'W, 600 m, *A. Questel 2269* (P, US). St. Claude, 16°02'07"N 061°40'02"W, *R. Howard 19521* (A, NY, US); Bains Chauds du Matouba, 16°02'N 061°41'W, 1100 m, *H. Stehle 1513* (GH, MICH, P, UC, US).VENEZUELA. **Aragua:** Parque Nacional Henri Pittier, 10°22'22"N 067°35'31"W, 1140 m, A. *L. Weitzman 112* (NY, US). **Distrito Federal:** Parque Nacional El Avila, 0°35'N 066°55'W, 1650 - 1800 m, S. *Knapp 6869* (BH, K, US). **Falcón**: Cerro Cerón, 10°25'N 070°40'W, 1800-2000 m, R. *Liesner 8173* (HUA, MO, NY). **Miranda**: 10°30'N 066°45'W, 1200-1300 m, *G. N. Morillo 1735* (NY). **Lara:** Fila Potreritos, Parque Nacional Yacambu, 09°41'N 069°35'W - 09°42'N 069°37'W, 1800 - 2100 m, *G. Davidse 21150* (F, HUA, M, MO, NY). **Trujillo:** Distr. Boconó, 09°17'N 070°18'W, 2000-2400 m, *L. Dorr 7552* (MO).

3.3 *Passiflora jorullensis* var. jorullensis and *Passiflora jorullensis* var. *salvadorensis* are separate species

In 2004, MacDougal lumped *Passiflora jorullensis* K. and *Passiflora salvadorensis* J. D. Smith into a single species: *Passiflora jorullensis* K. and proposed

that these former species be treated as varieties: *Passiflora jorullensis* var. *salvadorensis* (J. D. Smith) J.M. MacDougal and *Passiflora jorullensis* K. var. *jorullensis*. In the most recent phylogenetic reconstruction of section *Decaloba*, the single sample of *Passiflora jorullensis* was not placed as the closest relative to *Passiflora salvadorensis*, although both species were placed in the same larger Central American clade (Clade CA1 Fig. 2 in Acha et al. in prep.). Instead, *Passiflora salvadorensis* appears as the sister to *P. aff. mexicana* in the phylogeny. Based on these phylogenetic results, we propose recognizing the two varieties as separate species, as they were originally treated by Killip (1938).

3.4. Notes about taxonomy of *Passiflora biflora* Lam.

Acha et al.'s analysis (in prep.) clearly separated specimens identified as "P. biflora" into two groups in clade CA2, but not particularly close to each other in the phylogeny. This phylogenetic finding and previous morphological observations support recognizing more than one species in what has been called *P. biflora* Lam. (Fig. 2 in Acha et al., in prep. and supplementary Fig. S1). One lineage (including accessions P.biflora052 from Guatemala, *P. biflora* from Mexico, and P.biflora613 from Costa Rica) was included in a strongly supported clade that also included *P. nubicola* MacDougal and *P. yucatanensis* Killip and represents a generally inland or montane variant that we propose to be called *P. biflora* s. str. The flowers in this species have a nearly white androgynophore and numerous laminar glands that rarely are enlarged near the base of the main secondary veins. Additionally, as noted by Vanderplank and Edwards (2014), the mature arils are translucent yellow to orange in the limited number of specimens that document aril color; based on outgroup comparison, this appears to be an autoapomorphy of the species.

Geographic range is from NE and central Mexico to NE Ecuador and Venezuela, from sea level to 1500 m.

