



The evolutionary history of vines in a neotropical biodiversity hotspot: Phylogenomics and biogeography of a large passion flower clade (*Passiflora* section *Decaloba*)

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ARTICLE INFO

Keywords:

2b-RAD
Vines
Passion flowers
Neotropics
Andes
Biogeography

ABSTRACT

Because of their extraordinary flower and leaf morphology, passion flowers (Passifloraceae) have fascinated naturalists since their discovery. Within the large, diverse (600 species) genus *Passiflora* is an especially enigmatic and species-rich (120 spp.) subclade, Section *Decaloba*, which occurs in the Neotropics and has its center of diversity in Andean montane forests. A recent phylogenetic study of Passifloraceae showed that Section *Decaloba* was monophyletic, but was unable to resolve relationships within the clade, thus preventing inferences of evolutionary history and biogeography. The goal of this study was to elucidate the phylogeny and biogeography of Section *Decaloba*. We sampled 206 accessions representing 91 of the ~120 known species in section *Decaloba* and four outgroups, with samples derived predominantly from herbarium specimens. We generated DNA sequences using a high-throughput DNA sequencing technique called 2b-RAD, reconstructed the phylogeny, and conducted ancestral area reconstructions to infer the biogeographic history of the group. We recovered predominantly well-supported trees in which species were grouped into two main clades: 1) the Central American clade, within which the majority of nodes well supported and species were monophyletic and 2) the South American clade, a large clade that showed overall lower resolution and included several polyphyletic species and species complexes that need additional research. RASP analysis showed that section *Decaloba* originated in Central America around 10.4 Ma, and then dispersed to South America, the Greater Antilles, and the Bahamas. The South American clade diversified in the Northern Andes and then dispersed to the rest of South America, and Lesser Antilles. Results suggest that both long-distance dispersal and colonization of newly available habitats (i.e., in the Andes) likely promoted diversification of this clade. This study also illustrates how using herbarium specimens and a RAD-seq approach can produce phylogenies for broadly distributed, highly diverse, and poorly accessible groups of plants where field collections would be unfeasible.

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 regions in the world. In total, 15 of the 25 biodiversity hotspots identified by Myers et al. (2000) occur in the Neotropics, making it the biogeographic region with the highest plant diversity (Gentry, 1982; 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, even though the species-level diversity of herbaceous plants and vines is thought to be comparable to that of trees and epiphytes in many Neotropical ecosystems (e.g. Linares-Palomino and Kessler, 2009), species with a non-woody habit are often even more poorly understood (Cicuzza et al., 2013) because many large biodiversity inventory studies in the Neotropics have focused primarily on

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<https://doi.org/10.1016/j.ympev.2021.107260>

Received 29 November 2020; Received in revised form 8 July 2021; Accepted 12 July 2021

Available online 14 July 2021

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woody tree species (e.g. Gentry, 1988; Simon et al., 2009; DRYFLOR et al., 2016). In this study, we focus on a highly diverse group of vines in the passion flower family (Passifloraceae) as a model system for improving our understanding of the evolutionary and biogeographic history of vines in the Neotropics.

Passifloraceae, the passion flower family, is a highly diverse family of vines that has its center of diversity in the Neotropics (Christenhusz and Byng, 2016). With ca.750 species, Passifloraceae is one the largest families of vines (Gentry, 1991; Feuillet and MacDougal, 2007) and it includes *Passiflora*, a species-rich genus of angiosperms (Frodin, 2004) with over 600 species (Unpublished data MacDougal and Feuillet, 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; Boza et al., 2018). Only in the last decades have there been collaborative efforts to understand the evolutionary relationships of this diverse genus (Feuillet and MacDougal, 2003; Muschner et al., 2003; Yockteng and Nadot, 2004; Krosnick et al., 2013; Buitrago et al., 2018; Sader et al., 2019).

Within *Passiflora*, past efforts to understand the phylogeny of the group led to the delimitation of six subgenera, including *Passiflora*, *Deidamioides* (Harms) Killip, *Astrophea* (DC.) Mast, *Tryphostemmatoides* (Harms) Killip, *Tetrapathea* (DC.) P.S. Green, and *Decaloba* (DC.) Rchb. (MacDougal and Feuillet, 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). Although most previous studies revealed well-supported relationships at deeper levels (i.e., at the subgenus and supersection levels), they showed lower resolution among species at shallower phylogenetic levels.

One particularly diverse group within *Passiflora* whose evolutionary and biogeographic history remains poorly known is *Passiflora* section *Decaloba*, a group that contains around 20% of all *Passiflora* species (Krosnick et al., 2013). The greatest species diversity in section *Decaloba* occurs in the Northern Andes, but this group is widely distributed across the Neotropics and in some subtropical regions (Tropicos®, 2019). Although section *Decaloba* was shown to be monophyletic in a previous study that focused on understanding the broader relationships among the major groups of *Passiflora*, nearly all relationships among species within section *Decaloba* were poorly resolved (Krosnick et al., 2013). Furthermore, because only one individual per species was included in the previous study, the monophyly of species in section *Decaloba* has not been investigated.

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 and a 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*, the geographic origin of the section 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.

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 (Hughes and Eastwood, 2006; Lagomarsino et al., 2016; Pérez-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); these periods of uplift correspond well with previously estimated stem ages of section *Decaloba*: 6.5 Ma (Kozak, 2015) and 11.06 Ma (Abrahamczyk et al., 2014). Another biogeographic process identified as important driver of diversification is recent climate fluctuations during the Pleistocene (~2.6 Ma to 11,700 years ago) (Vuilleumier, 1971; Pisias and Moore, 1981; Taylor et al., 1993), as seen in Andean Bromeliads and other lineages (Rull, 2011; Jabaily and Sytsma, 2013; Nevado et al., 2018). 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.

In this study, we investigated the phylogeny and biogeography of *Passiflora* section *Decaloba*. To achieve the greatest taxon sampling and phylogenetic resolution possible, we obtained DNA samples predominantly 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 and Matz, 2014). We used the resulting genome-wide data to infer a time-calibrated phylogeny and to reconstruct the historical biogeography of the group. The two main goals of our study were to: 1) elucidate the evolutionary history of the group, including identifying major clades and testing the monophyly of species, and 2) 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 species level 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 contribute 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 aimed to sample all the ~ 120 species considered to be part of section *Decaloba* s. str. (clade W of Krosnick et al., 2013; Unpublished data MacDougal and Feuillet, 2019). We reviewed nearly 2000 herbarium specimens representing almost every species 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. All the taxonomic names used in this study represent the current and accepted determination for the specimens (see Appendix for more details), but a future publication will address some of the necessary taxonomic changes in some species within section *Decaloba* (Acha, 2019; Acha and MacDougal, 2021).

From these herbarium collections, we sampled the sheets with

enough material to obtain $\sim 2 \text{ cm}^2$ of leaf tissue, and on 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 <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. auriculata* 332, Appendix).

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 a modified CTAB DNA extraction protocol for plants, with an additional 95% ethanol wash of the DNA pellet (Doyle and Doyle, 1987). We quantified the DNA concentrations in each sample using a Qubit™ fluorometer (ThermoFisher) and cleaned the samples using a GENECLAN® turbo kit (MP Biomedicals). As expected for herbarium specimens, the quantity and quality of DNA varied among samples, with only 542 samples containing the $\geq 200 \text{ ng}$ of DNA required for 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://docs.google.com/document/d/1am7L_Pa5JQ4sSx0eT5j4vdNPy5FUAtMZRsJZ0Ar5g9U/edit), with some modifications. A total of 150–250 ng of DNA was digested using the restriction enzyme BclI (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 using agarose gel electrophoresis to confirm the success of digestion and ligation. Of the original 542 samples that met our minimum DNA concentration, only 219 successfully amplified.

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 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 the gel-purified PCR product using a Qubit fluorometer, pooled the eight PCR reactions at a concentration of 10 nM, and sequenced them for 1x50 cycles on an Illumina HiSeq 2500 or 4000 sequencer (2017–2018) at Duke University. After including 6 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 conducted initial quality assessments of the resulting sequences using FastQC (Babraham Bioinformatics). We then applied the 2bRAD_denovo script written by M. Matz (available at: https://github.com/zoon/2bRAD_denovo) to demultiplex the sequencing reads (sorting them into individuals), and remove barcodes and Illumina adaptors.

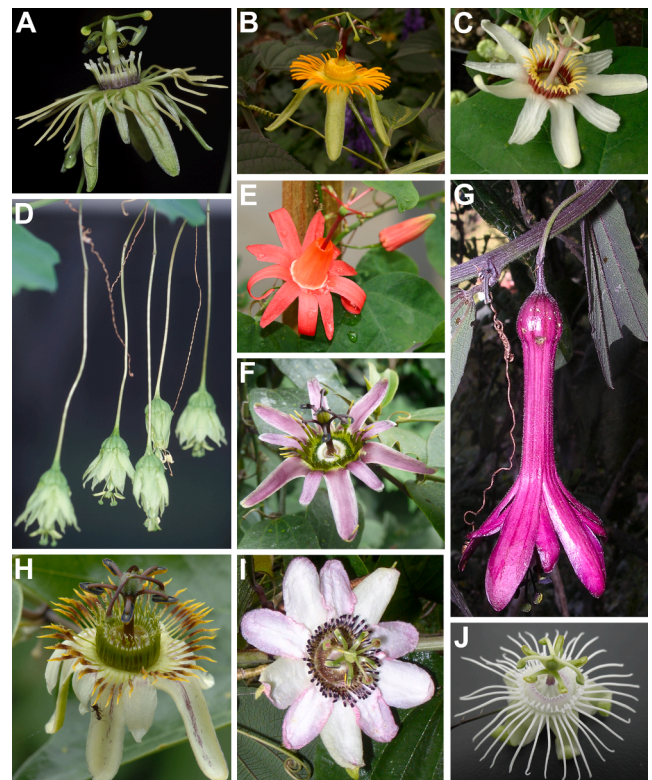


Fig. 1. Examples of floral morphology diversity of the lineages included in this study. A) *P. lutea* (photo credit: J. Richard Abbott), B) *P. gilbertiana* (photo credit: Barry Hammel), C) *P. yucatanensis* (photo credit: Elizabeth Peters), D) *P. penduliflora* (photo credit: Ronald Boender), E) *P. murucuja* (photo credit: Ronald Boender), F) *P. aff. andreana* (photo credit: Ronald Boender), G) *P. hyacinthiflora* (photo credit: Alexandra Hernández), H) *P. alnifolia* (photo credit: RJR Vanderplank), I) *P. pascoensis* (photo credit: Tatiana Erika Boza Espinoza), J) *P. misera* (photo credit: Jorge Ochoa). All the pictures are reproduced with the authors permits.

