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New Insights into the Evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): Phylogenetic Relationships and Morphological Synapomorphies

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Abstract—Phylogenetic relationships of *Passiflora* subgenus *Decaloba* were examined using 148 taxa and four molecular markers: nuclear nrITS, ncpGS, cp *trnL-F,* and *ndhF*. Relationships of subgenus *Decaloba* to the other four *Passiflora* subgenera (*Astrophea*, *Deidamioides*, *Tetrapathea*, and *Passiflora*) are investigated, as are relationships among the eight supersections within subgenus *Decaloba*. Results indicate that subgenus *Deidamioides* is not monophyletic. Subgenus *Astrophea* + subgenus *Deidamioides* (section *Tryphostemmatoides*) together form the most basally branching lineage in the genus, followed by a clade comprised of subgenus *Passiflora* + subgenus *Deidamioides* (sections *Tetrastylis, Polyanthea,* and *Deidamioides*). *Passiflora obovata* (subgenus *Deidamioides* section *Mayapathanthus*) is resolved as part of subgenus *Decaloba*. The Old World subgenus *Tetrapathea* is supported as sister to subgenus *Decaloba*. Subgenus *Decaloba* is monophyletic and contains seven major lineages that generally correspond to currently recognized supersections. Within subgenus *Decaloba,* supersection *Pterosperma* is most basally branching, followed by supersection *Hahniopathanthus* + *P. obovata*. The New World species *Passiflora multiflora*, the type of supersection *Multiflora*, is resolved as sister to a monophyletic Old World supersection *Disemma*. The remainder of the former supersection *Multiflora* is paraphyletic with respect to supersection *Auriculata*. Supersections *Cieca*, *Bryonioides*, and *Decaloba* are monophyletic. Within supersection *Decaloba*, two main clades are resolved: 1) section *Xerogona* + section *Decaloba* pro parte and 2) the remainder of section *Decaloba*. The molecular phylogeny supports a New World origin for *Passiflora*, with two independent radiations to the Old World. Morphological synapomorphies are discussed for the major clades, documenting a pattern of remarkable evolutionary lability in several notable characters.

Keywords—*Astrophea, Deidamioides, Tetrapathea*, molecular phylogeny, New World, Old World.

Passiflora L. is a large and diverse genus of more than 560 species of vines, lianas, trees, and shrubs. The genus is primarily distributed throughout Mexico and Central and South America, but 24 species are endemic to Southeast Asia and the Pacific. In the most recent infrageneric classification of *Passiflora,* Feuillet and MacDougal (2003) recognized four subgenera: *Passiflora, Deidamioides* (Harms) Killip, *Astrophea* (DC.) Mast., and *Decaloba* (DC.) Rchb. Krosnick et al. (2009) recognized subgenus *Tetrapathea* (DC.) P. S. Green, raising the number of subgenera to five. Subgenus *Passiflora* includes ca. 250 species and is the largest and best-known of the subgenera, partly because some species have economic importance (Ulmer and MacDougal 2004). Subgenus *Passiflora* is generally characterized by having large flowers with multiple series of coronal filaments, and is most diverse in South America. Subgenus *Deidamioides* is a morphologically disparate group of 14 species found in Central and South America. Subgenus *Astrophea* consists of ca. 60 species of lianas and small to medium-sized trees that are most diverse in the lowlands of northern South America. Subgenus *Tetrapathea* is the smallest, with three species of dioecious lianas found in northeast Australia, Papua New Guinea, and New Zealand. Lastly, with ca. 230 recognized and more than a dozen as yet undescribed species, subgenus *Decaloba*, with its characteristically small flowers, rivals subgenus *Passiflora* in species diversity. The center of diversity for subgenus *Decaloba* is Mexico (59 species), followed by Colombia (56 species), and Guatemala (40 species). With species in the United States, Mexico, Central and South America, Asia, Australia, and the Pacific, subgenus *Decaloba* also has the broadest geographical distribution of any of the subgenera: it is the only subgenus to have both New World (NW) and Old World (OW) species. Although *Decaloba* is the second largest subgenus in *Passiflora,* comparatively little is known about the evolutionary history of these species.

Subgenus *Decaloba* was originally described by de Candolle (1822) as a section within *Passiflora*. He recognized this group as having five sepals and five petals, reduced or absent floral bracts, and single-flowered peduncles. Reichenbach (1828) later elevated the section to subgeneric rank. Masters (1871) established subgenus *Plectostemma* Mast. containing almost the same taxa (Masters 1872), not realizing subgenus *Decaloba* already existed. The most comprehensive treatment of NW Passifloraceae, including *Decaloba*, was completed by Killip (1938). He maintained subgenus *Plectostemma* and recognized 21 additional subgenera; of these, five contained species that are now recognized as part of subgenus *Decaloba*. The OW species were not revised until De Wilde (1972) formally treated them as part of subgenus *Decaloba.* One additional subgenus, *Porphyropathanthus* L. K. Escobar, was established in 1989, containing a single Colombian species (Escobar 1989). Feuillet and MacDougal (1999, 2003) and MacDougal and Feuillet (2004) recognized eight supersections within subgenus *Decaloba*: *Pterosperma* (L. E. Gilbert & J. M. MacDougal) J. M. MacDougal & Feuillet (four species), *Hahniopathanthus* (Harms) J. M. MacDougal & Feuillet (six species), *Auriculata* J. M. MacDougal & Feuillet (eight species), *Cieca* (Medik.) J. M. MacDougal & Feuillet (19 species), *Disemma* (Labill.) J. M. MacDougal & Feuillet (21 species), *Bryonioides* (Harms) J. M. MacDougal & Feuillet (22 species), *Multiflora* (Small) J. M. MacDougal & Feuillet (22 species), and *Decaloba* (DC.) J. M. MacDougal & Feuillet (130 species). The subgenus

has a suite of unique characteristics not found elsewhere in *Passiflora*: relatively small flowers (generally < 4 cm in diameter), a plicate membranous (vs. smooth or filamentous) operculum, and two to three series of coronal filaments (vs. usually four or more). Several groups may be distinguished within the subgenus based on additional unique character combinations such as variegation of juvenile leaves, gravitational orientation of shoot tips, trichome morphology, seed coat ornamentation, absence of petals, and position or absence of laminar or petiolar nectaries.

Phylogenetic knowledge of subgenus *Decaloba* at all taxonomic levels remains limited. Previous phylogenetic studies of the genus have included at most 39 of the 230 species in subgenus *Decaloba* (Muschner et al. 2003; Yockteng and Nadot 2004a; Krosnick and Freudenstein 2005; Hansen et al. 2006). Since Killip (1938), only four lineages within subgenus *Decaloba* have been studied in detail thus far: supersection *Bryonioides* (MacDougal 1994), supersection *Cieca* (Porter-Utley 2003, 2007, in press), supersection *Disemma* (Krosnick and Freudenstein 2005; Krosnick 2006), and supersection *Decaloba* section *Xerogona* (Raf.) Killip (Boza et al. in press). Relationships within and between the remaining supersections, including the largest, *Decaloba*, are essentially unknown. Phylogenetic analyses of DNA sequence data for *Passiflora* indicate that aspects of the classification of Feuillet and MacDougal (2003) may be problematic with regard to subgenus *Decaloba*. For example, Krosnick and Freudenstein (2005) showed that supersection *Multiflora* is paraphyletic with respect to supersection *Auriculata.*

Another issue that has not been fully addressed is the relationship of subgenus *Decaloba* to the remainder of the genus. This is complicated by the fact that infrageneric relationships in *Passiflora* are still poorly understood. The monotypic genera *Hollrungia* K. Schum. and *Tetrapathea* (DC.) Rchb. were shown to be part of *Passiflora* (Yockteng and Nadot 2004a; Hearn 2006; Krosnick et al. 2009). *Tetrapathea*, however, was resolved within subgenus *Decaloba* (Yockteng and Nadot 2004a), while in Hearn (2006), it was resolved as sister to *Decaloba*. In the results of Yockteng and Nadot (2004a), subgenus *Deidamioides* appears to be paraphyletic as currently defined, with *P. cirrhiflora* Juss. resolved as sister to the rest of *Passiflora* and *P. tryphostemmatoides* Harms resolved as sister to subgenus *Astrophea*. Hansen et al. (2006) resolved *P. deidamioides* Harms, *P. ovalis* Vell. ex M. Roem., and *P. cirrhiflora* (subgenus *Deidamioides*) as a monophyletic clade sister to subgenus *Decaloba.* Increased sampling of species placed in other subgenera, particularly *Astrophea, Tetrapathea,* and *Deidamioides*, is needed to test the monophyly of subgenus *Decaloba* and to identify appropriate outgroups for comparative studies.

The present study seeks to address several important goals in order to increase understanding of the evolutionary history of subgenus *Decaloba*. First, the monophyly of subgenus *Decaloba* is tested to determine the boundaries of the subgenus relative to the four other subgenera. Second, relationships among the subgenera are clarified, resulting in the identification of the sister lineage to subgenus *Decaloba*. Third, Feuillet and MacDougal's (2003) classification of subgenus *Decaloba* (supersections and sections) is tested with a dense taxon sample for the subgenus and recommendations are made for taxonomic revision of problematic lineages. Lastly, putative morphological synapomorphies are identified for the major lineages supported in the molecular analysis.