The other lineage (P.biflora244 Guatemala, P.biflora423 Honduras) represents a lowland and coastal variant that we propose to be identified as *P. brighamii* S. Watson. The flowers have a reddish purple androgynophore and there are two enlarged laminar glands at the base of the veins, as well as a reduced number of laminar glands overall. The fruit contains whitish arils when documented (Vanderplank and Edwards, 2014, identified as *P. transversa* Mast.). It was placed in a strongly supported clade that includes most of the West Indian species of *Decaloba*, including the red-flowered ones (Acha et al. (in prep.)). One of these samples, P.biflora423 (MacDougal 3458GR), was collected in the field relatively close to the type locality of *P. brighamii* and is well characterized because it was thereafter grown in the greenhouses at MO for many years, affording detailed documentation of the vegetative and floral morphology. The range of *P. brighamii* is from the Pacific coast of Mexico and Guatemala to the Atlantic coast of Yucatan, Belize, Guatemala, Honduras, (probably Nicaragua and Costa Rica), and Panama, generally in lowlands near the coast, sometimes in mangrove forest.

3.5. New circumscription of *Passiflora alnifolia*

Passiflora alnifolia Kunth Nova Genera et Species Plantarum (quarto ed.) 2: 136. 1817. Type: Colombia: Tolima, Rio Cuello, Quindio trail, 04°26'38"N 075°25'47"W, 1500-3000 m. Woody perennial. Floret August, F.W.H.A. von Humboldt s.n. (Type: P; Holotype: P!; Isotype: B (destroyed), G, MO! (photograph), P)

Passiflora chelidonea Mast. The Gardeners' Chronicle & Agricultural Gazette 12: 40. 1879. Type: no date; Colombia, Ecuador. Lehmann 3731 (Type: B!; Holotype: Ecuador: Pichincha, Sodiro (cult. by Anderson-Henry) s.n. K)

Passiflora tribolophylla Harms Repertorium Specierum Novarum Regni Vegetabilis 18: 297. 1922. Type: Colombia: Cauca, Popayán. In dichten sehr feuchten Wäldern and den mittleren Westhängen der West-Anden von Popayan, 02°26'39"N 076°37'17"W, 1300-1700 m, Jun. F.C. Lehmann 5420 (Holotype: B (photography); Isotype: F!, K, MO!, US!)

Passiflora bauhiniifolia Kunth Nova Genera et Species Plantarum (quarto ed.) 2: 132. 1817. Type: Ecuador: Pichincha, Crescit in temperatis Regni Quitensis et ad ripas fluminis Amazonum prope Tomependa, floret Novembri, 00°13'S 078°30'W, 2000-3100m. Humboldt & Bonpland s.n. (Type; P; Holotype: P!; Isotype: B (destroyed); photo: AAU(P))

Vines, tomentose to glabrerrimous; stems-angular. Stipules 2.3–9 X 0.4–1 mm, triangular, lingulate, falcate or subfalcate, apex acuminate; petioles 0.8–1.7 cm X 0.6–1.3 mm; leaf oblong-ovate, ovate or ovate-elliptic, laminas 5.2–13 X 2.4–7 cm, rounded or slightly cordate at the base, puberulent to tomentose below, glabrous above sometimes with puberulous nerves, 2- or 3-lobed or sometimes with no clear lobes but a truncate leaf apex, lateral lobes (0.2)0.4–2.5(3.1) X (0.3)0.5–1.3(2.1) cm, with a acute, rounded, or mucronulate apex; midvein 3.8–11.8 cm long, central lobe (0.1)0.2–0.4 (0.75) X (0.3)0.6–1.4 cm, with a obtuse, rounded or mucronulate apex or sometimes almost or totally absent, angle between the lateral veins 10°–23(30)°; (4)6–22(32) glands placed mostly between the main secondary veins, sometimes with few glands outside the main secondary veins, with a highly variable position placement; peduncles 2.5–5 cm X 0.5–1