Lastly, we used FastX toolkit (available at: http://hannonlab.cshl.edu/fastx_toolkit/) to remove low-quality sequences, retaining reads that had $\geq 90\%$ of the bases with a minimum quality score of 20 and an input quality ASCII offset of 33.

Next, we used iPyRAD v0.7.28 (Eaton and Overcast, 2020) with the parameters described in Supplementary Data table S1 to assemble loci *de novo*. For all analyses, the aligned dataset included the entire 36-bp RAD fragment for each locus, including both variable and invariable sites. 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 (supplementary material S2) and discarded any sample with $> 95\%$ missing data if there was another accession of the same species with less missing data. We also generated an additional data set where we employed a more restrictive filter, discarding samples with $>50\%$ missing data, but obtained lower resolution and support when using this data set in downstream phylogenetic analyses (data not shown).

2.4.2. Phylogenetic analyses

We initially used the full dataset containing 217 samples (including some duplicates) to reconstruct the phylogeny using RAxML v8.2.10 (Stamatakis, 2014). We used JModelTest2 (Darriba et al., 2012) to determine the optimal model of evolution, which was the GTRCAT model; we therefore conducted analyses using this model with 1000

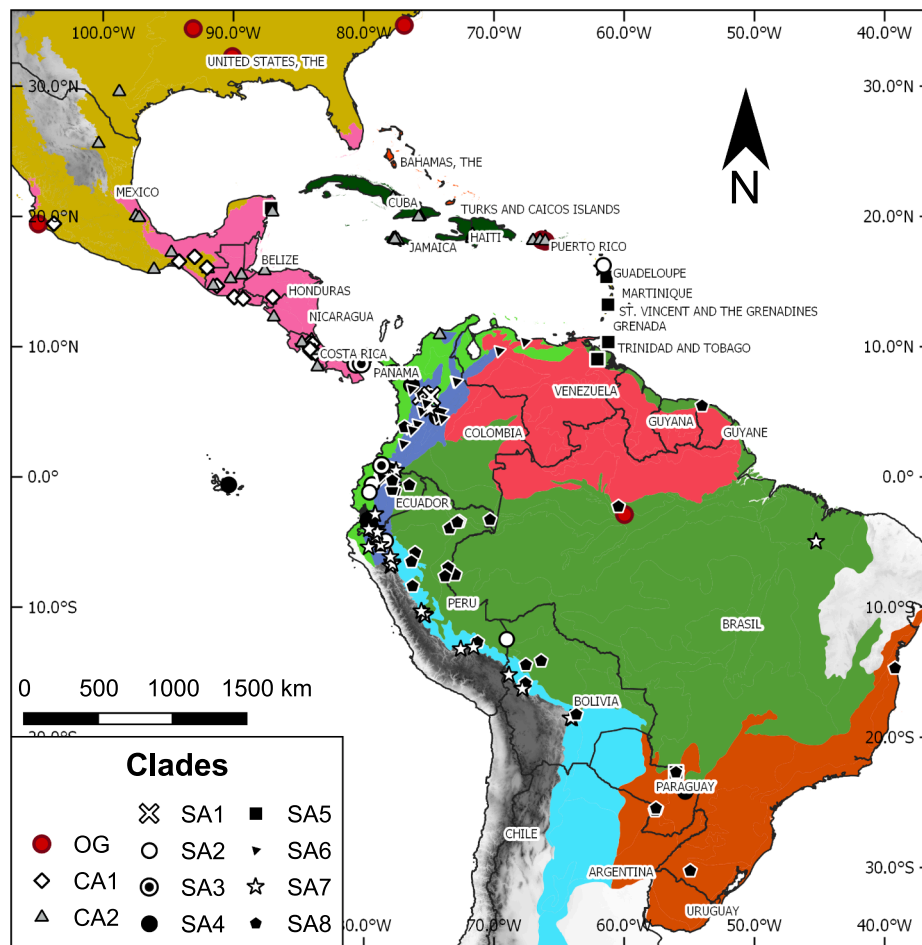


Fig. 2. Map of the collections and ecoregions used for biogeographical analyses in this study. The different symbols represent the clades in Fig. 3. For the ecoregion names and color legend, see Fig. 5. The base map shows a digital elevation model, with higher elevations indicated by progressively darker shading, and international borders.

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 phylogeny. The main advantage of TBE is that it allows for a degree of uncertainty or instability in 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 at medium-depth and deeper nodes, where frequently FBP fails. 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.

We also conducted parsimony phylogeny reconstruction of the full data set using PAUP* 4.0a169 (Swofford 2003). In all parsimony analyses, we conducted heuristic searches using 1000 random addition replicates and TBR branch swapping, saving 1 tree per replicate. Bootstrap analyses (1000 replicates; Felsenstein 1985) were used to assess branch support using a heuristic search with TBR branch swapping, with 10 random additions per replicate, saving no > 1 tree. per replicate.

2.5. Divergence time estimation and historical biogeographical reconstruction

Because all subsequent analyses required the inclusion of only one individual per species, we generated a reduced dataset that included one individual per lineage. We inspected phylogenies based on the full data

set (see results) and in each monophyletic species, we retained the individual in the analysis with the lowest percentage of missing data. When a species was 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 and 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 a chronogram of the reduced dataset produced using treePL v1 (Smith and O'meara, 2012) as our starting tree, while using the same calibration points described above. We explored the BEAST log results in Tracer v1.7.1, summarized posterior trees in TreeAnnotator v.2.5.2 (Rambaut et al., 2018), and visualized the phylogeny using R, the ggtree package (v.1.4.2), and its dependencies (R Core Team, 2021; Yu et al., 2017; Yu et al., 2018; Yu et al., 2020).

For the historical biogeographical reconstruction, based on general patterns of endemism and habitat types occupied by sect. *Decaloba*, we divided the range into ecoregions (Fig. 2) following the Commission for Environmental Cooperation (CEC) (1997) and Griffith et al. (1998). The 11 ecoregions were: 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

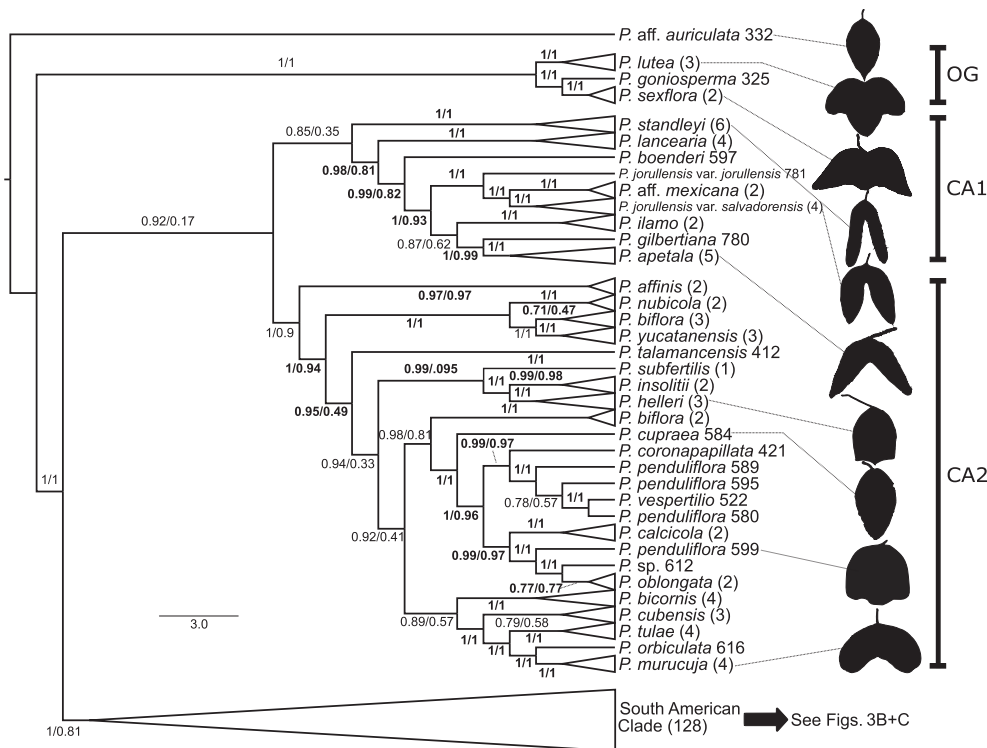


Fig. 3. Maximum likelihood phylogeny of *Passiflora* section *Decaloba* resulting from the RAXML analysis including parsimony analysis results and leaf image rendering from herbarium specimens. Support values generated using transfer bootstrap expectation (TBE) and Felsenstein bootstrap (FBP) are shown above branches in the format: TBE/ FBP. These values are in bold if the node had $\geq 70\%$ parsimony bootstrap support in the parsimony analysis. Collapsed clades include the number of total samples in parentheses next to the names. The leaf outlines represent some of the accessions sampled in this study (scale not proportional to real size). The Figure is divided into following three panels: A) the Central American clade, with the South American clade collapsed, B) South American clades 1–6, and the Central American, SA7 and SA8 clades collapsed, and C) South American Clades 7 and 8, with the 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.

(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) (Fig. 2). Although some of the ecoregions were discontinuous (e.g., North America and Central America, which differentiated between tropical dry forest and wet forest), we did not alter the ecoregions of Griffith et al. (1998) because none of our samples occupied one of these areas. Further, other studies of the region showed similar discontinuous patterns in the delimitations of ecoregions (Ricketts et al., 1999; Olson et al., 2001). 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 ecoregions, we collated information on species geographical ranges using several available data sources. We mapped all section *Decaloba* accessions in the Tropicos® database using QGIS (QGIS Development Team, 2019) and complemented it with all the available information about their distribution. The Tropicos® database also provided us with elevation and collection information from non-georeferenced specimens that was used in further interpretation of the results.