Materials and Methods

***Taxon Sampling and Outgroup Selection*—**Recent analyses strongly support a monophyletic *Passiflora* (Yockteng and Nadot 2004a; Krosnick and Freudenstein 2005; Hansen et al. 2006). Six taxa were chosen outside of *Passiflora*, including three Passifloraceae (*Adenia heterophylla* (Blume) Koord., *Paropsia madagascariensis* (Mast.) H. Perrier, and *Basananthe triloba* (Bolus ex Schinz) W. J. de Wilde), two Malesherbiaceae (*Malesherbia lanceolata* Ricardi, *M. weberbaueri* Gilg), and one Turneraceae (*Turnera ulmifolia* L.). Within *Passiflora,* subgenera *Passiflora* and *Astrophea* were sampled to ensure that all major lineages within *Passiflora* were represented. Six species (of 250 species) from subgenus *Passiflora* were included, as were seven (of 60) from *Astrophea.* Since the primary focus of this study was to address relationships within subgenus *Decaloba* and its position relative to the questionably placed subgenera *Deidamioides* and *Tetrapathea,* sampling was most extensive in these groups. Subgenus *Decaloba* was represented by all eight supersections, including *Auriculata* (four of eight species), *Bryonioides* (11 of 22), *Cieca* (7 of 19), *Decaloba* (64 of ca. 130), *Disemma* (13 of 21), *Hahniopathanthus* (three of five), *Multiflora* (10 of 22), and *Pterosperma* (three of four). All three species of subgenus *Tetrapathea* were included. Subgenus *Deidamioides* was represented by seven of 14 species, including representatives from all five sections within the subgenus. A total of 148 taxa were sequenced across four loci. Sequences were generated via direct sequencing of DNA from leaf material (see DNA Extraction and Purification). In cases where herbarium samples or older DNA isolations did not amplify for all four loci, alternative accessions of the same taxon were used to ensure that sequence data for a given taxon were as complete as possible for all loci sampled. If alternative accessions were not available, those taxa were included with missing data in the multi-locus analyses but no taxa were included in the analyses unless sequences from at least three of the four loci were available. A total of 19 sequences were obtained from GenBank. Appendix 1 includes herbarium specimen voucher information and GenBank accession numbers for all taxa used in this analysis.

***DNA Extraction and Purification*—**Total genomic DNA was isolated from fresh leaf material, tissue preserved in silica gel, or herbarium specimens (sampled with permission of the lending institution). Total genomic DNA was extracted using the CTAB method (Doyle and Doyle 1987) or DNeasy Plant Mini kits (Qiagen Inc., Valencia, California). DNA from herbarium specimen material was isolated using the CTAB protocol with the following modifications: dry leaf tissue was homogenized using a Mini-Beadbeater-8 (BioSpec Products Inc., Bartlesville, Oklahoma) in 1.5 ml microcentrifuge tubes filled to 1/3 volume with 2.3 mm diameter silicon beads or in standard mortars and pestles; following a 24:1 chloroform-isoamyl alcohol precipitation, DNA was precipitated in 0.04 volume of 3 M sodium acetate and 0.65 volume of 100% isopropanol for 3–5 wk at –20°C. When necessary, DNA samples were further purified using the Elu-Quik DNA purification kit (Whatman Inc., Piscataway, New Jersey), the QIAquick PCR purification kit (QIAGEN Inc.), or by precipitating the DNA 2–3 times with 10 mM NH_4OAc in 76% EtOH.

***DNA Amplification and Sequencing*—**The nuclear ribosomal internal transcribed spacer region (nrITS) including ITS1, the 5.8S gene, and ITS2, was directly amplified using primers 5 and 4 of White et al. (1990). In cases where direct amplification was not readily achieved, the primers 17SE and 26SE (Sun et al. 1994) were used in an initial round of PCR, and 0.2–1 μl of PCR product was used as a template in a subsequent reaction using primers 5 and 4. The PCR reaction protocols for nrITS followed Krosnick and Freudenstein (2005). Nuclear expressed glutamine synthetase (ncpGS) was amplified using primers 687 and 994 (Emshwiller and Doyle 1999) in *Adenia* Forssk., *Paropsia* Noronha ex Thouars, *Basananthe* Peyr., *Turnera* L., and *Malesherbia* Ruiz & Pav. samples as well as subgenera *Passiflora, Deidamioides, Astrophea,* and *Tetrapathea.* In subgenus *Decaloba,* these same primers amplify the multi-copy nuclear encoded cytosol-expressed glutamine synthetase (cytGS; Yockteng and Nadot 2004b) instead of ncpGS (Yockteng and Nadot 2004a). In these species, ncpGS was specifically targeted using primers 839F and 1056R designed by Yockteng and Nadot (2004a) for use in subgenus *Decaloba*. The internal primers ncpGS-IntF (5′ CATCAAACTCACCTTTTCTTTCC 3′) and ncpGS-IntR (5′ ACATCACCTCAATTGGTTTTG 3′) were designed for use in nested PCR reactions. The ncpGS amplification reactions contained 40 μl HPLC water, 1 μl each of 10 μm primer, 5 μl 10 × buffer (100 mM Tris-HCl pH 8.8, 35 mM $MgCl_2$, 250 mM KCl), 0.5 μl of 0.20 μm dNTPs, 0.5 μl *Taq* polymerase, and 0.5 μl of 10 μg/μl of bovine serum albumen. The amplification program for ncpGS was a single initial cycle of 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min, followed by a final 5 min extension at 72°C.

The *trnL-F* region of chloroplast DNA was amplified with primers "c" and "f" of Taberlet et al. (1991). Internal primers "d" and "e" (Taberlet et al. 1991) were used as necessary for nested PCR to obtain complete sequences for the region. The PCR amplification conditions followed those outlined in Krosnick and Freudenstein (2005). The *ndhF* region of chloroplast DNA was amplified using primers 5.5F and 10.2R of R. Nyffeler (Davis et al. 2001). For DNAs that would not amplify readily, the primers Pass-*ndhF*-BAK-1F (5′ TGGTTGTATTCACCTATTTTCGC 3′) and Pass-*ndhF*-BAK-2R (5′ ACAGAGTAAATTCTACAAACTCTCTTAT ACCC 3′) were used. The *ndhF* amplification reactions contained 40 µl HPLC water, 1 µl of each of 10 µm primer, 5 µl 10 × buffer (100 mM Tris-HCl pH 8.8, 35 mM $MgCl_2$, 250 mM KCl), 0.5 µl of 0.20 µm dNTPs, 0.5 µl *Taq* polymerase, and 0.5 µl of DMSO. The amplification program for *ndhF* was a single initial cycle of 94°C for 5 min, followed by 25 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min, followed by a final 7 min extension at 72°C.

Amplification products of nrITS, ncpGS, *trnL-F*, and *ndhF* were purified by precipitating with 50 µl of 20% polyethylene glycol-2.5 M NaCl followed by two ethanol precipitations or by using ExoSAP-IT (Affymetrix/USB, Santa Clara, California) or Qiagen PCR purification kits (Qiagen Inc.). Dideoxy cycle sequencing reactions used BigDye Terminator version 3.1 chemistry (Applied Biosystems, Foster City, California) scaled down to quarter reaction volume. Sequencing reactions were analyzed on an Applied Biosystems 3100 automated sequencer at Rancho Santa Ana Botanic Garden (Claremont, California), or using an Applied Biosystems 3730XL automated sequencer at the High-Throughput Sequencing Solutions lab at University of Washington, Department of Genome Sciences (Seattle, Washington). Bidirectional sequence contigs were assembled and edited using Geneious Pro v. 5.0.3 (Drummond et al. 2011) or Sequencher v. 4.1.1 (Gene Codes Corporation 2000).

Dataset Congruence, Phylogenetic Analyses, and Branch Support—All nrITS, ncpGS, *trnL-F*, and *ndhF* sequences were initially aligned using Clustal W (Thompson et al. 1994) using the default alignment parameters. The ncpGS, *trnL-F*, and *ndhF* matrices were adjusted manually using Se-Al (Rambaut 2000). The nrITS alignment used for phylogenetic analysis was as output by Clustal without further adjustment as manual adjustments did not improve the alignment. Both individual and combined datasets used were deposited in TreeBASE (study number 12855) and all sequences were deposited in GenBank (see Appendix 1).

The incongruence length difference (ILD) Test of Farris et al. (1994), as implemented in WinClada and submitted to NONA, was performed among all pairwise combinations of individual single gene matrices as well as between the nuclear and chloroplast datasets. All parsimony uninformative characters were deactivated prior to the ILD analyses. Following the recommendations of Davis et al. (2004), five hundred paired replicate analyses (# of replications) of random character partitions were performed, with each replicate comprising four search initiations (# of mult reps/replication) and up to 20 trees retained (# trees to hold/mult rep) during TBR swapping after each initiation. This was followed by TBR of all shortest trees from each set of four initiations, including those generated during this phase of swapping, with up to 100 trees retained (# trees for hold*).

Unweighted maximum parsimony (MP) analyses were undertaken using Winclada (BETA) ver. 1.00.08 (Nixon 2002). All characters were treated as non-additive, and uninformative characters were deactivated for all analyses. Datasets were analyzed separately (nrITS, ncpGS, *ndhF*, and *trnL-F*) and in combination (chloroplast loci, nuclear loci, and all loci combined). The parsimony ratchet of Nixon (1999), as implemented in Winclada and submitted to NONA, was used as the primary search strategy for both the individual and combined matrices. For single locus analyses, the following parameters were used: 20 sequential ratchets were performed, each with 500 iterations per replication, holding two trees per replication, in which 10% of the characters were re-weighted, using a random constraint level of 10. All trees obtained as a result of the Ratchet runs were retained in the tree buffer within Winclada, and these were then submitted to NONA for additional swapping (max out) with the upper limit on the number of trees retained set to 100,000 trees (max 100,000). For combined locus analyses (chloroplast, nuclear, and all loci combined), trees obtained as a result of the Ratchet runs were retained in the tree buffer within Winclada, and these were then submitted to NONA for additional swapping (max out) with the upper limit on the number of trees retained set to 500,000 trees (max 500,000). All MP trees were saved and summarized through strict consensus. The consistency (CI) and retention (RI) indices for each dataset were calculated including both informative and uninformative characters. Branch support for the MP analyses was assessed using 10,000 jackknife replicates (MP JK) in Winclada, with random character removal set at 37%. Heuristic searches were performed using two starting trees per replicate (mult*2), with two trees held per replicate (hold/2). Only clades with a frequency of 50% or higher were retained in the jackknife consensus tree.

For maximum likelihood (ML) and Bayesian inference (BI) analyses, molecular evolution model parameters were estimated from the individual nrITS, ncpGS, *ndhF*, and *trnL-F* datasets using the Akaike information criterion (AIC) in jModelTest v. 0.1.1 (Posada 2008). All models selected incorporated the gamma distribution for rate heterogeneity. The Q-matrices selected were all variants of TIM, TPM, TVM, or GTR. ML analyses were performed for the combined nrITS, ncpGS, *ndhF*, and *trnL-F* using GARLI v. 2.0 (Zwickl 2006). Model parameters and rate multipliers obtained for each locus from jModelTest were used to configure independent partitions in GARLI for maximum likelihood analyses of the concatenated alignment. Starting topologies for tree searches were generated in GARLI starting from random trees and using 5,000,000 generations per search (five search replicates), with indels treated as missing. Maximum likelihood bootstrap values (ML BS) were estimated from 100 bootstrap replicates in GARLI. Saved trees were summarized as majority rule consensus trees in PAUP* v. 4.0b10 (Swofford 2002).