mm, usually in pairs; floral bracts 2.7–6 X ~0.3 mm, linear or setaceous. floral stipe 1.5–4 mm long. Flowers white, sometimes with pink or purple parts, 3–4.6 cm diameter; sepals 15–22 X 4.5–6.5 mm, oblong-triangular, apex obtuse, white or white-pinkish; petals 8–12 X 3–4 mm, obelliptic, oblong, or rhomboidal, apex rounded or obtuse, white or white-pinkish; corona filaments in 2 series, filaments of outer series 32–49, 5–8.5 X 0.3–1 mm, linear to cultrate, usually white with yellow tips and a purple band(s) in the middle or base; filaments of inner series 3.5–6 mm, filiform; operculum 2–4 mm long, plicate; limen 7–14 mm diameter, androgynophore 8–11 X 1.1–1.4 mm; staminal filaments 4–7 mm long, anthers 3–4.8 X 1.3–2.4 mm, ovary globose to ellipsoid, densely pubescent; styles 4.5-7 mm long, stigmas 1.5-1.8 mm width. Fruits 1.3–1.4 cm in diameter, globose; seeds 3.5–4.8 mm long, 1.5 mm tick broadly ovoid, testa transversely grooved, with 7–8 sulci and rugulose ridges.

Distribution (Fig. 4). This species is distributed from the Central and Western Andes of Colombia's to montane forests in the Ecuadorean Andes.

Discussion

We propose a wider geographical and morphological range for *Passiflora alnifolia* based on our observations and those of other collaborators. The morphological characteristics used to distinguish *P. alnifolia* from *P. chelidonea* and *P. tribolophylla* are not consistent and do not show any phylogenetic signal (Acha et al., in prep.). Furthermore, population genomics methods discovered that the genetic structure in this group does not correspond to geography or support the recognition of more than one species. Instead, the latest studies in this group showed signs of genetic admixture

between populations that matched with the individuals identified previously as *P. tribolophylla.*

Specimens examined

ECUADOR. **Carchi**: Along unfinished road from El Chical to El Carmen 00°59'01"N 078°11'37"W, 1350 m, *Thomas B. Croat & G. Ferry 93100* (MO, QCNE). **Imbabura:** NW of Otavalo: Cuicocha-Apuela road. Along roadside 30.0-30.5 km from Cuicocha toward to Alapuela, 00°21'34"N 078°14'54"W, 7690 - 7720 ft, J. M. MacDougal & J. Couch 1909 (DUKE, HUA, MICH, MO, QCA). **Pichincha:** Road Tandayapa-Bella Vista Lodge, 00°00'28"S 078°40'36"W, 1930 m, *P.M. Jørgensen & C. Ulloa 2484* (MO, QCNE), *2485* (MO, QCNE);Along road from Tandayapa to Mindo, vicinity Bellavista, 0.5 km S of Bellavista Cloud Forest Reserve, 00°01'02"S 078°40'52"W, 2180 m, *Thomas B. Croat et al.96490* (MO, QCNE); Bellavista lodge, cerca de la estación científica, 00°00'47"S 078°41'12"W, 2350 m, *C. Ulloa & J. M. Carrión 2182* (MO, QCA); Km 36 on Quito-Santo Domingo road via Chiriboga, 00°16'48"S 078°41'54"W, 2400 m, *C. H. Dodson et al. 10887* (MO, QCNE); Along winding road from Quito to Chirboga in wet montane forest in the watershed of the Rio Saloya. 10.8 km above Chiriboga, 00°16'S 078°42'W, 7500 ft, *J. M. MacDougal & J. Couch 1940* (DUKE, QCA); Along winding road from Quito to Chiriboga in wet montane forest in the watershed of the Rio Saloya. 11.4 km above Chiriboga, 7700 ft, 00°17'S 078°40'W, *J. M. MacDougal & J. Couch 1930* (DUKE, MO, QCA); Road Quito-Nono, 1 km before reaching Nono, 00°04'32"S 078°34'30"W, 2888 m, *P.M. Jørgensen et al. 2438* (AAU, K, MO, QCA, QCNE, US); Quito, Road from Quito to Tandapaya, 00°01'11"S 078°40'56"W, 2340 m, *J. F. Smith 1954* (QCA, WIS). COLOMBIA. *F. C. Lehman BT859* (NY). **Antioquia**: Medellin,