Ancestral range reconstruction analysis was conducted with the program RASP v4.0 (Reconstruct Ancestral State in Phylogenies; Yu et al., 2015), using our maximum clade credibility chronogram generated in BEAST, which incorporates the following models: DEC (Dispersal-Extinction-Cladogenesis; Ree and Smith, 2008; Massana et al., 2015), DEC + J (DEC + Jump parameter; Matzke, 2014), DIVA-LIKE (Dispersal Vicariance Analysis with Likelihood implementation; Ronquist, 1997; Matzke, 2014), BAYAREALIKE (Bayesian inference for discrete Areas with Likelihood implementation; Landis et al., 2013; Matzke, 2014), BAYAREALIKE + J (BAYAREALIKE + Jump parameter; Matzke, 2014), as implemented in BioGeoBEARS (Matzke 2013a, 2013b, 2014; R Core Team, 2021). We ran our ancestral range

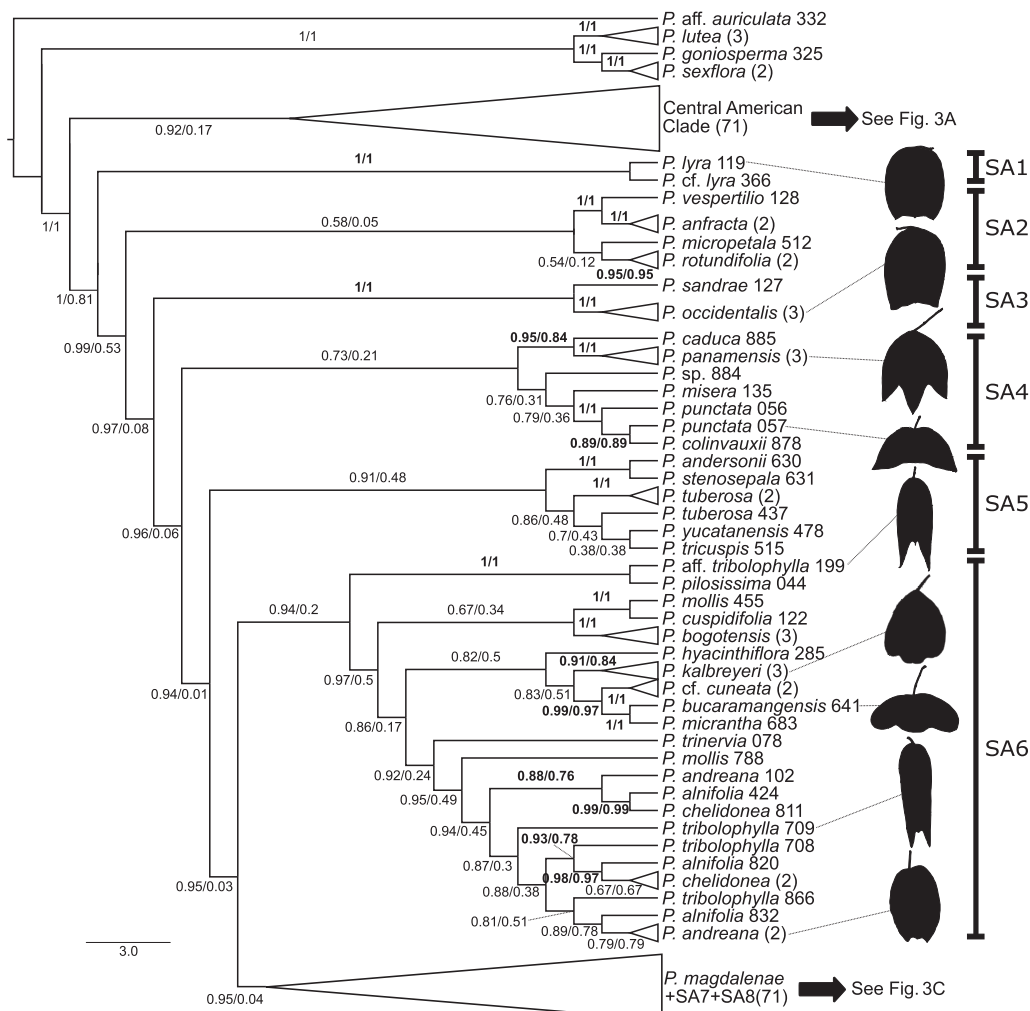
reconstruction analyses using the maximum clade credibility chronogram generated in BEAST and the previously mentioned six models (Supplementary Table S4A) to improve our ability to detect erroneous reconstructions. We interpreted congruent ancestral range reconstruction results for a given node across different models as indicative of high support for that node's reconstruction state(s). In RASP analyses, we ran the non-stratified ancestral range reconstruction using a maximum of 2–5 areas per node and 250 bootstrap pseudo-replicates. Additionally, we excluded range combinations that included disjunct distributions and considered the node reconstructions to be well supported when they reached a probability $\geq 75\%$. We used the model likelihood-ratio test (Supplementary Table S4 B) in RASP to select the best model based on comparisons of Akaike information criterion (AIC) values using standard information-theoretic approaches (Burnham and Anderson 2004).

3. Results

3.1. Locus assembly and SNP calling

After removing accessions with poor sequence quality, the full data set included 206 unique samples (Appendix, GenBank BioProject ID: PRJNA681354, study ID: SRP295475), including sequences from: 1) seven accessions representing four outgroup taxa, 2) 181 accessions representing 91 of the ~ 120 known species in section *Decaloba*, including both species with accepted published names as well as unpublished names, and 3) 18 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 full dataset, the total number of loci passing the initial sequencing quality control filter was 513,321. After applying quality control filters in iPyRAD as described in Supplementary Table S1, the final filtered data set contained 11,778 concatenated loci, with each locus 36 bp in length, resulting in a total alignment of 424,008 bp, which included both variant and invariant sites. Missing data per sample



ranged from 48 to 98% (Supplementary Table S2), with most samples having between 62 and 92% missing information.

3.2. Results of phylogenetic analyses

The maximum likelihood (ML) tree is presented in Fig. 3A–C, including results for the parsimony analysis. 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. auriculata* 332) and section *Xerogona* s. lat. (*P. goniosperma*, *P. lutea* (Fig. 1A), *P. sexflora*), section *Decaloba* was strongly supported as monophyletic with 100% support (Fig. 3), consistent with previous studies (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 71 accessions representing 28 taxa occurring mainly in Central America but also in South America (Fig. 3A), and a predominately South American clade (100% support), comprising 128 accessions in 76 taxa (Fig. 3B and 3C).

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. 3A, and Supplementary Figure S1), both of which showed medium to high values of support in most 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* ined., *P. gilbertiana* (Fig. 1B) and *P. apetala*) that are distributed from southwestern USA and western Mexico to the Isthmus of Panama. All 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 45 accessions from 19 taxa (*P. affinis*, *P. nubicola*, *P. yucatanensis* (Fig. 1. C), *P. biflora*, *P. talamancensis*, *P. subfertilis*, *P. insolitii*, *P. helleri*, *P. cupraea*, *P. coronapapillata* ined., *P. penduliflora* (Fig. 1D), *P. calcicola*, *P. oblongata*, *P. sp.* 612, *P. bicornis*, *P. cubensis*, *P. tulae*, *P. orbiculata* and *P. murucuja* (Fig. 1E)). This clade is distributed from the Edwards plateau in Texas, USA to the Ecuadorian Andes, the Bahamas, the Antilles, and northern Venezuela. In CA2, all species represented by multiple accessions were monophyletic except *P. biflora* and *P. penduliflora*.

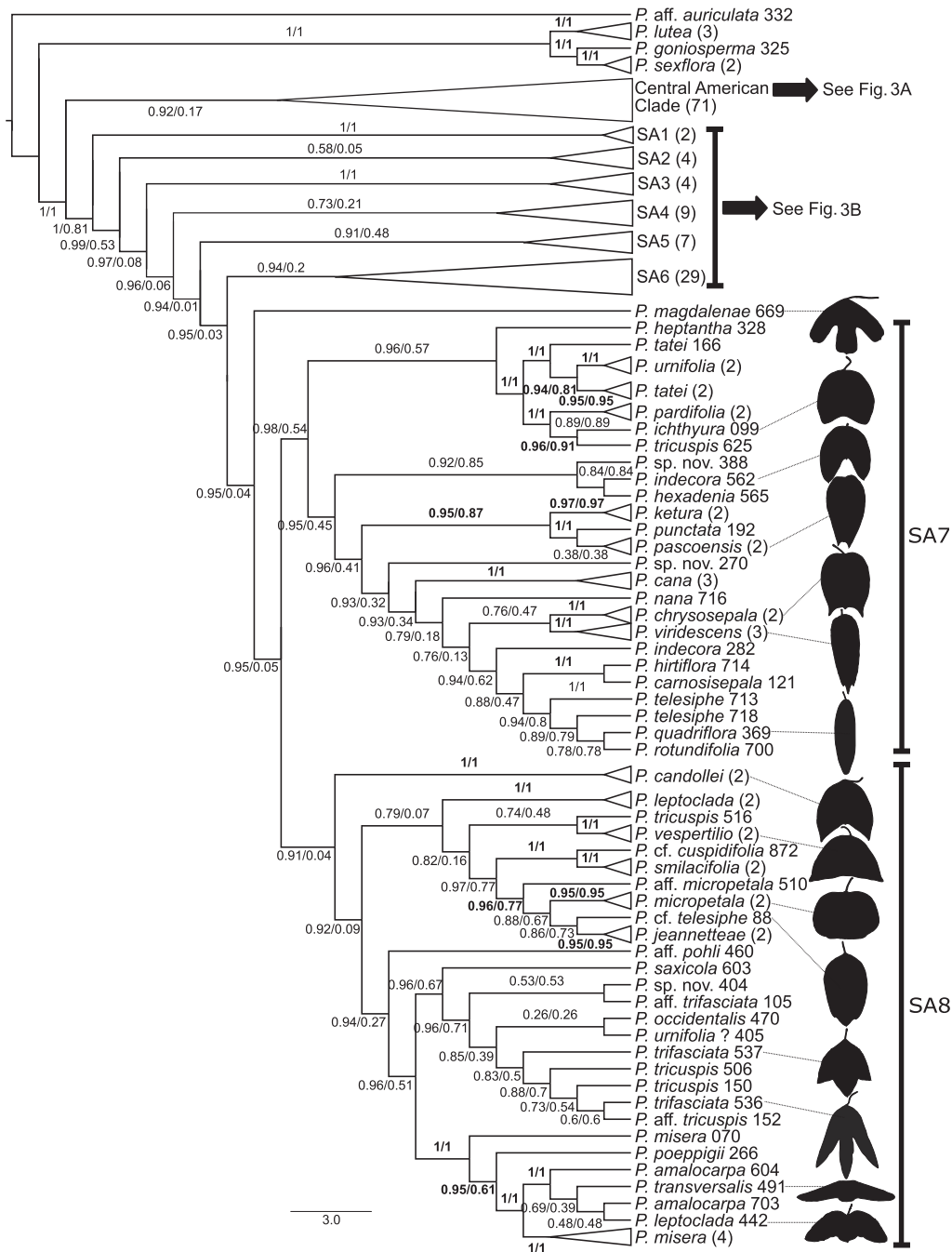


Fig. 3. (continued).