Bayesian analyses were performed in MrBayes on TG v. 3.1.2 (Huelsenbeck and Ronquist 2001) on the CIPRES cluster (Miller et al. 2010) for the combined molecular dataset with each gene region treated as a separate partition. Each analysis was done using the same models used for the ML analyses and consisted of one run of 10,000,000 generations from a random starting tree using a variable rate prior, and four Markov chains sampled every 1,000 generations. The resulting branch posterior probabilities and consensus topology were summarized using the sumt command in MrBayes, excluding trees from the initial 1,000 generations as burn-in. The combined posterior distribution was summarized using a 50% majority-rule consensus tree of all the post-burn-in trees.

Results

Molecular Dataset Characteristics—Seven datasets were assembled: individual nrITS, ncpGS, *trnL-F*, *ndhF*, combined nuclear loci (nrITS + ncpGS), combined chloroplast loci (*ndhF*, *trnL-F*), and four loci combined (Table 1). Owing to variable success in PCR amplifications across the loci, the individual datasets vary in the number of taxa included. nrITS was obtained for the greatest number of taxa, followed by *ndhF*, ncpGS, and *trnL-F*. Of the individual datasets, nrITS provided the greatest number of parsimony-informative characters (PICs), followed by ncpGS, *ndhF*, and *trnL-F*. The percentage of missing data was greatest for the *trnL-F* dataset, owing to large sequence differences between distantly related outgroup genera with the ingroup, and mononucleotide repeats that made obtaining full-length sequences difficult. Similarly, many data were missing for ncpGS owing to the use of primers 839/1056 (Yockteng and Nadot 2004a) for subgenus *Decaloba*. These primers amplify a shorter portion of the ncpGS gene compared to the primers used for taxa outside subgenus *Decaloba* (i.e. 687/994; Emshwiller and Doyle 1999). The combined four-locus dataset had most taxa included, with 148 accessions in total. The nuclear dataset had the most PICs (due to the inclusion of nrITS), followed by the four-locus dataset, with the fewest PICs observed in the chloroplast dataset.

Phylogenetic Analyses of Individual Datasets—Maximum parsimony analysis of the individual nrITS, ncpGS, *ndhF*, and *trnL-F* datasets revealed variable levels of resolution and support for relationships among the subgenera and within subgenus *Decaloba* (Table 1). The nrITS dataset produced 100,411 equally most parsimonious trees of 3,051 steps (Supplemental Fig. 1A; CI = 0.31, RI = 0.74); this locus provided the most resolution at the level of subgenus and supersection, with jackknife support ≥ 70% for most clades along the backbone of the tree and for most *Decaloba* supersections. The ncpGS dataset produced 100,188 equally

TABLE 1. Characteristics of the four individual (nrITS, ncpGS, *trnL-F* and *ndhF*) and three combined (nuclear, chloroplast, and four-gene combined) datasets. Taxon sampling differed between the four loci (see Appendix 1). Missing data values for combined datasets do not include placeholder taxa that were entirely lacking for that locus. CI and RI were calculated from parsimony analysis of individual and combined datasets.

Locus	Number of taxa	Aligned length	Constant sites	Variable sites (%)	Parsimony informative sites (%)	Missing data	# MP trees recovered	CI	RI	MP tree length
ITS	142	806	237	117 (14%)	452 (56%)	22%	100,441	0.31	0.74	3,051
ncpGS	133	760	269	201 (26%)	290 (38%)	34%	100,188	0.54	0.83	905
ndhF	135	751	347	170 (22%)	234 (31%)	10%	100,201	0.44	0.87	916
trnL-F	129	1,128	526	292 (25%)	310 (27%)	37%	100,264	0.59	0.71	1,362
ITS + ncpGS	148	1,566	506	318 (20%)	742 (47%)	26%	513,095	0.34	0.74	4,166
ndhF + *trnL-F*	147	1,879	873	462 (24%)	544 (29%)	23%	500,040	0.39	0.70	2,285
All loci combined	148	3,445	1,379	780 (22%)	1,286 (37%)	24%	41,024	0.34	0.71	6,797

parsimonious trees of 905 steps (Supplemental Fig. 1B; CI = 0.54, RI = 0.83). The ncpGS strict consensus showed most resolution at the level of supersection and below. Jackknife support was ≥ 70% for several supersections in subgenus *Decaloba.* The ncpGS dataset did not support expected outgroup relationships, as *Adenia* and *Basananthe* were resolved within *Passiflora,* and some members of subgenus *Decaloba* supersection *Decaloba* were placed basally within *Passiflora.* The combined nuclear dataset (nrITS + ncpGS) produced 513,095 equally parsimonious trees of 4,166 steps (Supplemental Fig. 1C; CI = 0.34, RI = 0.74). There was no resolution for outgroup relationships, but several supersections and sections within subgenus *Decaloba* were well resolved and supported as monophyletic with ≥ 70% jackknife support.

The *ndhF* dataset produced 100,201 trees of 916 steps (Supplemental Fig. 2A; CI = 0.44, RI = 0.87); this locus provided greatest resolution at the level of subgenus and supersection, providing good jackknife support (≥ 70%) for relationships among most outgroups (except *Malesherbia* and *Adenia,* which were placed within subgenus *Decaloba*). The *trnL-F* dataset produced 100,264 equally most parsimonious trees of 1,362 steps (Supplemental Fig. 2B; CI = 0.59, RI = 0.71). This region did not provide strong signal for resolution of relationships within subgenus *Decaloba,* but did provide jackknife support of ≥ 70% for relationships among outgroups *Malesherbia*, *Turnera*, and *Paropsia.* In addition, subgenus *Astrophea* and portions of subgenus *Deidamioides* were resolved by these data. The remaining taxa were generally unresolved. The combined chloroplast dataset (*ndhF* and *trnL-F*) produced 500,040 equally most parsimonious trees of 2,285 steps (Supplemental Fig. 2C; CI = 0.39, RI = 0.70). The chloroplast data provided greatest resolution for relationships among the outgroup genera (*Malesherbia, Turnera, Basananthe,* and *Paropsia*), as well as for relationships among subgenera *Passiflora, Deidamioides, Astrophea,* and *Tetrapathea*. Outgroup relationships were resolved as expected with the exception of *Adenia,* which was resolved within subgenus *Decaloba.* Clade support was greatest along the backbone of the tree, although some terminal clades were also supported with jackknife values of 70% or higher. Thus, whereas the nuclear data provide greatest resolution and support for relationships at the level of supersection and section in subgenus *Decaloba*, the chloroplast data provided most signal with respect to the deeper clades (genera and subgenera). Across the four datasets, nrITS provided the greatest resolution, followed by ncpGS, *ndhF*, and *trnL-F*.

Evaluation of Incongruence Between the Datasets—All pairwise comparisons of loci, as well as between the combined chloroplast vs. combined nuclear loci revealed significant incongruence ($p = 0.002$). The ILD test has been shown to be susceptible to several factors that increase the chance of detecting significant differences erroneously (type I error) including differences in taxon sample and size, matrix size, amount of homoplasy within each dataset, and amount of missing data (Dolphin et al. 2000; Barker and Lutzoni 2002; Hipp et al. 2004). To test for impact of differences in taxon sample size, additional analyses were run including only taxa for which there were no missing data in either of the matrices being compared. In these cases, incongruence was still significant at $p = 0.002$, suggesting that the incongruence detected by the ILD is due to weak signal and/or homoplasy within the individual datasets, or genuine incongruence. The strict consensus of MP trees generated from the individual analyses were, with the exception of the nrITS dataset, poorly resolved (Supplemental Figs. 1, 2), suggesting that the individual datasets have limited signal. Comparisons of jackknife support were made among the topologies present in the strict consensus trees produced by individual loci and revealed no strongly supported differences in resolution of the deeper nodes in *Passiflora* (subgenera, supersections). However, a few well-supported conflicts exist among terminal taxa. For example, *Adenia heterophylla* was sister to *P. multiflora* L. in the *ndhF* dataset (MP JK = 99%), *P. helleri* Peyr. was sister to *P. vespertilio* L. (MP JK = 97%) in the ncpGS topology. *Passiflora tenella* Killip was sister to section *Xerogona* (Raf.) Killip (MP JK = 93%) in the nrITS dataset, but sister to *P. sagasteguii* Skrabal & Weigend (MP JK = 83%) in the ncpGS dataset. Further study revealed that these cases of conflicting placement were primarily due to either high levels of sequence divergence which made unambiguous alignment difficult, or shorter sequence lengths that introduced additional missing data into the matrix. However, any taxa that behaved anomalously were prioritized for cloning to rule out additional factors such as heteroplasmy or hybridization. Data from all four loci were analyzed together to permit full interaction of characters and to allow secondary signal within the datasets to be revealed in a robust phylogeny constructed from the combined data.

Combined Molecular Dataset—The combined dataset contained 148 taxa and 3,445 nucleotide characters, of which 1,286 (37%) were parsimony informative (Table 1). The percentage of missing data within the combined dataset was 34%. Parsimony analyses produced 41,024 equally parsimonious trees of 6,797 steps (CI = 0.34, RI = 71). For the ML and BI analyses, models of sequence evolution were: nrITS—TPM2uf + I + G; ncpGS—TIM2 + G; *trnL-F*—TVM + G;

ndhF—GTR + G. Maximum likelihood analyses yielded a single most-likely tree (−lnL score = 46,517). The Bayesian MCMC runs retained 8,000 post burn-in trees. Comparison among the independent runs showed that all had mixed adequately, and stability was achieved by 350,000 generations.