Municipios Medellin y Guarne:Parque Ecológico Piedras Blancas, sector Lajas, 06°18'N 075°29'W, 2350 m, *R. Fonnegra et al. 5631* (COL, HUA, MO, QCNE, UPCB). **Cauca**: El tambo, Cerro Munchique, Oeste de Popayán, 02°32'N 076°57'W, 8000 ft, *L. K. Albert de Escobar & D.L. Escobar Uribe 1022* (TEX). **Nariño:** Pasto, Reserva Natural La Planada, 01°08'07"N 077°50'53"W, 1650-1800 m, *J. Betancur et al. 2578* (COL, HUA, MO, NY, TEX); La Planada, S of Ricaurte, 7 km from Tumaco-Pasto road, 01°10'N 077°58'W, 1800 m, *A. H. Gentry et al.55126* (HUA, MO, NY), Reserva La Planada. Quebradas: El Mar - La Calladita, *O. Benavides 9594* (HUA, MO); **Tolima**: Murillo, 04°52'37"N 075°10'26"W, 1500-3000 m, *M.T. Dawe 771* (K, NY, US).

3.6 Updated description of *Passiflora andreana*

Passiflora andreana Mast. Journal of the Linnean Society, Botany 20: 37. 1883. Type: Colombia: Nariño La Laja near Ipiales, 00°48'25"N 077°35'09"W, 2900 m. 1 Jun 1876, Édouard-François André 3478 (Holotype: K!, Isotype: NY).

Vines, glabrerrimous; stems-angular. Stipules 6–8 X 1 mm, triangular, lingulate, falcate or subfalcate, apex acuminate; petioles 1.4–2.6 cm X 0.9–1 mm; leaf ovate or ovate-elliptic, laminas 5.7–7.2 X 5.2–5.7 cm, rounded or slightly cordate at the base, glabrescent 2- or 3-lobed, lateral lobes 0.4–0.5 X 0.7–1.1 cm, with an acute, rounded, or mucronulate apex; midvein 5.7–6.4 cm long, central lobe 0.1–0.2 X 0.8–0.9 cm, with a obtuse, rounded or mucronulate apex or sometimes almost or totally absent, angle between the lateral veins 12°–25°; 6–8(–16) glands placed between the main secondary veins, at the base or all along the lamina; peduncles 3.6–5.5 cm X 0.9–1 mm, usually in pairs; floral bracts 4-6 mm long, linear or setaceous. floral stipe 2–2.5 mm long. Flowers

pink or purple, 5.3–6 cm diameter; sepals 20–27 X 3.5–8 mm, oblong-triangular, apex obtuse, pink or purple petals 5–20 X 2–5 mm, obelliptic, oblong, apex rounded or obtuse, pink or purple; corona filaments in 2 series, filaments of outer series 35–59, 7.3–9 X 0.5 mm, linear to cultrate, usually white or pink with yellow tips; filaments of inner series 4.1–7 mm long, filiform; operculum 2.3–3.5 mm long, plicate; androgynophore 13–17 X ~1 mm; staminal filaments ~5 mm long, anthers ~3.8 X ~1.4 mm, ovary globose to ellipsoid, ~1 X ~0.8 mm, densely pubescent; styles ~5.5 mm long, stigmas ~1 mm width.

Distribution. This species occurs in the mountain forest in the border between Colombia and Ecuador, around the Tulcan-Rumichaca road in the Carchi province.