3.2.2. South American clades (SA1-SA8)

In the large South American clade (Fig. 3B, 3C and Supplementary Figure S1), five early diverging, small subclades (SA1-SA5) were placed as successive sisters to the remainder of the SA clade. The first, Clade SA1, had 100% support and comprised a pair of accessions (*P. lyra* and *P. cf. lyra*) from Colombia and northwest Ecuador (400–900 m) (Fig. 3B). The clade containing the remaining accessions in the SA clade was strongly supported (99%) and was divided into two groups, SA2, which was weakly supported (58%) and a large clade containing clades SA3-8, which was strongly supported (97%). The SA2 clade 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. Within the clade containing SA3-SA8, SA3 was strongly supported (100%) and placed as sister to a strongly supported clade (96%) containing clades SA4-8. SA3 contained four accessions representing two species: *P. sandrae*, from the central-eastern regions of Panama and *P. occidentalis* ined. from the central region of Panama to the northernmost Ecuadorian coast (Fig. 3B).

Within the clade containing clades SA4-8, clade SA4 (Fig. 3B; 73%) included 9 accessions representing 6 species (*P. caduca* ined., *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, coastal Ecuador, and the Galapagos Islands. Two species represented by multiple accessions in clade SA4 were not monophyletic:

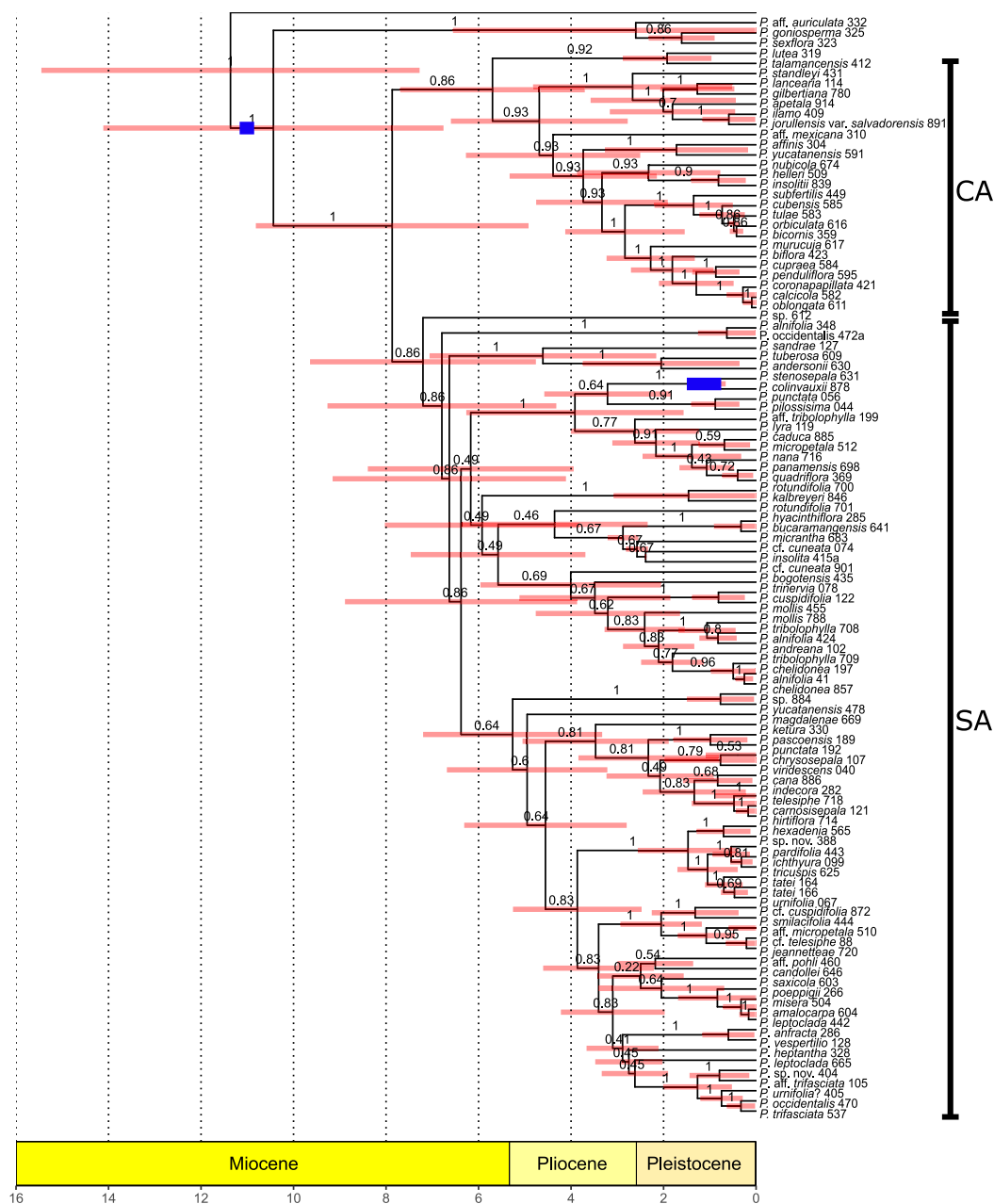


Fig. 4. BEAST maximum credibility chronogram. Values above the branches indicate Bayesian posterior probabilities and the bars at each node represent 95% credibility interval for the clade age in millions of years. Geological epochs limits are shown in the x axis. The two calibration points are highlighted in blue on the node to which they were assigned. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

P. colinvauxii was nested within a clade containing the two *P. punctata* accessions (*P. punctata*056 and *P. punctata*057), rendering *P. punctata* paraphyletic (Fig. 3B). *Passiflora misera* 135 from Paraguay was not grouped with the remaining *P. misera* samples, which were placed in the SA7 clade (Fig. 3C and Supplementary Figure S1). Sister to SA4 was a strongly supported (94%) group that contained clades SA5–SA8. Within this group, a well-supported (91%) SA5 clade (Fig. 3B) contained 7 accessions representing 5 species (*P. andersonii*, *P. stenosepala*, *P. tuberosa*, *P. yucatanensis* and *P. tricuspsis*) that are mostly distributed from the Lesser Antilles (*P. andersonii* and *P. stenosepala*) to Trinidad and the Venezuelan Andean and coastal regions (*P. tuberosa*). Two species placed in clade SA5 were not monophyletic: *Passiflora yucatanensis* 478 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.

Sister to SA5 was a strongly supported group (95%) containing clades SA6–SA8. Within this clade, the strongly supported (94%) clade SA6 (Fig. 3B) 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* (Fig. 1H), *P. chelidonea* and *P. tribolophylla*). Species in clade SA6 typically occupy Andean humid montane forest ranging from Colombia to Bolivia, but can also be found in Venezuela. Most species represented by more than one sample placed in this clade were not monophyletic, except for *P. bogotensis*, *P. kalbreyeri*. Sister to clade SA6 was the strongly supported (95%) *P. magdalenae* + SA7 + SA8 clade containing 71 accessions and 46 taxa (Fig. 3B and C). In this group, the single accession of *P. magdalenae*, which is found in the central inter-Andean Valleys of Colombia, was placed as a strongly supported sister (95%) to two strongly supported groups, the SA7 clade (98%) and the

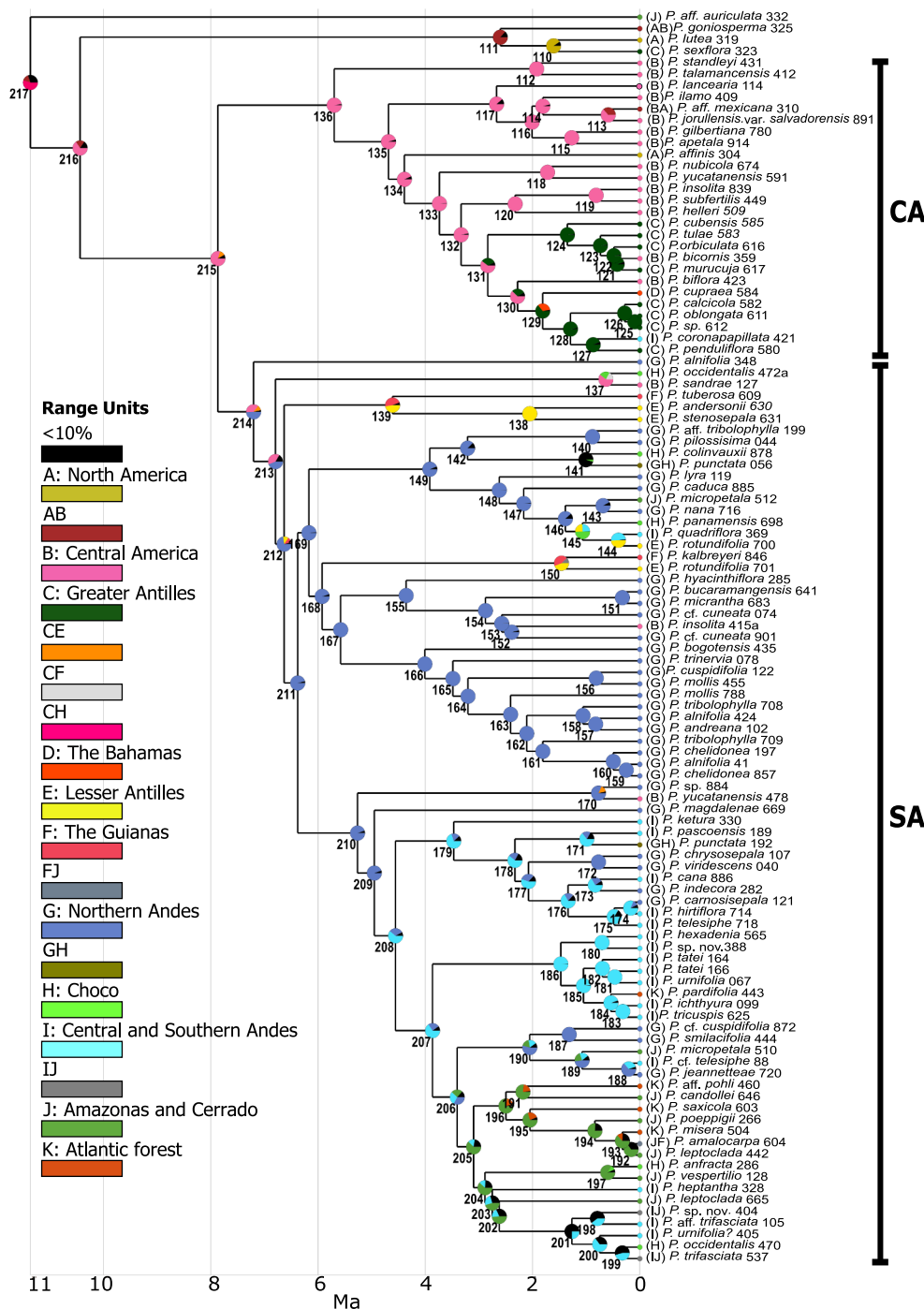


Fig. 5. Ancestral range reconstruction of *Passiflora* section *Decaloba*. The tips contain the current area assigned to the taxa in parentheses together with a colored circle representing the assigned area. Pie charts at each node represents the probability of an ancestral area. Colors for biogeographical areas that we modeled (ecoregions coded as biogeographical provinces) match those in Fig. 2, except for the categories that represented a two-area range combination. The ecoregions are the following: 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 * symbol and black color represent areas with < 10% probability of ancestry. The x-axis represents time in millions of years ago (Ma).