The ML topology was chosen to represent and discuss results from all three analytical approaches for two reasons. First, the MP, ML, and BI topologies are congruent, the only differences being in the amount of resolution (ML being the most resolved, followed by BI, then MP). Second, the ML topology was the most resolved, thus yielding the greatest insight into relationships within subgenus *Decaloba* supersection *Decaloba*, the least well-understood group in the subgenus. A simplified diagram of phylogenetic relationships resolved by ML analysis of the combined molecular dataset is shown as Fig. 1. Clades of particular importance were assigned letters to facilitate their identification across all figures and in the text. The detailed ML phylogeny is divided into two figures with Fig. 2 spanning the outgroup genera through clade M, and Fig. 3 presenting clades N−Y. Support values shown on the ML topology include ML bootstrap (ML BS), MP jackknife (MP JK) and Bayesian posterior probabilities (BPP) with thresholds for presentation of ≥ 50% for ML BS and MP JK, and ≥ 0.50 for BPP. Incongruences between analytical methods are discussed below.

With regard to outgroup genera, the MP, ML, and BI analyses resolved relationships similarly. With *Malesherbia* designated as the root, *Turnera* was sister to the remainder of the taxa in the analysis. In the ML and BI analyses, *Basananthe* and *Paropsia* were weakly resolved as sisters (ML BS = < 50%, BPP = 0.84), whereas in the MP analysis, *Paropsia* (99% MP JK) was resolved below *Basananthe* (54% MP JK) in a basal grade that leads to the rest of the family. In all analyses *Adenia* was strongly supported as sister to *Passiflora* (ML BS = 98%, BPP = 1.0, MP JK = 93%; support values will be presented in this same order subsequently); similarly, the genus *Passiflora* was well supported as monophyletic (95%, 1.0, 99%).

Subgeneric relationships in *Passiflora* were congruently resolved across the MP, ML and BI trees. Subgenera *Astrophea* and *Passiflora* were supported as monophyletic lineages in all three analyses. Subgenus *Deidamioides* was polyphyletic, with species placed in three different locations in the tree. The basally branching clade in *Passiflora* was comprised of subgenus *Astrophea* + subgenus *Deidamioides* section *Tryphostemmatoides* Harms (clade A: 92%, 1.0, 99%). The next branching lineage consisted of subgenus *Passiflora* sister to clade B, which contained three sections of subgenus *Deidamioides*: sections *Polyanthea, Tetrastylis,* and *Deidamioides,* though with somewhat weak support (clade C: 64%, 0.99, 77%). Next, subgenus *Tetrapathea* + subgenus *Decaloba* formed a well-supported clade (clade D: 100%, 1.0, 98%). Subgenus *Tetrapathea* was weakly supported as monophyletic by both ML and BI analyses (clade E: ML BS = <50%; BPP = 0.79). In the MP analysis, *P. kuranda* Krosnick & A. J. Ford and *P. aurantioides* (K. Schum.) Krosnick were well-supported as sisters (MP JK = 97%) while *P. tetrandra* Banks ex DC. was unresolved relative to these other two members of subgenus *Tetrapathea,* forming a polytomy with *P. kuranda* + *P. aurantioides* and subgenus *Decaloba.*

Within subgenus *Decaloba* (clade F), all supersections recognized by Feuillet and MacDougal (2003) were supported as monophyletic with the exception of supersections *Multiflora* and *Auriculata,* which were resolved as paraphyletic with respect to one another in all three analyses. In addition, *Passiflora multiflora* L., the type of supersection *Multiflora,* was resolved as sister to supersection *Disemma* (clade K). As a result, supersection *Multiflora* becomes monotypic excluding the remainder of the species placed in that section by Feuillet and MacDougal (2003). Within *Decaloba,* supersection *Pterosperma* was well-supported as the most basal clade (clade G: 100%, 1.0, 100%). *Passiflora obovata* Killip (subgenus *Deidamioides* section *Mayapathanthus* Killip ex J. M. MacDougal & Feuillet) + the remainder of subgenus *Decaloba* formed a strongly supported clade (clade I: 100%, 1.0, 99%). *Passiflora obovata* was weakly supported as sister to supersection *Hahniopathanthus* in the ML and BI topology (ML BS = 63%, BPP = 0.77), but was unresolved at the base of clade H in the MP tree. Whereas the ML and BI analyses provided resolution for relationships between the remaining supersections, the MP topology was unresolved with regard to the backbone relationships between those same clades.

The next lineage to diverge was the OW supersection *Disemma* + NW *P. multiflora* L. (clade K: 73%, 1.0; 75%). Supersection *Disemma* was strongly supported as monophyletic (99%, 1.0, 92%). After clade K, the next lineage resolved was clade M, which included all sampled members of supersections *Auriculata* and *Multiflora* (exception *P. multiflora*; ML BS = 57%, BPP = 0.97), with *P. holosericea* L. as the basal-most member. *Passiflora holosericea* was not placed with confidence by MP. Supersection *Cieca* diverged next along the *Decaloba* backbone, and was strongly supported as monophyletic (clade O: 100%, 1.0, 100%), followed by supersection *Bryonioides,* also well supported as monophyletic (clade Q: 95%, 1.0, 93%).

Supersection *Decaloba,* the largest in subgenus *Decaloba,* was well supported as monophyletic (clade P: 100%, 1.0, 92%). The BI tree was similar to the ML tree with respect to relationships within supersection *Decaloba,* though with less resolution overall; the MP topology was much less resolved and had several polytomies. The ML tree for supersection *Decaloba* indicated that two main clades, designated as clades S and W, were strongly supported as monophyletic (clade S: 93%, 1.0, 83%; clade W: 100%, 1.0, 99%). Within clade S, the first clade to diverge consisted of *P. lutea* L., *P. filipes* Benth., and *P. pavonis* Mast., and was weakly supported (ML BS = 56%, BPP = 1.0); this clade was unresolved at the base of clade S in the MP analysis. *Passiflora tenella* Killip was weakly supported as sister to the remainder of clade S (ML BS = 54%, BPP = 1.0) but was also unresolved at the base of the clade in the MP analysis. Section *Xerogona* (clade T: 80%, 1.0; 58%) and clade U (71%, 1.0, 99%) were both moderately supported as monophyletic. Clade V, which consists of *P. berteroana* Balb. ex DC. and relatives, was strongly supported as monophyletic (100%, 1.0, 99%). In the ML topology, *P. tricuspis* Mast., *P. urnifolia* Rusby, and *P. misera* Kunth formed a weakly supported basal grade within clade W, but this arrangement was not supported in the BI or MP analyses. The remaining relationships within clade W were not well resolved or strongly supported. The ML and BI analyses provided moderate support for clade X (75%, 1.0, MP unresolved) and Y (62%, 1.0, <50%) as monophyletic lineages.

Discussion

The study presented here represents the largest investigation into evolutionary relationships within *Passiflora* to date,

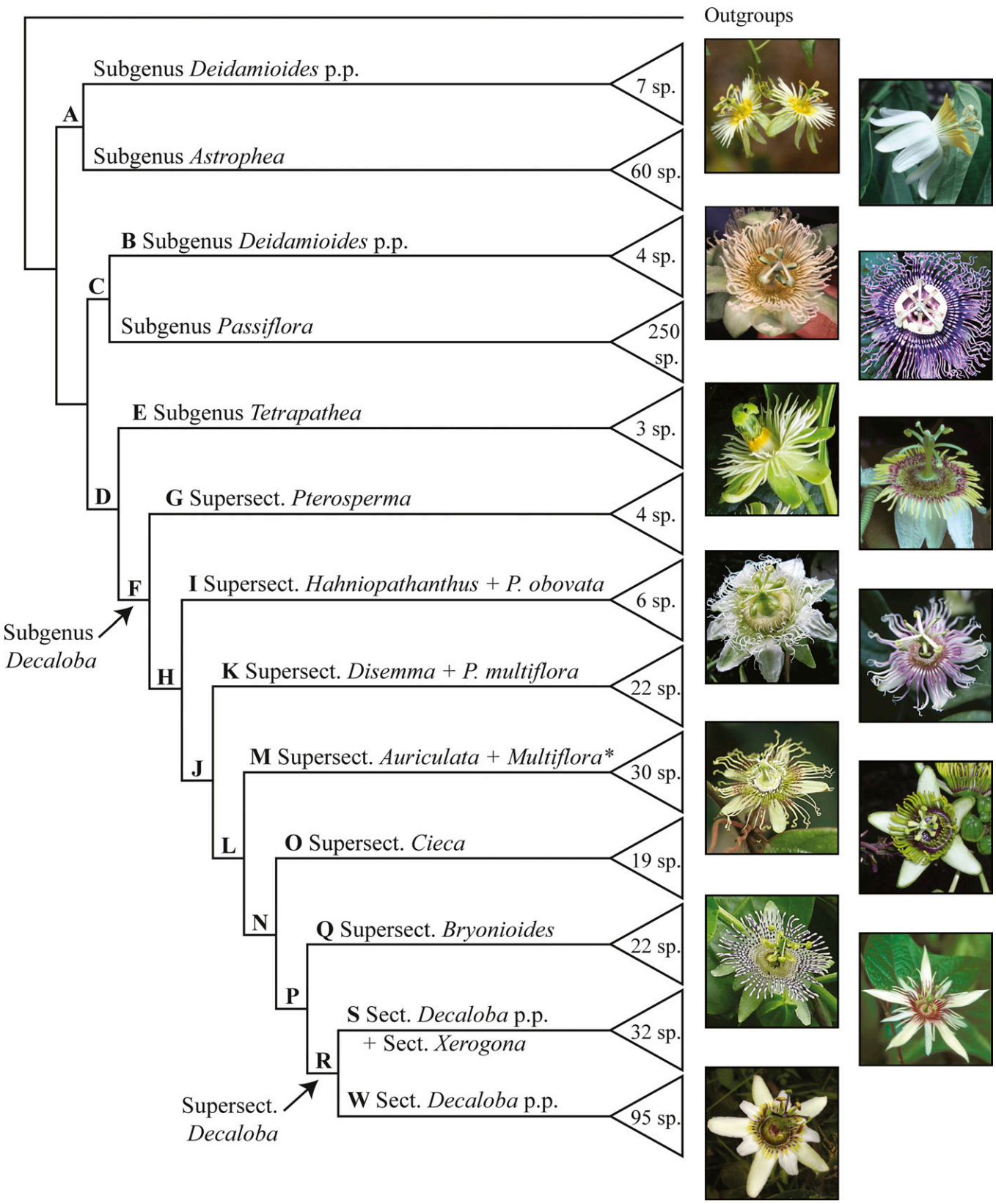

FIG. 1. Overview of relationships in *Passiflora* based on the combined nrITS, ncpGS, *trnL-F*, and *ndhF* dataset. Subgenera and supersections are lettered following descriptions in text; numbers of species per clade indicated within each triangle (p. p. = pro parte; supersect. = supersection); * indicates supersection *Multiflora* minus *P. multiflora*. Image of flower for species typical of each lineage shown to the right of each clade. Photos labeled from left to right, top to bottom, listed with species and photo credit: *P. arbelaezii* (R. Boender); *P. arborea* (L. Escobar); *P. deidamioides* (L. Gilbert); *P. incarnata* (E. Leiter); *P. aurantioides* (A. Ford); *P. microstipula* (J. MacDougal); *P. quetzal* (J. MacDougal); *P. cochinchinensis* (S. Krosnick); *P. rufa* (R. Boender); *P. sexocellata* (A. Hernández); *P. pterocarpa* (A. MacVean); *P. cisnana* (R. Boender); *P. sp.* (A. Hernández).