Specimens examined

ECUADOR. **Carchi**: Tulcan-Rumichaca road, side road towards Urbina and Las Pulcas, km 7 from main road, 00°48'20"N 077°41'19", m 2956 m, *P. M. Jørgensen & S. Chimbolema 2478* (MO!, QCA!, QCNE!); Road Rumichaca (border crossing between Colombia and Ecuador) Tulcan, km 2, 00°49'17"N 077°40'36"W, 2885 m, *P. M. Jørgensen & S. Chimbolema 2476* (MO, QCA, QCNE). **Napo**: Road Puyo-Baeza, km 131, between Jondachi and Cosanga, 00°39'06"S 077°47'30"W, 1792 m, *P. M. Jørgensen & S. Chimbolema 2474* (MO!, QCA, QCNE) (MO, QCA, QCNE).

Discussion

We accept *Passiflora andreana* as a species and propose flower size as the only diagnostic character for the species. In previous works, flower color was also used as a diagnostic character, but genetic studies (Acha et al., in prep) have shown that that character is very labile in closely related species. This name has been applied to specimens originating throughout the northern Andes with purplish flowers, but the only

cohesive genetic group has large flowers and occurs only in northern Ecuador and southern Colombia. Specimens in other areas of the Andean region and that were identified as *P. andreana* based on the color of the flower should be identified as another species. Based on our observations of the flower morphology in this species and its close relatives, we think this species could be evolving towards a shift in pollination syndrome from insect to hummingbird, as we observed a degree of fusion of the corolla elements that resembled a floral tube that has not been seen in close related species and its big pink flowers with the longest androgynophore in the whole clade .

3.7 *P.* aff. *andreana:* a putative new species

We found two accessions that formed a strong clade and a genetic cluster (12, mustard color) in Acha in prep. (2019) that did not match any of the existing species descriptions. These specimens are morphologically similar to *P. alnifolia* but are clustered together with *P. andreana* in the phylogenetic and population structure analyses. Additionally, these specimens correspond to samples from the Eastern slope of the Ecuadorian Andes at a lower elevation than other *P. andreana* or most of *P. alnifolia* accessions. Here we include a preliminary diagnosis.

Vines, glabrerrimous; stems-angular. Stipules 2.8–3 X 1 mm, triangular or subfalcate, apex acuminate; petioles 1–1.8 cm X –1 mm; leaves ovate or ovate-elliptic, laminas 4.4–11.9 X 2.6–5.5 cm, rounded or slightly cordate at the base, glabrescent 2- or 3-lobed, lateral lobes 0.2–2.7 X 0.6–1.3 cm, with an acute, mucronulate apex; midvein 3.8–8.4 cm long, central lobe 0.1–0.37 X 0.1–0.2 cm, with a obtuse, rounded or mucronulate apex or sometimes almost or totally absent, angle between the lateral veins 14.4°–26°; 6–12(–17) glands placed between and outside the main secondary veins, at the

base and tip of the lamina, with a gap in the middle; peduncles ~2.1 cm X ~0.75 mm, usually in pairs; floral bracts 3 mm long, linear or setaceous. floral stipe 5 mm long. Flowers white with purple shades, ~4 cm diameter; sepals ~19 X 6 mm, oblong-triangular, apex obtuse, whitish petals ~12 X ~4 mm, oblong, apex rounded or obtuse; corona filaments in 2 series, filaments of outer series ~33, ~7.5 X 0.4 mm, linear to cultrate, usually white with yellow tips; filaments of inner series ~3 mm long, filiform; operculum ~2 mm long, plicate; androgynophore ~7 X ~1.2 mm; staminal filaments ~4.5 mm long, anthers ~5.4 X ~3.2 mm, ovary globose to ellipsoid, ~3.7 X ~2.8 mm, densely pubescent; styles ~5.5 mm long, stigmas ~2.8 mm width.

Specimens examined

ECUADOR. **Napo**: Road Puyo-Baeza, km 131, between Jondachi and Cosanga, 00°39'06"S 077°47'30"W, 1792 m, *2475* (MO, QCA, QCNE); Cascada San Rafael (Coca Falls), turn off (INECEL Campamento Quipos, Proyecto Coca). 71.3 km Ne of Baeza on Baeza/lago Agrio (Nuevo Loja) road**,** 00°10'S 077°40'W, 1200-1400 m, *S. Knapp & J. Mallet 6204* (BH, CU, G, K, MO, NY, QCA, QCNE, US).