SA8 clade (91%).

The SA7 clade comprised 34 accessions representing 22 taxa (*P. heptantha* ined., *P. tatei*, *P. urnifolia*, *P. pardifolia*, *P. ichthyura*, *P. tricusps*, *P. sp. nov.*, *P. indecora*, *P. hexadenia* ined., *P. ketura* ined., *P. punctata*, *P. pascoensis* (Fig. 11), *P. sp. nov.*, *P. cana* ined., *P. nana*, *P. chrysosepala*, *P. viridescens*, *P. hirtiflora*, *P. carnosiseppala*, *P. telesiphe*, *P. quadriflora* and *P. rotundifolia*). Species in this clade are distributed from the Ecuadoran Andes to central Bolivian mountain forests and southern Brazil. Within Clade SA7, most internal branches are well-supported. The clade was subdivided into two groups that correspond to geography, with the smaller clade (7 taxa., 96% TBE) ranging from the eastern slopes of the Andes to the Amazon basin and the Atlantic forest, and the larger clade (15 taxa., 95% TBE) occurring only in the

Andes. Most species in clade SA7 represented by multiple accessions were supported as monophyletic except for *P. tatei*, *P. telesiphe* and *P. indecora*. Additionally, three accessions were unexpectedly included in the SA7 clade: the sample *P. punctata* 192 was not placed with the other *P. punctata* accessions in SA4 (Fig. 3A and B), *P. rotundifolia* 700 was placed in this clade whereas the rest of *P. rotundifolia* accessions were found in SA3 (Fig. 3B) and *P. tricusps* 625 was not placed with other *P. tricusps* accessions in SA8.

The SA8 clade contains 37 accessions and 24 taxa (*P. candollei*, *P. leptoclada*, *P. tricusps*, *P. vespertilio*, *P. cf. cuspidifolia*, *P. smilacifolia*, *P. aff. micropetala*, *P. micropetala*, *P. cf. telesiphe*, *P. jeannetteae* ined., *P. aff. pohlii*, *P. saxicola*, *P. sp. nov.*, *P. aff. trifasciata*, *P. occidentalis*, *P. urnifolia*?, *P. trifasciata*, *P. tricusps*, *P. aff. tricusps*, *P. misera* (Fig. 1J),

P. transversalis, *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 Andes, 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. vesperitilo* and *P. tricuspidis*) (Fig. 3C and suppl. Fig. S1).

3.3. Divergence time estimation and biogeographic analysis

After removing duplicate accessions of monophyletic species as identified by phylogenetic analyses based on the full data set, the reduced data set contained 109 unique samples (marked by an asterisk in the Appendix), including: 1) four accessions representing four out-group 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 passing the sequencing quality filters was 301,700. Applying the filters in iPyRAD as described in supplementary table S1, resulted in a final data set of 7,299 loci, each of which was 36 bp in length, such that the final assembly was 262,764 total bp in length.

Results of BEAST analyses of the reduced data set are shown in Fig. 4. We recovered section *Decaloba* as monophyletic (posterior probability = 1). Although half the nodes in the BEAST tree had posterior probabilities (PP) ≥ 0.95 , relationships in this tree overall showed lower resolution and support than those found in the RAXML tree. Like the RAXML tree, BEAST recovered the main two Central American (CA) and South American (SA) clades. Within the CA clade, accessions formed two strongly supported, smaller clades that largely corresponded to Clades CA1 and CA2 in the RAXML tree. In contrast, relationships among taxa in the SA clade differed significantly between the RAXML and BEAST trees. The BEAST tree did not recover RAXML clades SA1-SA5 and instead placed these samples in other large clades. Both RAXML and BEAST topologies recovered the SA6 clade, but RAXML showed greater support values for this clade (94% TBE) and its internal nodes (67–100%) than BEAST (0.49 PP clade, 0.46–1.00 internal nodes). Clades SA7 and SA8 from the the RAXML tree were not recovered in BEAST, which instead placed the samples into one large clade, with lower support for relationships than the RAXML tree.

We used the BEAST chronogram and RASP to analyze the biogeography of section *Decaloba*. AIC scores showed that the best-supported model for our dataset in all four RASP analyses was the DEC + J model (Supplementary table S4 B). Results were similar when the maximum number of areas allowed per node/tip ranged from 2 to 5 (data not shown), except for an increase in uncertainty in some nodes as the maximum number of areas increased; we therefore present only the results of analyses allowing a maximum of two areas per node (Figs. 2, 5 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 between the Central–Southern Andes mountains (I) and the Northern Andes mountains (G), with five dispersal events; these were also the areas with the highest numbers of speciation events (I:14, G:27), along with Central America (B) (13).

Results of biogeographic analyses showed that the most recent common ancestor of all section *Decaloba* diverged from all other passion flowers in the late Miocene around 10.4 Ma (95% HPD: 6.5–13.8 Ma) and that its range most likely occurred in mainland Central America (Fig. 5, node 215). The Central American clade (CA) and the South American Clade (SA) diverged 7.8 Ma (95% HPD: 5–10.9 Ma) in the late Miocene–Pliocene. The common ancestor of the CA clade (Fig. 5, node 136) showed a highly supported (93%) origin in mainland Central America around 5.7 Ma (95% HPD: 3.7–7.7 Ma), and most early-diverging nodes in the CA clade (i.e., nodes 132–135) showed ancestral ranges in Central America. In the CA clade, range reconstructions

showed the following range shifts: 1) a range expansion of *P. aff. mexicana* into North America (i.e., from B to AB; Fig. 5) around 0.58 Ma (95% HPD: 0.2–1.3 Ma), 2) an early dispersal into North America (i.e., from B to A; Fig. 5) 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 clades occurring solely in the Major Antilles (i.e., nodes 121–128; Fig. 5), 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 diverged around 7.2 Ma (95% HPD: 4.9–9.8 Ma). The ranges of the first early diverging lineages in this clade (nodes 212–214) were reconstructed as occurring either in Central America or the northern Andes, and several dispersal events or range expansions were also inferred at these nodes, 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 Ma) and had an ancestral range inferred in the north Andes, followed by two major clades (nodes 169 and 210), also with ancestral ranges in the north Andes. Beginning at node 169, most nodes had ancestral ranges in the north Andes, but we observed several subsequent range shifts within the clade, all of which occurred in the last 2 Ma: 1) two independent dispersal events to the Choco/Galapagos region (H) (nodes 141 and 145), 2) two independent dispersal events to the Lesser Antilles (nodes 144 and 150) and, 3) a colonization event into the Guianas (node 150).

The other SA clade corresponded mostly to the accessions in clades SA7-8 from the RAXML tree. Several range shifts were inferred in this group: 1) dispersal from the northern to the central and southern Andes that occurred 4.5 Ma (95% HPD: 2.9–6.4 Ma), which gave rise to several clades with ranges predominantly in the southern Andes, and 2) a colonization of Amazonas and Cerrado around 3 Ma (95% HPD: 1.9–4.3 Ma) (node 205) and 3) two or more instances of colonization of the Atlantic forest: one that occurred > 1 Ma, giving rise to *P. pardifolia* (node 184), and several possible dispersal events from Amazonas into the Atlantic forest (at nodes 191, 193 and 195) around 2.5 Ma (95% HPD: 1.6–3.5 Ma).

4. Discussion

In this study, we reconstructed the phylogeny of *Passiflora* section *Decaloba* (Passifloraceae) using samples obtained almost exclusively from herbarium specimens, and 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 the genus *Passiflora*. 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, a substantial portion of the nodes in the phylogenies were well resolved despite many samples having a high percentage of missing data, and like other previous studies (e.g., Tripp et al., 2017), found that resolution increased to a certain point as the percentage of missing data increased. Additionally, the use of TBE (Lemoine et al., 2018) provided support for phylogenetic relationships that FBP and the parsimony analysis 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. The overall approach employed in this study 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 (e. g., the uplift of the Andes and fluctuations in climate during the Pleistocene). 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 containing subgenus *Decaloba* (Abrahamczyk, 2014), similar to some Bromeliaceae (Givnish et al., 2011, 2014), Solanaceae (Dupin et al., 2017) and Bignoniaceae clade's (Lohmann et al., 2013; Carvalho Francisco and Lohmann, 2020). However, our analyses revealed a Central American origin to the clade, comparable to South American Valerianaceae (Bell and Donoghue, 2005; Bell et al. 2012) and Guettardeae (Manns et al., 2012). Another possible scenario for the biogeographical history of section *Decaloba* is that it originated in North America followed by a progressive dispersal southward, like American Stachydeae (Roy et al., 2013) or the neotropical *Prunus* (Chin et al., 2014). The hypothesis of a North American origin is moderately supported by the available fossil records for Passifloraceae (Hermesen, 2021), the location of which suggests a possible European origin for the family and a dispersal in the Eocene across the North Atlantic Land Bridge to North America (Tiffney, 1985), with a subsequent colonization from North America to South America. However, 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 in the next section.

4.1. Central America

After diverging from the SA clade 7.8 Ma, the CA clade initially remained species-poor for several million years. Diversification began to occur around 4 Ma, possibly as the result of the geologic and environmental changes resulting from the formation and closure of the Isthmus of Panama. The number of species in the CA clade rapidly increased from 4 Ma until the present, with a large increase in diversification occurring in the last 2 Ma, likely in response to the rapid, cyclical fluctuations in temperature, aridity, and sea level that occurred during the Pleistocene (e.g., Leyden, 1984). Diversification also occurred as the result of the colonization of new habitats through dispersal to the Greater Antilles (clade CA2). Interestingly, phylogenies indicate that the species in the Greater Antilles likely originated from two separate dispersal events, resulting in a pattern where the species on an island are more closely related to those on different islands than to those that co-occur on the same island. It also appears that one species in mainland Central America likely originated via dispersal back to the Central American mainland from the Greater Antilles (*P. bicornis*).