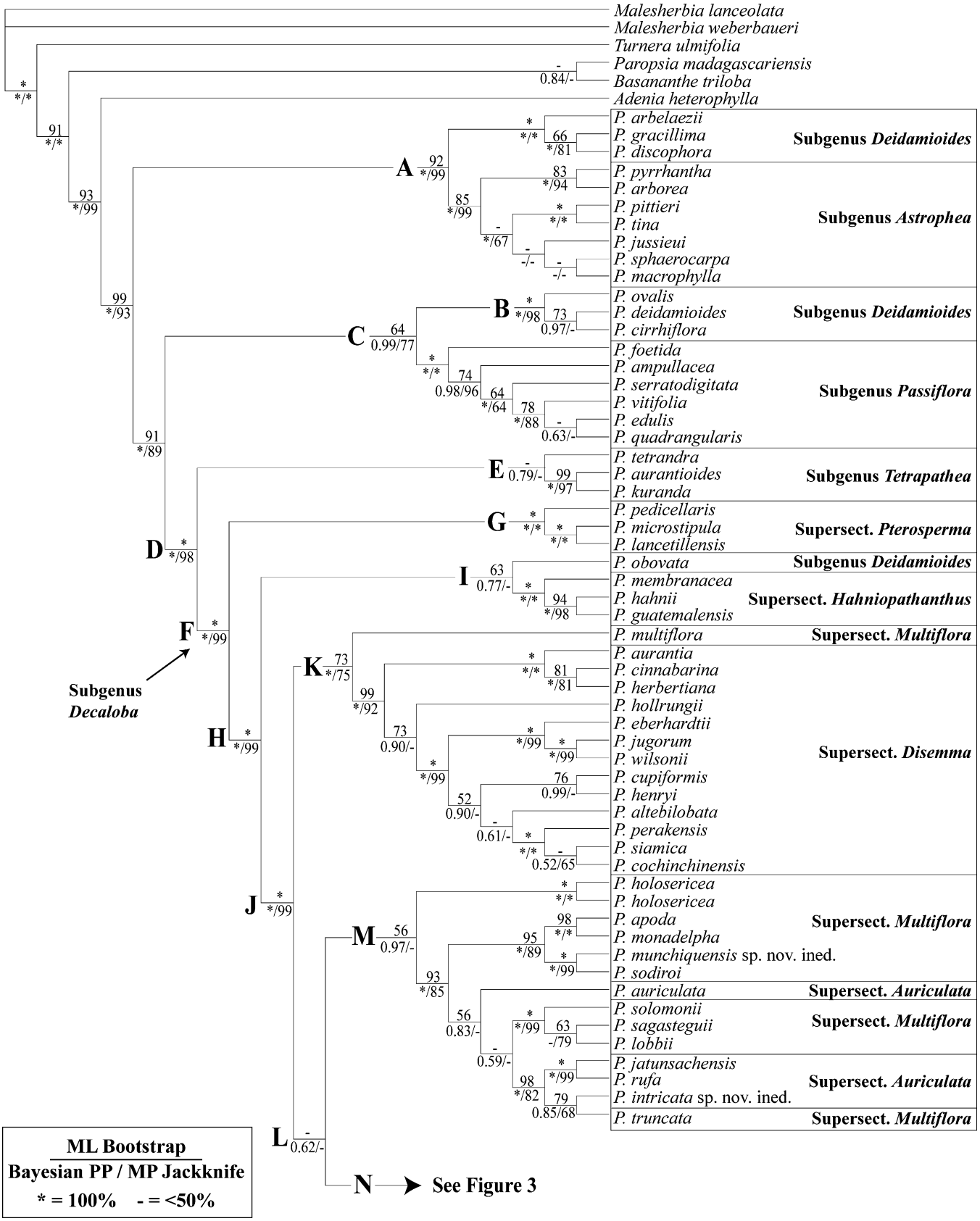

FIG. 2. Phylogenetic relationships in *Passiflora* based on the combined nrITS, ncpGS, *trnL-F*, and *ndhF* dataset: clades A-M. Maximum likelihood topology shown with maximum likelihood bootstrap values (≥ 50%) above branch; under branch, Bayesian inference posterior probabilities (≥ 0.50) to the left, and parsimony jackknife values (≥ 50%) to the right (* = 100% ML BS, BPP, or MP JK support; – = < 50% ML BS, BPP, or MP JK support). Subgenera and supersections indicated to the right of the terminals (supersect. = supersection). See text for full explanation of each lettered clade.

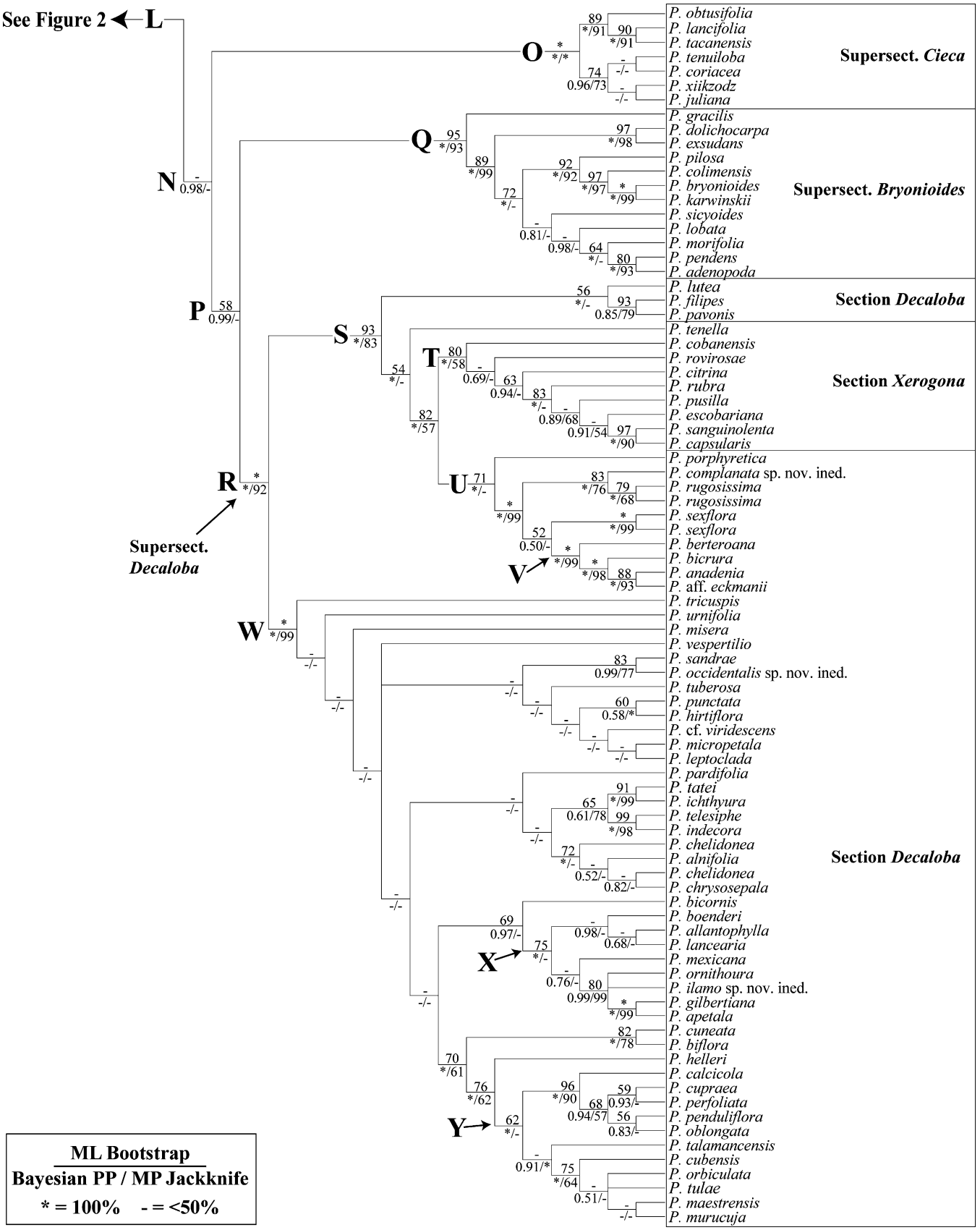

FIG. 3. Phylogenetic relationships in *Passiflora* subgenus *Decaloba* based on the combined nrITS, ncpGS, *trnL-F*, and *ndhF* dataset: clades N–Y. Maximum likelihood topology shown with maximum likelihood bootstrap values (≥ 50%) above branch; under branch, Bayesian inference posterior probabilities (≥ 0.50) to the left, and parsimony jackknife values (≥ 50%) to the right (* = 100% ML BS, BPP, or MP JK support; – = < 50% ML BS, BPP, or MP JK support). Supersections and sections indicated to the right of the terminals (supersect. = supersection). See text for full explanation of each lettered clade.

with 139 species of *Passiflora* included. It is also the first study to focus on resolving the placement of subgenera *Deidamioides* and *Tetrapathea* relative to subgenus *Decaloba,* as well as on relationships within subgenus *Decaloba*. Compared to earlier studies, data for the most commonly used molecular markers in *Passiflora* (nrITS, *trnL-F*, and ncpGS) is expanded by 30–80 species per locus; the *ndhF* dataset is entirely new. This expanded matrix (i.e. both taxa and characters) has allowed for a more comprehensive analysis of relationships in subgenus *Decaloba* and allied subgenera, providing a new framework for examination of morphological character transformations.