Discussion

P. aff. *andreana* has a unique combination of vegetative characters that distinguish it from *P. andreana* and *P. alnifolia:* It has glands all along the lamina with a clear gap in the middle and, these glands are placed inside the secondary veins with few outside the main secondary veins in the base of the lamina.

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Figures

Figure 1. A) *P. kalbreyeri* (Photo: A. M. Orellana). B) *P.* rotundifolia (plate CCXC from Cavanilles, 1790). C) *P. bucaramangensis* var. *micrantha* (Photo: K. Kingma). D) *P. bucaramangensis* var. *bucaramangensis* (Photo: M. Molinari).

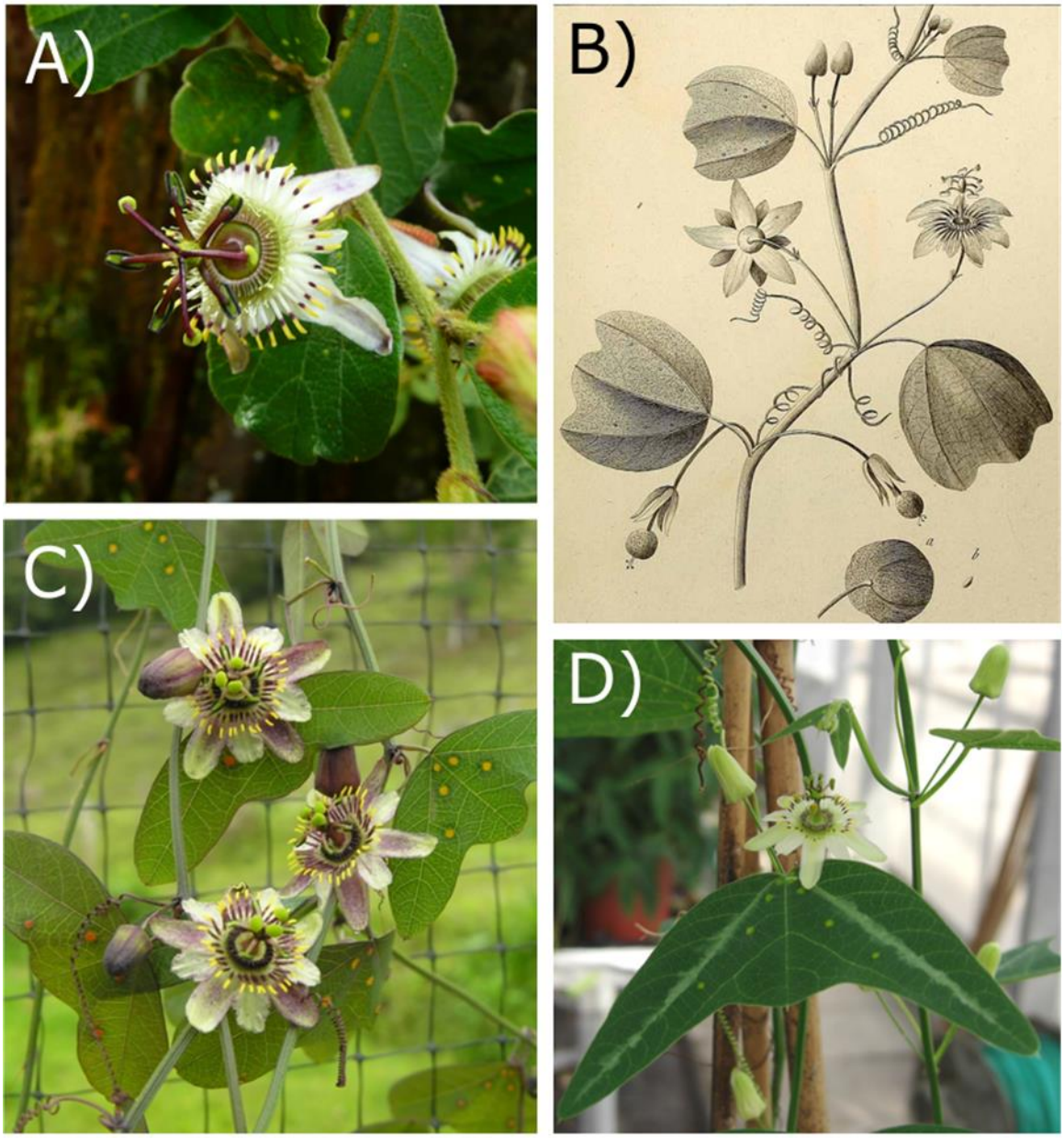

Figure 2. Distribution of *P. bucaramangensis*. Triangles represent *P. bucaramangensis* var. *micrantha* and circles P. *bucaramangensis* var. *bucaramangensis.*

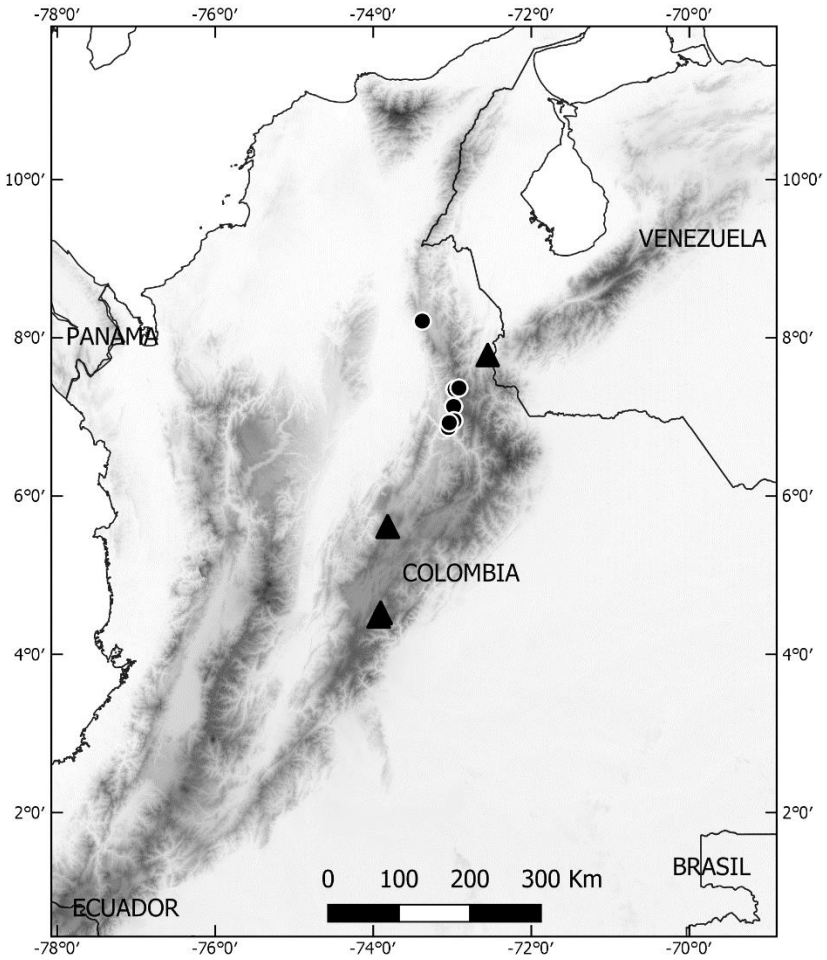

Figure 3. Distribution of *P. rotundifolia*. The triangles mark specimens previously identified as *P. rotundifolia* and the circles mark the specimens previously identified as *P. kalbreyeri.*