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 (Fig. B) with white, yellow or red corona elements and bilobed leaves (Fig. 3A), except for *P. lancearia*, which is early diverging within the clade and has elliptic leaves. Species in clade CA2 are distributed from the Edwards plateau in Texas, USA to the Colombian Andes, the Bahamas, the Antilles, and northern Venezuela. Several of the species in clade CA2 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 (Fig. 1D and E). The phylogenetic relationships and pollination biology of the two clades occupying the Greater Antilles, which generally have elongated flowers, were studied in depth by Kay (2003), and we obtained a similar topology but with higher support values for relationships among species.

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). One possible way this colonization was achieved is through seed dispersal, which is a strong driver of plant speciation and therefore of biogeographical patterns (Givnish, 2010). Yet, very little is known about seed dispersal in Section *Decaloba*. Seed dispersal ecology in *Passiflora* varies across clades, with some clades likely dispersed by small mammals (Cáceres, 2002), birds (Macedo and Prance, 1978; Carlo and Morales, 2016) and even crocodilians (Platt et al., 2013). Section *Decaloba* and many species of Subgenus *Decaloba* share the same type of fruits: small berries, black when mature, which are suspected of being dispersed by birds (Ulmer and MacDougal, 2004), which may have facilitated dispersal across the isthmus of Panama; however, additional research on the seed dispersal ecology in this clade is needed to evaluate this hypothesis.

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 new habitats made available through the uplift of the Andes as well as fluctuations in environmental conditions during the Pleistocene (Baker et al., 2020). In particular, our results showed that most speciation events in the South American clade of section *Decaloba* occurred during the Pleistocene. In the northern Andes, instead of the latitudinal shifts in vegetation that occurred in North America and Europe, Pleistocene glacial cycles largely resulted in vertical shifts in montane vegetation zones, with plants moving higher in elevation during the warmer interglacial periods and lower in elevation during the colder glacial periods; for example, the forest line was between 1200 and 1400 m lower in elevation during the Last Glacial Maximum (van der Hammen and Cleef, 1986; Hooghiemstra and Van der Hammen, 2004; Hooghiemstra et al., 2006; Graham, 2009; Jomelli et al., 2014; Nevado et al., 2018). These vertical displacements resulted in the expansion of available habitat and increased habitat connectivity when vegetation zones shifted lower in elevation during glacial periods and a contraction of available habitat and connectivity during interglacial periods as suitable habitat became isolated to higher-elevation areas (Simpson, 1974; Flantua et al., 2014; Flantua and Hooghiemstra, 2018). Thus, populations of Andean plants such as *Passiflora* may have experienced cyclical isolation during the Pleistocene, leading to high rates of diversification via repeated allopatric speciation.

After the colonization of the northern Andes, the SA clade of *Passiflora* subsequently dispersed 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 (Santiago-Valentin and Olmstead, 2004; Maunder et al., 2011), such as the modern colonization events of the Lesser Antilles observed in birds (Ricklefs and Bermingham 2008).

In clades SA1-SA4, many of the observed relationships 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 of low

levels of informative data, as it is composed primarily of samples with high to moderate transfer index values such as accession *P. micropetala* 512, which has the greatest transfer index value of all samples. In particular, the placement of *P. micropetala* 512 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 material with no type specimen, 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. yucatanensis* 478 (which was collected in Quintana Roo, Mexico) is correctly placed, then it would indicate that a long-distance dispersal event from the Venezuelan coast to Quintana Roo-Mexico occurred, although a possible incorrect placement is also plausible given its relative high transfer index. One accession of the polyphyletic species *P. tricuspis* 515 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 of most of these species a challenge. Furthermore, we recovered strong vicariance patterns that support phylogenetic allopatric breaks in the Andean species (e.g. Marañón river valley, see Fig. 1 in Hazzi et al., 2018). 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 (Fig. 3B). Their flowers are small, white, disc-shaped with some traces of purple (similar to Fig. 1 H), except *P. hyacinthiflora* (Fig. 1G) and *P. trinervia*, which both have tubular pink flowers thought to be an adaptation for hummingbird pollination (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017). However, despite the strong geographic and morphological characters uniting clade SA6, most species within the clade are highly polyphyletic; extensive additional research is necessary to clarify species limits (Appendix).

Most species with multiple accessions in the SA7 clade are monophyletic except *P. tatei*, *P. telesiphe* and *P. indecora*. Given that this is one of the youngest clades, one explanation for the paraphyly in these and other species is incomplete lineage sorting (ILS), as found in other cultivated (Yockteng et al., 2011) and wild (Turchetto et al., 2018) *Passiflora*. ILS is common in rapid radiations and can cause genealogical discordance as seen in Malpighiales (Cai et al., 2020) and cichlid fishes (Takahashi et al., 2001). Another explanation is hybridization, as the distribution of these species also overlaps with members of clade SA6. Hybridization in *Passiflora* within and outside section *Decaloba* has been proved several times under cultivation (Fischer, 2004; Yockteng et al., 2011; Braglia et al., 2014). Future research is needed to evaluate whether these processes have occurred 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 recent 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 one of the largest and most well-resolved phylogenies of Neotropical vines to date. The phylogeny has uncovered new relationships within *Passiflora* section *Decaloba* that were not known previously, confirmed relationships that were previously proposed, and resolved many important questions that arose through previous morphological and taxonomic studies. However, we also identified several groups (clades SA1-SA4, SA6) 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 to 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.

Funding

Funding was provided by the Whitney R. Harris World Ecology Center, the Missouri Botanical Garden, and the International Association for Plant Taxonomy. Declarations of interest: none.

CRedit authorship contribution statement

Serena Acha: Conceptualization, Methodology, Writing - review & editing, Formal analysis, Investigation, Visualization, Funding acquisition. **Alexander Linan:** Writing - review & editing, Formal analysis, Investigation, Visualization. **John MacDougal:** Conceptualization, Methodology, Writing - review & editing, Data curation. **Christine Edwards:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

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

Acknowledgments

Thanks to Peter Jørgensen, Nathan Muchhala, Robert Ricklefs and

Justin Bagley for comments on previous versions of the manuscript. Most analyses were conducted using the Saint Louis University and University of Missouri high performance computing clusters. We also thank all the institutions (MO, F, US, NY, DUKE, TEX and LPB) and

people that provided us with herbarium specimens, permits and samples, especially Shawn Krosnick and John Vanderplank. Finally, we thank Sara Cogburn and James Solomon for curatorial assistance.

Appendix

List of samples and collection information. (*) marks samples included in the reduced dataset and (¹) indicates nonpublished names. Cult.: from cultivation source.

Accession	Institution	Collection		Country	Locality	Name status	SRA accession
P. aff. auriculata 332*	MO	Costa	439	Brazil	Amazonas	Accepted	SRR13180839
P. aff. mexicana 310*	MO	Boyle	632	Mexico	Oaxaca	Accepted	SRR13180838
P. aff. mexicana 311	MO	Tajia Yocupicio	MOID: 2238530	Mexico	Sinaloa	Accepted	SRR13180637
P. aff. pohlii 460*	MO	Basualdo	6410	Paraguay	Amambay	Accepted	SRR13180762
P. aff. micropetala 510*a & b	MO	Grandez	5801	Peru	Loreto	Needs re-circumscription	SRR13180751
P. aff. tribolophylla 199*	MO	Fonnegra	4960	Colombia	Antioquia	Accepted	SRR13180804
P. aff. trifasciata 105*	MO	Schunke	7642	Peru	San Martín	Accepted	SRR13180782
P. affinis 304*	MO,F,US	Webster	11193	Mexico	Nuevo Leon	Accepted	SRR13180739
P. affinis 363	MO,TEX	Lott	4393	United States	Texas	Accepted	SRR13180728
P. alnifolia 41*	MO	Croat	96520	Ecuador	Pichincha	Needs re-circumscription	SRR13180837
P. alnifolia 424*	MO	Jorgensen	2475	Ecuador	Napo	Needs re-circumscription	SRR13180826
P. alnifolia 820	MO	Dodson	10887	Ecuador	Pichincha	Needs re-circumscription	SRR13180815
P. alnifolia 832	US	Drew	E-265	Ecuador	Imbabura	Needs re-circumscription	SRR13180714
P. amalocarpa 604*	MO	MacDougal	6337	Cult.	Cult.	Accepted	SRR13180703
P. amalocarpa 703	MO	Silveira	1185	Brazil	Acre	Accepted	SRR13180692
P. andersonii 630*	DUKE	Webster	13379	Dominica	NA	Accepted	SRR13180681
P. andreana 049	MO	Jorgensen	2476	Ecuador	Carchi	Needs re-circumscription	SRR13180670
P. andreana 102*	MO	Jorgensen	2478	Ecuador	Carchi	Needs re-circumscription	SRR13180659
P. andreana 256	MO	Jorgensen	2477	Ecuador	Carchi	Needs re-circumscription	SRR13180648
P. anfracta 280	MO	Dodson	6673	Ecuador	Los Ríos	Accepted	SRR13180636
P. anfracta 286*	MO	Dodson	14452	Ecuador	Los Ríos	Accepted	SRR13180625
P. apetala 594	MO	Kay	194	Costa Rica	Heredia	Accepted	SRR13180770
P. apetala 632	F,MO	Rodriguez	1583	Costa Rica	San José	Accepted	SRR13180769
P. apetala 633	MO	Morales	2180	Costa Rica	San José	Accepted	SRR13180768
P. apetala 887	MO	Grayum	8085	Costa Rica	Cartago	Accepted	SRR13180767
P. apetala 914*	MO	Fernandez	1472	Costa Rica	Heredia	Accepted	SRR13180766
P. bicornis 141	MO	Coronado	4866	Nicaragua	León	Accepted	SRR13180765
P. bicornis 359*	MO	Gonzalez	385	El Salvador	La Libertad	Accepted	SRR13180764
P. bicornis 695	NY	Thorne	7216	United States	Hawaii	Accepted	SRR13180763
P. bicornis 696	NY	Albert de Escobar	3482	Colombia	Magdalena	Accepted	SRR13180761
P. biflora 052	MO	Avila	3717	Guatemala	Izabal	Needs re-circumscription	SRR13180760
P. biflora 244	MO	Morales	2997	Guatemala	Izabal	Needs re-circumscription	SRR13180759
P. biflora 288	MO	Pascual	999	Mexico	Oaxaca	Needs re-circumscription	SRR13180758
P. biflora 423*	MO	MacDougal	3458GR	Honduras	Atlantida	Needs re-circumscription	SRR13180757
P. biflora 613	MO	Kay	197	Costa Rica	Heredia	Needs re-circumscription	SRR13180756
P. boenderi 597	MO	Kay	196	Costa Rica	Heredia	Accepted	SRR13180755
P. bogotensis 435*	MO	Krosnick	405	Cult.	Cult.	Accepted	SRR13180754
P. bogotensis 439	MO	Krosnick	383	Cult.	Cult.	Accepted	SRR13180753
P. bogotensis 441	MO	Krosnick	503	Cult.	Cult.	Accepted	SRR13180752
P. bucamangensis 641*	NY,US	Killip	17046	Colombia	Santander	Accepted	SRR13180750
P. caduca 885 ^{*1}	MO	Vanderplank	2398/17	Cult.	Cult.	Accepted	SRR13180749
P. calicicola 600; P. calicicola 582*	MO	Kay	131	Jamaica	Clarendon	Accepted	SRR13180747; SRR13180748
P. calicicola 610	MO	Kay	105	Jamaica	Trelawny	Accepted	SRR13180746
P. cana 558 ¹	MO	Gentry	23238	Peru	Amazonas	Accepted	SRR13180745
P. cana 643 ¹	F	Weigend	98/374a	Peru	Amazonas	Accepted	SRR13180744
P. cana 886 ^{*1}	MO	Vanderplank	2449/18	Cult.	Cult.	Accepted	SRR13180807