Phylogenetic Utility of Molecular Markers—NUCLEAR LOCI—nrITS has been one of the primary sources of data for reconstructing relationships in Passifloraceae (Muschner et al. 2003; Krosnick and Freudenstein 2005; Hearn 2006). However, the problem of intra-individual variation in nrITS sequences has been well documented both in *Passiflora* (Porter-Utley 2003; Hansen 2004; Mader et al. 2010) and in other vascular plant genera (Alvarez and Wendel 2003; Bailey et al. 2003). The problem of non-concerted evolution of nrITS copies in *Passiflora* appears to be most problematic in cases of hybridization and/or polyploidization (Porter-Utley 2003; Hansen 2004). *Passiflora* is characterized by significant differences in genome size across the subgenera. For example, Yotoko et al. (2011) showed that subgenus *Passiflora* has a genome approximately three times larger than the average sized genome in subgenus *Decaloba.* The genus displays substantial variation in chromosome number as well. Subgenus *Passiflora* is generally $n = 9$ (meiotic chromosome count), but may be $n = 10$ or 11 in a few species, whereas subgenus *Astrophea* is $n = 12$ (Hansen et al. 2006). Subgenus *Deidamioides* section *Tryphostemmatoides* is known from one count of $2n = 22$ (mitotic chromosome count; J. MacDougal, unpubl. data), and subgenus *Tetrapathea* is $n = 12$ (Hair and Beuzenberg 1959). Subgenus *Decaloba* is generally $n = 6$, though one count in a basal lineage is $n = 9$ (Snow and MacDougal 1993). Polyploidy has been documented in subgenus *Decaloba* supersection *Cieca*, supersection *Bryonioides*, and supersection *Decaloba* (Snow and MacDougal 1993; de Melo et al. 2001; Hansen et al. 2006), and also in subgenus *Passiflora* (Hansen 2004).

For a large taxon sample such as that included here, it is not practical to clone nrITS amplicons for each taxon to ensure that all unique copies have been identified. To account for the possibility of non-concerted evolution among nrITS copies owing to polyploidy in *Passiflora*, known polyploid taxa were eliminated from the analysis if sequence contigs displayed frequent nucleotide polymorphisms or regions of significant disagreement. Following these criteria, nrITS data were omitted for two taxa (*P. suberosa* ssp. *suberosa* and *P. suberosa* ssp. *litoralis*). These accessions were removed from the analysis and prioritized for cloning. Proceeding cautiously in this way, the nrITS dataset proved to be a valuable source of phylogenetic signal for the current analysis, with 56% phylogenetically informative characters. While the use of nrITS as a molecular marker in *Passiflora* carries some risk, it also has the advantage that a great deal of sequence data already exists for this locus. If the acquisition and use of nrITS data is done cautiously (i.e. excluding known polyploids and/or hybrids), and sequences are carefully screened for polymorphisms or other indication of presence of multiple copies, nrITS data should continue to serve as a valuable tool for phylogenetic reconstruction in *Passiflora.*

Yockteng and Nadot (2004a) first used the chloroplast-expressed copy of glutamine synthetase (Emshwiller and Doyle 1999; Doyle et al. 2003) for phylogenetic reconstruction within *Passiflora.* In that initial study, ncpGS provided 40% parsimony informative characters. The high proportion of parsimony informative characters, coupled with the fact that it is apparently a single copy nuclear gene, strongly support the use of ncpGS for reconstruction of phylogenetic relationships within *Passiflora*. It is also straightforward to align across outgroup genera as well as within *Passiflora* itself, with discrete gaps that could be useful as indel characters, though that was not explored in the current analysis. In Yockteng and Nadot's (2004a) analysis, data from this gene provided the strongest signal at the subgeneric and supersectional levels rather than among the terminals. In the current analysis, the ncpGS data matrix provided 38% informative characters with most variation among supersections in subgenus *Decaloba*. A few taxa were resolved in unexpected positions by the ncpGS dataset; these are hypothesized to be due to shorter sequence lengths caused by differences in primer location and/or large gap regions. Any taxa with unexpected placements were selected for future cloning to further characterize these sequences (e.g. presence of multiple copies or evidence of hybridization).

CHLOROPLAST LOCI—The use of chloroplast markers is complex in *Passiflora* because the mode of inheritance of the chloroplast may be maternal, paternal, or biparental (Muschner et al. 2006; Hansen et al. 2007). The three modes of inheritance have the potential to complicate interpretation of phylogenetic relationships in *Passiflora* by yielding intra-individual variation or heteroplasmy that may or may not stem from hybridization events. Hansen et al. (2007) cited several examples of *Passiflora* species resolved in unexpected phylogenetic positions in prior analyses due to suspected heteroplasmy, and noted that these cases are most easily detected when the placement of the specific taxon is in direct conflict with morphology. Thus, similar to the use of nrITS data, chloroplast loci must be used cautiously as phylogenetic markers in *Passiflora.*

This is the first study to use *ndhF* as a phylogenetic marker for reconstructing relationships in *Passiflora.* This marker was chosen due to its relatively high rate of evolution compared to other chloroplast regions (Olmstead and Reeves 1995; Scotland et al. 1995; Kim and Jansen 1995; Wurdack and Davis 2009). The region is quite variable and generally straightforward to align with single nucleotide substitutions being the primary source of parsimony informative characters. The *ndhF* alignment yielded 31% parsimony informative characters and provided substantial resolution at the level of outgroup genera and subgenera within *Passiflora* (Supplemental Fig. 2A). However, two of the outgroup taxa, *Malesherbia weberbaueri* and *Adenia heterophylla,* were spuriously resolved within subgenus *Decaloba* by this gene. In addition, there were several examples of likely erroneous placements within subgenus *Decaloba.* For example, *P. altebilobata* Hemsl. (supersection *Disemma,* China) is resolved with "P. intricata" sp. nov. ined. (supersection *Auriculata,* Dominican Republic), and *P. citrina* J. M. MacDougal (section *Xerogona,* Honduras and Guatemala) is resolved within supersection *Disemma* (SE Asia, Austral Pacific). In addition, supersection *Cieca* is not supported as monophyletic by the

ndhF data, while all other single-locus topologies examined here do support the monophyly of this group.

These results are unexpected based on resolution provided by other markers and morphology but are well supported with jackknife values of > 70% in the single-locus MP analysis of *ndhF*. These results were most likely due to missing data; alternatively, these apparent anomalies may reflect true cases of heteroplasmy or a paralogous copy of the region that was selectively amplified. Taxa placed unexpectedly were prioritized for cloning of *ndhF* amplicons to investigate the cause of their erroneous placement. Controlled pollinations to determine the mode of plastid inheritance would be extremely interesting for these taxa. Because relationships resolved by the *ndhF* region are overall congruent with relationships established based on other data at deeper nodes in the genus, the marker will still be valuable for phylogenetic reconstruction in *Passiflora* if used in concert with additional sources of data.

The chloroplast region *trnL-F* was first utilized for phylogenetic reconstruction in *Passiflora* by Muschner at al. (2003), and later by Krosnick and Freudenstein (2005) and Krosnick et al. (2006). In the current analysis, the *trnL-F* dataset included 27% parsimony informative characters, yet the strict consensus was generally unresolved. The *trnL-F* data appear to be most valuable in reconstructing relationships at the level of outgroup genera. *Passiflora* is largely unresolved in the strict consensus (Supplemental Fig. 2B), with the exceptions of subgenus *Astrophea,* parts of subgenus *Deidamioides,* and some isolated terminal clades in subgenus *Decaloba* and subgenus *Tetrapathea.*

Combined Molecular Data—While the individual datasets provide resolution and support at varying taxonomic levels across the genus, none satisfactorily resolves relationships simultaneously at all levels within the tree. It is important to investigate the individual datasets, but a combined approach allows signal to interact among datasets, often resulting in new topologies (Kluge 1989; Nixon and Carpenter 1996). Gatesy et al. (1999) suggested that hidden signal could be quantified by looking at the differences between branch support for a particular clade in the individual analysis and support for the same clade in the combined analysis.

The individual analyses of the molecular nrITS, ncpGS, *ndhF*, and *trnL-F* datasets each produced more than 100,000 MP trees, whereas the combined dataset produced 47,032 MP trees. This suggests that the total signal present in the combined analysis is strong enough to eliminate many of the alternative topologies present in the single locus analyses. In general, the combined molecular analysis had higher jackknife values for identically resolved clades than did the individual partitions. For example, the nrITS dataset, which provided the greatest support and resolution for relationships at all levels across the phylogeny, resolves subgenus *Decaloba* as monophyletic with 83% jackknife support whereas in the combined analysis, this same clade has 99% jackknife support (Fig. 2, clade F). Support for *Astrophea* + *Deidamioides* from the nrITS dataset (81%) increases to 99% in the combined analysis, and support for subgenus *Tetrapathea* as sister to subgenus *Decaloba* increases from 83% (nrITS alone) to 98% in the combined analysis.

In several cases, strict consensus of the individual datasets provided no resolution or weak support for placement of individual species, whereas these same species were resolved with strong support in the combined analysis. For example, the combined nuclear dataset (nrITS and ncpGS; Supplemental Fig. 1C) shows no resolution of relationships among outgroup genera, whereas these same taxa are fully resolved with strong jackknife support in the combined analysis (Figs. 2, 3). The increase in jackknife support and resolution suggests that hidden signal within the individual datasets is combining to produce a strongly-supported phylogeny when all four loci are analyzed together. The combined dataset is robust to the three different analyses methods used (MP, ML, and BI). Of the three, the MP tree is the least resolved with respect to the backbone of subgenus *Decaloba,* while the BI and ML trees have much greater levels of resolution, much of it with strong support.