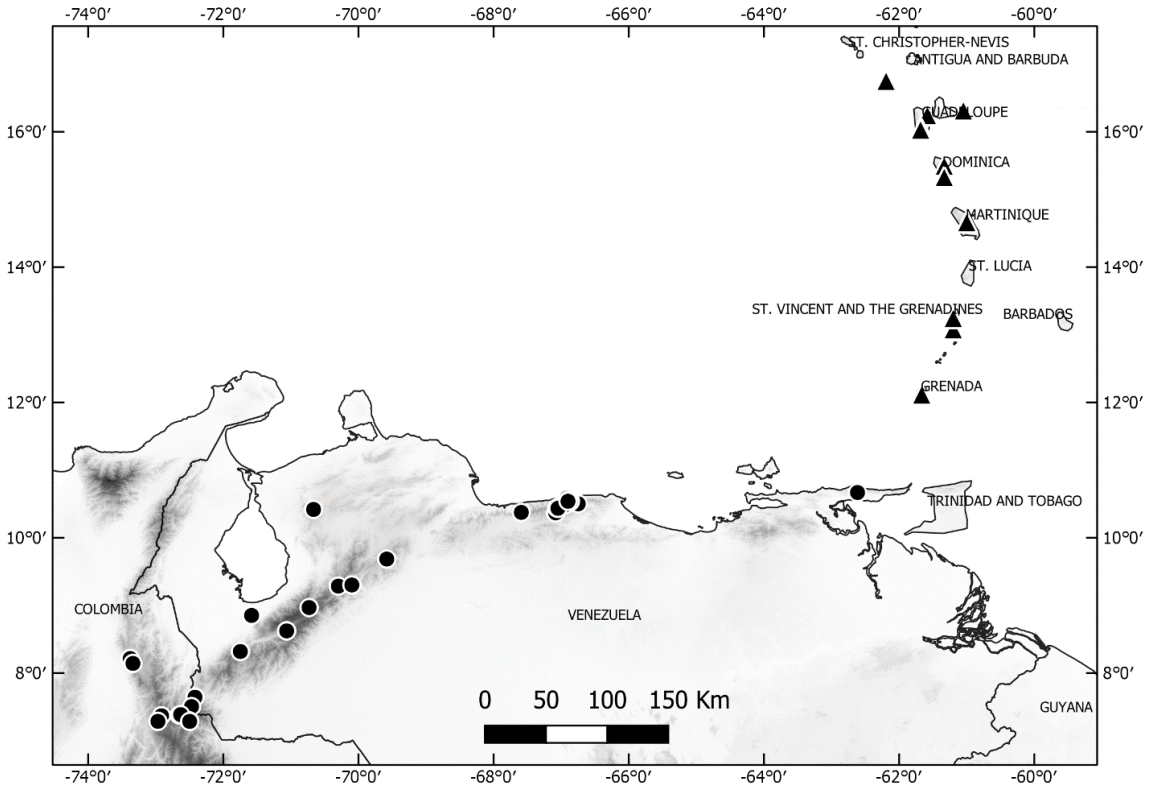
-74°0′
-72°0′
-70°0′
-68°0′
-66°0′
-64°0′
-62°0′
-60°0′
16°0′
14°0′
12°0′
10°0′
8°0′
ST. CHRISTOPHER-NEVIS
ANTIGUA AND BARBUDA
GUADALOUPE
DOMINICA
MARTINIQUE
ST. LUCIA
ST. VINCENT AND THE GRENADINES
BARBADOS
GRENADA
TRINIDAD AND TOBAGO
COLOMBIA
VENEZUELA
GUYANA
0 50 100 150 Km

Figure 4. Distribution of *P. alnifolia*. Each species lumped into the new *P. alnifolia* circumscription is showed with a different symbol, this map includes all the records from MO Tropicos database. The dashed polygon shows the proposed distribution for *P. alnifolia* new circumscription.