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Accession	Institution	Collection		Country	Locality	Name status	SRA accession
P. candollei 646*	MO,US	Betancur	2836	Colombia	Amazonas	Accepted	SRR13180806
P. candollei 782	MO	Nunez	14616	Peru	Madre de Dios	Accepted	SRR13180805
P. carnosiseipala 121*	MO	Matezki	342	Ecuador	Zamora-Chinchipe	Accepted	SRR13180803
P. cf. cuneata 074*	MO	Ramos	3588	Colombia	Valle del Cauca	Accepted	SRR13180802
P. cf. cuneata 901*	US	Daniel (Hermano)	147	Colombia	Antioquia	Accepted	SRR13180801
P. cf. cuspidifolia 872*	MO	Krosnick	367	Cult.	Cult.	Accepted	SRR13180800
P. cf. lyra 366	MO	Clark	4920	Ecuador	Esmeraldas	Accepted	SRR13180799
P. cf. telesiphe88*	MO	Campos	6273	Peru	Cajamarca	Accepted	SRR13180798
P. aff. tricuspis 152	MO	Fuentes	4395	Bolivia	La Paz	Accepted	SRR13180793
P. chelidonea 811	MO	Knapp	6204	Ecuador	Napo	Needs re-circumscription	SRR13180796
P. chelidonea 857*	MO	Ulloa	2213	Ecuador	Pichincha	Needs re-circumscription	SRR13180794
P. chelidonea 197	MO	Fonnegra	5631	Colombia	Antioquia	Needs re-circumscription	SRR13180797
P. chelidonea 812	MO	Jorgensen	61638	Ecuador	Pichincha	Needs re-circumscription	SRR13180795
P. chrysosepala 106	MO	Alvarez	1982	Ecuador	Napo	Accepted	SRR13180792
P. chrysosepala 107*	MO	Schwerdtfeger	95022134	Ecuador	Sucumbios	Accepted	SRR13180791
P. colinvauxii 878*	MO	Krosnick	539	Ecuador	Galapagos	Accepted	SRR13180790
P. coronapapillata 421* ¹	MO	Campos	3901	Peru	Cajamarca	Accepted	SRR13180789
P. cubensis 585*	MO	Kay	233	Cuba	Camagui	Accepted	SRR13180788
P. cubensis 586	MO	Kay	231	Cuba	Santiago de Cuba	Accepted	SRR13180787
P. cubensis 601	MO	Kay	232	Cuba	Santiago de Cuba	Accepted	SRR13180786
P. cupraea 584*	MO	Kay	227	Cuba	Las Tunas	Accepted	SRR13180785
P. cuspidifolia 122*	MO	Stein	3686	Colombia	Cundinamarca	Accepted	SRR13180784
P. gilbertiana 780*	MO,US	Hammel	18530	Costa Rica	San José	Accepted	SRR13180783
P. goniosperma 325*	MO	Lott	3785	Mexico	Jalisco	Accepted	SRR13180781
P. helleri 108	MO	Mendoza	1382	Mexico	Puebla	Accepted	SRR13180780
P. helleri 143	MO	Sevilla DJS	1033	Mexico	Veracruz	Accepted	SRR13180779
P. helleri 509*	MO	Ventura	19556	Mexico	Veracruz	Accepted	SRR13180778
P. heptantha 328* ¹	MO	Rojas	3955	Peru	Pasco	Accepted	SRR13180777
P. hexadenia 565* ¹	MO	Vasquez	28889	Peru	Pasco	Accepted	SRR13180776
P. hirtiflora 714*	MO	Perea	2982	Peru	Cajamarca	Accepted	SRR13180743
P. hyacinthiflora 285*	MO	Hernandez	195	Colombia	Santander	Accepted	SRR13180742
P. ichthyura 099*	MO	Nee	36203	Bolivia	Santa Cruz	Accepted	SRR13180741
P. ilamo 407a & b ¹	MO	MacDougal	6201	Guatemala	Solola	Accepted	SRR13180740;
							SRR13180738
P. ilamo 409* ¹	MO	MacDougal	6203	Guatemala	Solola	Accepted	SRR13180737
P. indecora 282*	MO	Lewis	2413	Ecuador	Loja	Accepted	SRR13180736
P. indecora 562	MO	Jorgensen	1136	Ecuador	Loja	Accepted	SRR13180735
P. insolitii 415a*; P. insolitii 415b	MO	MacDougal	6213	Guatemala	Baja Verapaz	Accepted	SRR13180734;
							SRR13180733
P. insolitii 839* ¹	MO	Vanderplank	sn	Mexico	Chiapas	Accepted	SRR13180732
P. jeannettae 469 ¹	MO	Giraldo Canas	593	Colombia	Antioquia	Accepted	SRR13180731
P. jeannettae 720* ¹	MO	MacDougal	4160	Colombia	Antioquia	Accepted	SRR13180730
P. jorullensis var. salvadorensis 660	MO	Sandoval	112	El Salvador	Ahuachapán	Needs re-circumscription	SRR13180727
P. jorullensis var. salvadorensis 661	MO	Fidel Lopez	MOID: 2243361	El Salvador	Ahuachapán	Needs re-circumscription	SRR13180726
P. jorullensis var. salvadorensis 663	MO	Toledo	1	El Salvador	Ahuachapán	Needs re-circumscription	SRR13180725
P. jorullensis var. salvadorensis 891*	MO	Breedlove	27627	Mexico	Chiapas	Needs re-circumscription	SRR13180724
P. jorullensis var. jorullensis 781	MO	Vazquez	1227	Mexico	Jalisco	Needs re-circumscription	SRR13180729
P. kalbreyeri 283	MO	Davidse	21150	Venezuela	Lara	Accepted	SRR13180723
P. kalbreyeri 553	MO	Porter-Utley	415	Cult.	Cult.	Accepted	SRR13180722
P. kalbreyeri 846*	NY,US	Weitzman	112	Venezuela	Aragua	Accepted	SRR13180721
P. ketura 330* ¹	MO,US	Woytkowski	7804	Peru	Amazonas	Accepted	SRR13180720
P. ketura 710 ¹	MO	de Cevalco	MOID: 2877363	Peru	Amazonas	Accepted	SRR13180719
P. lancearia 114*	MO	MacDougal	6276	Panama	Colón	Accepted	SRR13180718
P. lancearia 115	MO	MacDougal	6268	Panama	Coclé	Accepted	SRR13180836
P. lancearia 251	MO	MacDougal	6263	Panama	Coclé	Accepted	SRR13180835
P. lancearia 399	MO	Morales	4078	Costa Rica	Heredia	Accepted	SRR13180834
P. leptoclada 442*	MO	Krosnick	491	Cult.	Cult.	Accepted	SRR13180833
P. leptoclada 665*	F,US	Williams	5252	Peru	Loreto	Accepted	SRR13180832
P. leptoclada 666	US	Williams	2737	Peru	Loreto	Accepted	SRR13180831
P. lutea 319*	MO	Thomas	150563	United States	Mississippi	Accepted	SRR13180830
P. lutea 320	MO	Stone	1532	United States	North Carolina	Accepted	SRR13180829
P. lutea 322	MO	Christy	MOID: 34151736	United States	Arkansas	Accepted	SRR13180828
P. lyra 119*	MO	Miller	5884	Colombia:	Antioquia	Accepted	SRR13180827