Phylogenetic Relationships Among Outgroup Genera—The placement of *Malesherbia* and *Turnera* has varied across phylogenetic studies. In some studies, they are sister groups (Chase et al. 2000; Chase et al. 2002; Sosa et al. 2003), whereas in others, Malesherbiaceae is sister to Passifloraceae + Turneraceae (Davis et al. 2005; Korotkova et al. 2009; Tokuoka 2012). In addition, the APG III classification recognizes Malesherbiaceae and Turneraceae as part of Passifloraceae s.l. (Bremer et al. 2009). Although *Malesherbia* was designated as the outgroup in the present analysis, confident resolution of the relationships of Malesherbiaceae and Turneraceae to Passifloraceae will require much greater sampling. Within Passifloraceae, two tribes are recognized: Passifloreae and Paropsieae (De Wilde 1971, 1974; Feuillet and MacDougal 2007). *Paropsia* belongs to tribe Paropsieae, while *Adenia, Basananthe* and *Passiflora* belong to Passifloreae. Relationships among the 17 genera currently recognized (Feuillet and MacDougal 2007) in Passifloraceae have been poorly understood. However, Tokuoka (2012) provides the densest sampling to date for Passifloraceae s.s., with 15 genera represented. In that analysis, *Paropsia* is resolved as sister to a larger clade containing *Basananthe, Passiflora* and *Adenia.* In the current study, *Basananthe* and *Paropsia* are weakly supported as sisters in the ML and BI topologies, but are resolved as a basal grade with *Paropsia* below *Basananthe* in the MP tree. In Tokuoka (2012), *Adenia* is resolved as sister to a clade containing *Passiflora, Basananthe* and several additional genera. In the present study, *Adenia* is strongly supported as sister to *Passiflora*. The ML topology does not support the monophyly of tribes Paropsieae and Passifloreae, as *Basananthe* (Passifloreae) is resolved as sister to *Paropsia* (Paropsieae). As the limited generic-level sampling in Passifloraceae was primarily used to test the monophyly of *Passiflora,* it is not possible to confidently resolve the positions of subfamilies and genera relative to one another in the present analysis.

The Genus Passiflora—With a taxon sample that included 139 species representing all recognized subgenera, the MP, ML, and BI topologies strongly support monophyly of the genus. Characteristics that unite the genus as a whole may include the liana habit, chromosome number of $n = 12$, plants nearly glabrous, presence of two prophylls on the vegetative bud, small stipules, two petiolar nectaries, laminar nectaries none or marginal, presence of a primary peduncle (sensu Krosnick and Freudenstein 2005) with two small and unrecaulesced floral bracts, subequal calyx and corolla, reticulate seed coat, and mammal dispersal of seed from large fragrant fruits.

Passiflora Subgenus Astrophea Sister to Subgenus Deidamioides Section Tryphostemmatoides—*Passiflora* subgenus *Astrophea* (Fig. 2 Clade A) consists of 60 species of

woody or shrubby lianas, shrubs, and small to medium trees endemic to South America with chromosome counts of $n = 12$ or $2n = 24$ (Berry 1987; De Melo et al. 2001). Some tree species in *Astrophea* have higher order branching, a synapomorphy for tribe Passifloreae (De Wilde 1971). In the current analysis, subgenus *Astrophea* is supported as monophyletic. Subgenus *Astrophea* + subgenus *Deidamioides* section *Tryphostemmatoides* is strongly supported as a monophyletic lineage and is resolved as sister to the remainder of the genus. Yockteng and Nadot (2004a) also found a similar result in their analysis, with *P. tryphostemmatoides* sister to subgenus *Astrophea*. These results are not unexpected as the monophyly of subgenus *Deidamioides* has been in doubt for some time (Ulmer and MacDougal 2004). However, plants in clade A are extremely divergent with respect to morphology: whereas *Astrophea* are lianas with unbranched tendrils or small–medium trees without tendrils, species in section *Tryphostemmatoides* have branched tendrils with terminal adhesive disks and are generally slender climbers with small leaves. Only one chromosome count has been made within clade A (section *Tryphostemmatoides*: *P.* aff. *gracillima*, $2n = 22$, *MacDougal 4752GR*; J. MacDougal, unpubl. data). Shared morphological characteristics for clade A may include scar-like sessile petiolar nectaries at the blade base, absent or reduced pubescence, retention of the primary peduncle, coronal filaments in 2–3 series, and outer coronal filaments mostly yellow in color.

Subgenus Passiflora Sister to Subgenus Deidamioides Sections Polyanthea, Deidamioides, and Tetrastylis—Subgenus *Passiflora* (Fig. 2 Clades B, C) is strongly supported as monophyletic in the current analysis, with potential synapomorphies including loss of the primary peduncle, presence of large stipules, bracts, and flowers, and recaulescence of floral bracts resulting in three bracts per pedicel. Subgenus *Deidamioides* appears again in clade B, this time as a well-supported lineage consisting of three sections (*Polyanthea, Deidamioides,* and *Tetrastylis*). Section *Polyanthea* is monotypic (*P. cirrhiflora*), as is section *Deidamioides* (*P. deidamioides*). Section *Tetrastylis* contains two species, *P. ovalis* and *P. contracta* Vitta. The three species sampled in the current analysis are extremely divergent from one another morphologically, and have therefore been difficult to place with confidence. In Muschner et al. (2003), only *Passiflora ovalis* was included and it was resolved as sister to subgenus *Passiflora.* Yockteng and Nadot (2004a) included only *P. cirrhiflora,* which was resolved as sister to the rest of *Passiflora* with moderate support. Hansen et al. (2006) included all three species, and found that *P. ovalis, P. cirrhiflora,* and *P. deidamioides* together were monophyletic with moderate bootstrap support.

Within clade B, *Passiflora cirrhiflora* is moderately supported as sister to *P. deidamioides* in both the ML and BI analyses presented here. Both species are completely glabrous, and interestingly, these two species have pedunculate simple cymes ending in a tendril and a plicate operculum (C. Feuillet, pers. comm.); they also have compound leaves, a rare condition in the genus as a whole. Compound leaves are also observed in a few species in subgenus *Passiflora* and in one species in subgenus *Decaloba*. Clade B, which contains *P. ovalis, P. cirrhiflora,* and *P. deidamioides,* is moderately supported as sister to subgenus *Passiflora.* This differs from the placement of the same clade in Hansen et al. (2006) where these species were resolved as sister to subgenus *Decaloba. Passiflora ovalis* and the closely allied *P. contracta* (not sampled here) have both evolved bat pollination syndromes (Jørgensen et al. 2012); pollinators for the others in clade B are unknown.

Synapomorphies that unite *P. cirrhiflora, P. ovalis,* and *P. deidamioides* with subgenus *Passiflora* may include the loss of higher order branching in the inflorescence, and the presence of a well-developed corona in five or more series (corona reduced in *P. ovalis*). The base chromosome number for subgenus *Passiflora* is $n = 9$ (Snow and MacDougal 1993), but no chromosome counts are available for any of the species in clade B. Obtaining chromosome counts for the four species in sections *Polyanthea, Deidamioides,* and *Tetrastylis* may further clarify our understanding of relationships among these highly divergent taxa. The phylogeny presented here suggests that hummingbird pollination evolved multiple times in clade C, and bat pollination at least twice. Much greater sampling in subgenus *Passiflora* would be required to document these patterns completely.

Subgenus Tetrapathea Sister to Subgenus Decaloba—The combined molecular dataset (Fig. 2 Clades D, E) strongly supports placement of OW subgenus *Tetrapathea* sister to subgenus *Decaloba* (clade D). Subgenus *Tetrapathea* comprises three species (*P. kuranda, P. aurantioides,* and *P. tetrandra*) from Australia, Papua New Guinea, and New Zealand. This subgenus is readily distinguished from subgenus *Decaloba* as the three species are dioecious canopy lianas that lack a plicate operculum (instead, the operculum has vertical striations). The chromosome number for this subgenus (n = 12) is known from a single count of *P. tetrandra* (Hair and Beuzenberg 1959). Supersection *Disemma* (subgenus *Decaloba,* Fig. 2 clade K) is the only other OW clade in *Passiflora.* Clade D thus contains both of the OW lineages in the genus, but these two groups are only distantly related. This same result was obtained by Krosnick and Freudenstein (2005), who included *P. kuranda* and *P. tetrandra* as well as nine species from supersection *Disemma* in a preliminary analysis focused on testing the monophyly of supersection *Disemma.* It appears that two separate dispersals to the OW have occurred within *Passiflora:* one that resulted in subgenus *Tetrapathea* in the Austral-Pacific and a second event that resulted in supersection *Disemma* in Indochina, Southeast Asia, and the Austral-Pacific. Clade D also includes species with higher order branching within their inflorescences. Compound cymes are common in the Passifloraceae, but are relatively rare in *Passiflora:* some tree species in subgenus *Astrophea* (clade A) have branched inflorescences, but this character is not observed in clade C (subgenus *Passiflora* + subgenus *Deidamioides* sections *Tetrastylis, Deidamioides,* and *Polyanthea*). Higher order branching is again observed in subgenus *Tetrapathea,* and is then absent in other lineages of subgenus *Decaloba,* implying multiple gains or losses of this character. Clade D is also marked by reduction of coronal filaments from four or more series to just two or three: subgenus *Tetrapathea* has two series as do most members of subgenus *Decaloba.* However, the basally branching supersections *Pterosperma* and *Hahniopathanthus* have 3–10 series, as do a small number of species nested elsewhere in the subgenus.

Subgenus *Tetrapathea* (clade E) is resolved as monophyletic in the ML and BI analyses, though support is low. In the MP analysis, the individual trees show two alternative resolutions for these plants, one as depicted in Fig. 2, the other indicating a non-monophyletic subgenus *Tetrapathea* with

P. kuranda + *P. aurantioides* basal to *P. tetrandra,* which is in turn sister to the remainder of subgenus *Decaloba*. These alternative resolutions result in a trichotomy in the MP strict consensus (i.e. *P. kuranda* + *P. aurantioides*, *P. tetrandra*, remainder of subgenus *Decaloba*). When included as the lone representative of subgenus *Tetrapathea, P. tetrandra* has been similarly unstable in recent analyses: Yockteng and Nadot (2004a) found that this species was resolved within subgenus *Decaloba,* Hearn (2006) showed *P. tetrandra* as unresolved at the base of a clade containing subgenera *Astrophea, Deidamioides,* and *Decaloba,* and Hansen et al. (2006) showed that BI analyses supported *P. tetrandra* as sister to the genus *Dilkea* Mast. The fact that the ML and BI analyses resolve subgenus *Tetrapathea* as monophyletic in the current analysis, while the MP analysis does not, may indicate that *P. tetrandra* behaves as a long branch due to many autapomorphies, thus reducing support for monophyly of the clade.