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Accession	Institution	Collection		Country	Locality	Name status	SRA accession
<i>P. magdalenae</i> 669*	NY,US	Uribe	2568	Colombia	Tolima	Accepted	SRR13180825
<i>P. micrantha</i> 683*	NY	Fosberg	22018	Colombia	Cundinamarca	Accepted	SRR13180824
<i>P. micropetala</i> 385	MO	Bass	377	Ecuador	Napo	Accepted	SRR13180823
<i>P. micropetala</i> 512*	MO	Jaramillo	1335	Peru	Amazonas	Accepted	SRR13180822
<i>P. micropetala</i> 721	MO	MacDougal	4982	Ecuador	Napo	Accepted	SRR13180821
<i>P. misera</i> 070	MO	MacDougal	6281	Panama	Canal Area	Accepted	SRR13180820
<i>P. misera</i> 135	MO	Zardini	60751	Paraguay	Canindeyú	Accepted	SRR13180819
<i>P. misera</i> 257	MO	Beck	3292A	Bolivia	Beni	Accepted	SRR13180818
<i>P. misera</i> 501	MO	Zardini	31610	Paraguay	Central	Accepted	SRR13180817
<i>P. misera</i> 503	MO	Zardini	34670	Paraguay	Central	Accepted	SRR13180816
<i>P. misera</i> 504*	MO	Zardini	36019	Paraguay	Central	Accepted	SRR13180814
<i>P. mollis</i> 455*	MO	Gentry	48035	Colombia	Valle del Cauca	Needs re-circumscription	SRR13180813
<i>P. mollis</i> 788*	TEX	Escobar	420	Colombia	Caldas	Needs re-circumscription	SRR13180812
<i>P. murucuja</i> 592	MO	Kay	217	Dominican Republic	Distrito Nacional	Accepted	SRR13180811
<i>P. murucuja</i> 617*	MO	Kay	211	Dominican Republic	Baoruco	Accepted	SRR13180810
<i>P. murucuja</i> 618	MO	Kay	206	Dominican Republic	Independencia	Accepted	SRR13180809
<i>P. murucuja</i> 619	MO	Kay	212	Dominican Republic	Independencia	Accepted	SRR13180808
<i>P. nana</i> 716*	MO	Campos	2921	Peru	Cajamarca	Accepted	SRR13180717
<i>P. nubicola</i> 674*	DUKE	MacDougal	1244	Costa Rica	Cartago	Accepted	SRR13180716
<i>P. nubicola</i> 676	TEX	Knapp	857	Costa Rica	Alajuela	Accepted	SRR13180715
<i>P. oblongata</i> 587	MO	Kay	107	Jamaica	Trelawny	Accepted	SRR13180713
<i>P. oblongata</i> 611*	MO	Kay	183	Jamaica	Trelawny	Accepted	SRR13180712
<i>P. occidentalis</i> 261 ¹	MO	MacDougal	6303	Panama	Coclé	Accepted	SRR13180711
<i>P. occidentalis</i> 337 ¹	MO,US	MacDougal	6302	Panama	Coclé	Accepted	SRR13180710
<i>P. occidentalis</i> 470* ¹	MO	Taylor	12192	Colombia	Valle del Cauca	Accepted	SRR13180709
<i>P. occidentalis</i> 472a* & b ¹	MO	Onore	MOID: 100987836	Ecuador	Esmeraldas	Accepted	SRR13180708; SRR13180707
<i>P. orbiculata</i> 616*	MO	Kay	214	Dominican Republic	Independencia	Accepted	SRR13180706
<i>P. panamensis</i> 698*	DUKE	MacDougal	444	Panama	Darién	Accepted	SRR13180705
<i>P. panamensis</i> 787	MO	Zarucchi	5107	Colombia	Antioquia	Accepted	SRR13180704
<i>P. panamensis</i> 912	MO,US	Foster	2837	Panama	Darién	Accepted	SRR13180702
<i>P. pardifolia</i> 443*; <i>P. pardifolia</i> 126	MO	Vanderplank	MOID: 3330227	NA	NA	Accepted	SRR13180700; SRR13180701
<i>P. pascoensis</i> 189*	MO	Rodriguez	95	Peru	Pasco	Accepted	SRR13180699
<i>P. pascoensis</i> 190	MO	Rodriguez	42	Peru	Pasco	Accepted	SRR13180698
<i>P. penduliflora</i> 580*	MO	Kay	102	Jamaica	Trelawny	Accepted	SRR13180697
<i>P. penduliflora</i> 589	MO	Kay	230	Cuba	Santiago de Cuba	Accepted	SRR13180696
<i>P. penduliflora</i> 595	MO	Kay	104	Jamaica	Trelawny	Accepted	SRR13180695
<i>P. penduliflora</i> 599	MO	Kay	174	Jamaica	Claredon	Accepted	SRR13180694
<i>P. pilosissima</i> 044*	MO	Hernandez	291	Colombia	Antioquia	Accepted	SRR13180693
<i>P. poeppigii</i> 266*	MO	Boza	2139	Peru	Loreto	Accepted	SRR13180691
<i>P. punctata</i> 056*	MO	Jorgensen	2458	Ecuador	Azuay	Accepted	SRR13180690
<i>P. punctata</i> 057	MO	Jorgensen	2457	Ecuador	El Oro	Accepted	SRR13180689
<i>P. punctata</i> 192*	MO	Weigend	98/184	Peru	Piura	Accepted	SRR13180688
<i>P. quadriflora</i> 369*	MO	Galiano	6424	Peru	Cusco	Accepted	SRR13180687
<i>P. rotundifolia</i> 700*	US	Stehle	1513	Guadeloupe	NA	Accepted	SRR13180686
<i>P. rotundifolia</i> 701*	NY	Stehle	123	Caribbean,	Guadeloupe	Accepted	SRR13180685
<i>P. rotundifolia</i> 908	US	Stehle	2585	Caribbean,	Guadeloupe	Accepted	SRR13180684
<i>P. sandrae</i> 127*	MO	MacDougal	6290	Panama	Coclé	Accepted	SRR13180683
<i>P. saxicola</i> 603*	MO	MacDougal	6336	Brazil	Cult.	Accepted	SRR13180682
<i>P. sexflora</i> 323*	MO	Hansen	9185	Puerto Rico	Patillas	Accepted	SRR13180680
<i>P. sexflora</i> 324	MO, NY, US	Axelrod	6137	Puerto Rico	Barranquitas	Accepted	SRR13180679
<i>P. smilacifolia</i> 444*	MO	Schwerdtfeger	MOID: 2879562	Ecuador	Napo	Accepted	SRR13180678
<i>P. smilacifolia</i> 464	MO	Krosnick	500	Ecuador	Cult.	Accepted	SRR13180677
<i>P. sp.</i> 884*	MO	Vanderplank	sn	Cult.	Cult.	Accepted	SRR13180675
<i>P. sp. nov.</i> 270	MO	Raurau	91	Peru	Cusco	Accepted	SRR13180674
<i>P. sp. nov.</i> 388*	MO	Valenzuela	13876	Peru	Pasco	Accepted	SRR13180673
<i>P. sp. nov.</i> 404*	MO	Ferreira	7783	Peru	San Martin	Accepted	SRR13180672
<i>P. sp.</i> 612*	MO	Kay	108	Jamaica	Trelawny	Accepted	SRR13180676
<i>P. standleyi</i> 272	MO	Breedlove	37173	Mexico	Chiapas	Accepted	SRR13180671
<i>P. standleyi</i> 273	DUKE	MacDougal	855	Costa Rica	San Jose	Accepted	SRR13180669
<i>P. standleyi</i> 395	MO	Castillo	ISF00812	El Salvador	Ahuachapán	Accepted	SRR13180668
<i>P. standleyi</i> 426	MO	Renders	410	El Salvador	La Libertad	Accepted	SRR13180667
<i>P. standleyi</i> 431*	MO	Davidse	35029	Honduras	El Paraiso	Accepted	SRR13180666
<i>P. standleyi</i> 432	MO	Davidse	29971	Mexico	Chiapas	Accepted	SRR13180665
<i>P. stenosepala</i> 631*	US	Morton	6140	St. Vincent	NA	Accepted	SRR13180664
<i>P. subfertilis</i> 449*; <i>P. subfertilis</i> 263	MO,DUKE	MacDougal	597GR	Guatemala	Quetzaltenango	Accepted	SRR13180662; SRR13180663

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Accession	Institution	Collection		Country	Locality	Name status	SRA accession
P. talamancensis 412*	MO	Kernan	120	Costa Rica	Puntaremas	Accepted	SRR13180661
P. tatei 068	MO	Boza	2113	Bolivia	La Paz	Accepted	SRR13180660
P. tatei 164*	MO	Fuentes	8025	Bolivia	La Paz	Accepted	SRR13180658
P. tatei 166*	MO	Delanoy	398	Bolivia	La Paz	Accepted	SRR13180657
P. telesiphe 173a & b	MO	Grant	958976	Ecuador	Zamora-Chinchipe	Accepted	SRR13180656; SRR13180655
P. telesiphe 718*	MO	Knapp	9124	Ecuador	Zamora-Chinchipe	Accepted	SRR13180654
P. transversalis 491	MO	Pedersen	15696	Brazil	Rio Grande do Sul	Accepted	SRR13180653
P. tribolophylla 708*	NY	Luteyn	12480	Colombia	Antioquia	Needs re-circumscription	SRR13180652
P. tribolophylla 709*	NY	Lehmann	BT859	Colombia	NA	Needs re-circumscription	SRR13180651
P. tribolophylla 866	TEX	Albert de Escobar	1022	Colombia	Valle del Cauca	Needs re-circumscription	SRR13180650
P. tricuspis 150	MO	Delanoy	154	Bolivia	La Paz	Accepted	SRR13180649
P. tricuspis 506	MO	Boza	2104	Bolivia	La Paz	Accepted	SRR13180647
P. tricuspis 515	MO	Zardini	46427	Paraguay	Amambay	Accepted	SRR13180646
P. tricuspis 516	MO	Zardini	46426	Paraguay	Amambay	Accepted	SRR13180645
P. tricuspis 625a* & b	NY	Nee	37485	Bolivia	Santa Cruz	Accepted	SRR13180644; SRR13180643
P. trifasciata 536	MO	Krosnick	506	Cult.	Cult.	Accepted	SRR13180642
P. trifasciata 537*	MO	Krosnick	460	Cult.	Cult.	Accepted	SRR13180641
P. trinervia 078*	MO	Ramos	3000	Colombia	Valle del Cauca	Accepted	SRR13180640
P. tuberosa 437	MO	Krosnick	484	Cult.	Cult.	Accepted	SRR13180639
P. tuberosa 445	MO	Kay	223	Cult.	Cult.	Accepted	SRR13180638
P. tuberosa 609*	MO	Kay	223	Trinidad	Cult.	Accepted	SRR13180635
P. tulae 581	MO	Kay	225	Puerto Rico	Maricao	Accepted	SRR13180634
P. tulae 583*	MO	Kay	224	Puerto Rico	Maricao	Accepted	SRR13180633
P. tulae 590	MO	MacDougal	6030	Cult.	Cult.	Accepted	SRR13180632
P. tulae 614	MO	Kay	202	Puerto Rico	Patillas	Accepted	SRR13180631
P. urnifolia 067*	MO	Delanoy	190	Bolivia	La Paz	Accepted	SRR13180630
P. urnifolia 783	LPB	Beck	14905	Bolivia	La Paz	Accepted	SRR13180628
P. urnifolia? 405	MO	Villarroel	1494	Bolivia	Santa Cruz	Accepted	SRR13180629
P. vespertilio 128*	MO	Valenzuela	2488	Peru	Madre de Dios	Accepted	SRR13180627
P. vespertilio 598; P. vespertilio 549	MO	MacDougal	6022	French Guiana	NA	Accepted	SRR13180624; SRR13180626
P. viridescens 039	MO	Ulloa	2522	Ecuador	Azuay	Accepted	SRR13180623
P. viridescens 040*	MO	Ulloa	1887	Ecuador	Azuay	Accepted	SRR13180622
P. viridescens 125	MO	Schwerdtfeger	96090602	Ecuador	Loja	Accepted	SRR13180775
P. yucatanensis 478*	MO	Cabrera	6470	Mexico	Quintana Roo	Accepted	SRR13180774
P. yucatanensis 92	MO	Aniuk	36	Mexico	Quintana Roo	Accepted	SRR13180771
P. yucatanensis 591*; P. yucatanensis 722	MO	MacDougal	4680	Mexico	Quintana Roo	Accepted	SRR13180773; SRR13180772

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2021.107260>.

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