Two reproductive characteristics support monophyly of subgenus *Tetrapathea.* First, the three species in subgenus *Tetrapathea* are all dioecious. Dioecy is observed in Passifloraceae outside of *Passiflora* (e.g. *Adenia*), but is rare within the genus. While some *Passiflora* species may be functionally andromonoecious (MacDougal 1994), subgenus *Tetrapathea* is the only clade to display dioecy. Green (1972) hypothesized that isolation of *P. tetrandra* facilitated the evolution of dioecy in this species, as the condition is relatively common in New Zealand endemics. Interestingly, *P. tetrandra, P. aurantioides* and *P. kuranda* display varying levels of dioecy (Krosnick et al. 2009). In *P. tetrandra,* male flowers have only rudimentary carpels and female flowers have aborted stamens. In *P. aurantioides,* both stamens and carpels are present, though these organs are more or less exaggerated depending on the sex of the plant. However, *P. kuranda* is functionally dioecious such that sex can only be determined by performing crosses and observing if fruit is produced. The second synapomorphy shared by these three species is variability in carpel number. Though this phenomenon is extremely rare in angiosperms (Endress 1994; Endress and Igersheim 1997; Krosnick et al. 2006), all three of these species show variation in carpel number among individual plants (*P. tetrandra*: 2–4 carpels; *P. aurantioides*: 3–5; *P. kuranda:* 5–8).

Subgenus Decaloba (Clade F)—The monophyly of subgenus *Decaloba* has been supported in recent analyses (Muschner et al. 2003; Yockteng and Nadot 2004a; Hansen et al. 2006) and is strongly supported here. Subgenus *Decaloba* is supported by a number of synapomorphies, including variegation in juvenile leaves, presence of a plicate membranous floral operculum, reduction of the corona to just two to three series (rarely more, then considered secondarily derived). Many species have multiple bands of red, purple, or violet on the corona. The chromosome number for this clade is normally $n = 6$, but $n = 9$ has also been reported (Snow and MacDougal 1993; Hansen et al. 2006). The lower count appears to be a synapomorphy for a large sublineage within the subgenus (see discussion of $n = 6$ group below). Interestingly, the current analysis places the poorly known *P. obovata* Killip in subgenus *Decaloba. Passiflora obovata* is a large liana from Mexico and Central America that Feuillet and MacDougal (2003) placed in subgenus *Deidamioides.* The phylogenetic position of this species was first examined by Krosnick (2006), who obtained results similar to those obtained here.

Supersection Pterosperma (Clade G)—Supersection *Pterosperma* is well supported as monophyletic in the current analysis, and is the most basally branching lineage within subgenus *Decaloba.* This placement is similar to results obtained in previous analyses (Muschner et al. 2003; Yockteng and Nadot 2004a; Hansen et al. 2006). Earlier classifications had placed this group in subgenus *Deidamioides* (Feuillet and MacDougal 1999; Gilbert and MacDougal 2000), but it has since been recognized as a member of subgenus *Decaloba* (MacDougal and Hansen 2003). This supersection consists of four species endemic to southeastern Mexico and Central America: *Passiflora lancetillensis* J. M. MacDougal & Meerman, *P. microstipula* L. E. Gilbert & J. M. MacDougal, *P. pedicellaris* J. M. MacDougal, and *P. eueidipabulum* Knapp & Mallet (*P. eueidipabulum* not sampled here). These species are easily recognized by a number of morphological features, including unlobed leaves, cernuous shoot tips, multiple pairs of extrafloral nectaries along the petiole (lost in *P. pedicellaris*), conspicuously winged seeds, retention of a primary peduncle, and (2) 3–4 series of coronal filaments. The fruits are believed to be dispersed by bats (Gilbert and MacDougal 2000). Chromosome number is $n = 9$ from a single count of *P. microstipula* (Snow and MacDougal 1993).

Supersection Hahniopathanthus + Passiflora obovata Sister to the Rest of Decaloba—Clade H (Fig. 2 Clades H, I) is strongly supported as monophyletic, and comprises clade I, supersection *Hahniopathanthus* + *P. obovata* (subgenus *Deidamioides* section *Mayapathanthus*), plus the remainder of subgenus *Decaloba.* Clade H is distinguished by the absence of the primary peduncle. Within clade I, the monophyly of supersection *Hahniopathanthus* itself is strongly supported by the molecular data. Species placed in supersection *Hahniopathanthus* have several notable morphological characters: presence of cernuous shoot tips; large, foliose stipules and floral bracts; loss or extreme reduction of one of three floral bracts on the pedicel, and absence of coronal banding. In the current analysis, *P. obovata* is weakly supported as sister to supersection *Hahniopathanthus* (clade I) by the ML and BI analyses, but is unresolved in the MP topology. *Passiflora obovata* is quite different from species in section *Hahniopathanthus* with regard to morphological characters. Krosnick (2006) found weak support for this same placement; in that study, possible long-branch attraction was investigated by removing species one by one from subgenus *Pterosperma* and *Hahniopathanthus* during phylogenetic analysis. Under all taxon inclusion schemes, *P. obovata* was resolved as sister to the remaining taxa in section *Hahniopathanthus.* In the current analysis, a second DNA isolation of *P. obovata* was sequenced to confirm that contamination was not the cause of this unexpected placement.

Although *P. obovata* is quite distinct relative to subgenus *Hahniopathanthus,* placement of this species in subgenus *Decaloba* is supported by presence of a plicate operculum and two series of coronal filaments. Superficially, *P. obovata* is similar to species in subgenus *Tetrapathea* based on growth form and presence of an erect operculum. Juveniles climb by adhesive disks, as in subgenus *Deidamioides* section *Tryphostemmatoides*, but tendrils are not branched in *P. obovata*. However, this species does share some notable features with supersection *Hahniopathanthus,* including absence of the primary peduncle and presence of marginal laminar nectaries. Juvenile leaves are peltate, as are the leaves in most species of *Hahniopathanthus*. The chromosome number

for supersection *Hahniopathanthus* was noted as $n = 11$ or 12 based on an ambiguous count by MacDougal (Hansen et al. 2006). No counts are available for *P. obovata,* but focusing on this species and those in supersection *Hahniopathanthus* for future cytological studies may help to clarify relationships in this clade.

The n = 6 Group—The remaining six supersections of subgenus *Decaloba* (Fig. 2 Clade J) are resolved within clade J. These taxa share a number of morphological similarities, including reduction of vegetative prophylls from two to one, submarginal or abaxial laminar nectaries, small purple-black fruits, small seeds, conspicuous pubescence, reduction of the corona to two rows with the inner row much shorter than the outer row, and reduction in habit from forest lianas to small vines and climbers at forest edges. Seeds are often dispersed by birds. Most notably, however, this clade is distinguished by having a chromosome number of $n = 6$, indicating a major historical shift in the chromosomal makeup of these species relative to the rest of the genus.

Recent molecular work has begun to confirm differences in genetic makeup of the $n = 6$ clade relative to the rest of *Passiflora*. For example, Yockteng and Nadot (2004b) found that a cytosolic-expressed copy of glutamine synthetase (cytGS) was preferentially amplified in the $n = 6$ members of subgenus *Decaloba,* while in the rest of the genus, the same primers amplified the chloroplast-expressed copy (ncpGS). Repeated attempts to amplify cytGS in the remainder of subgenus *Decaloba* (supersections *Pterosperma* and *Hahniopathanthus*) and subgenera *Tetrapathea, Deidamioides, Astrophea* and *Passiflora* have been unsuccessful, suggesting that the cytGS copy may be absent outside of the $n = 6$ lineage (K. Porter-Utley and S. Krosnick, unpubl. data). Similarly, a single copy of CRABS CLAW (CRC) is present in species of the $n = 6$ group that have been sequenced to date, whereas multiple copies have been detected outside of this clade (Krosnick, in prep.). These data, while preliminary, suggest that a significant rearrangement or reduction of genetic information has occurred in this lineage.

Passiflora multiflora* Sister to Supersection *Disemma*)—Within the $n = 6$ group (Fig. 2 Clade K, a clade containing *P. multiflora* + supersection *Disemma* is well supported as sister to the remainder of the lineage. The placement of *P. multiflora* + supersection *Disemma* suggests a New World origin for the $n = 6$ group (clade J), followed by a biogeographic event that led to the OW supersection *Disemma*. *Passiflora multiflora* is native to southernmost Florida and the Caribbean, and supersections *Pterosperma* and *Hahniopathanthus* are all endemic to Central America. Recent age estimates for the emergence of Passifloraceae s. s. range from ca. 71–65 MYA (Davis et al. 2005; Hearn 2006) to 37 MYA (based on the age of Malesherbiaceae; Gengler-Nowak 2002), whereas *Passiflora* itself appeared ca. 40 MYA (Hearn 2006). Several unconfirmed *Passiflora* fossils have been described from the Eocene and Miocene in Eastern Europe (Rásky 1960; Mai 1967; Gregor 1982), and Mexico (Graham 1976). Recently, an unambiguous fossil seed of *Passiflora* from the Miocene was discovered in Panama (M. Carvalho pers. comm.).

While an explicit examination of biogeographical relationships in *Passiflora* was not conducted as part of this study, given that two serial lineages of *Passiflora* (Fig. 2 clades A, C) are NW, as are Malesherbiaceae, it is most parsimonious to posit a NW ancestor for the genus and clades within it until clades E (subgenus *Tetrapathea*) and K (subgenus *Decaloba* supersection *Disemma*) which, as described above, are distributed throughout Indochina, Southeast Asia and the Austral Pacific. Taking the age estimates for Passifloraceae and *Passiflora* into account, it is possible that the ancestors of clades E and K arrived in the Old World by any of three possible mechanisms: 1) species may have moved from Central/South America to Asia/Austral Pacific via the North Atlantic Land Bridge during the Eocene/Oligocene (Boreotropics hypothesis: McKenna 1972; Wolfe 1975; Tiffney 1985; Lavin and Luckow 1993); 2) more recently via the Bering Land Bridge during the Pleistocene (Beringian hypothesis: Colinvaux 1981; Hopkins et al. 1981; Sher 1999; Waltari et al. 2007), or 3) via long-distance dispersal of seeds, given that most *Passiflora* are animal dispersed (Ulmer and MacDougal 2004). However, without strong fossil evidence, discerning among these possible hypotheses with confidence will be difficult.

Supersection *Disemma* consists of 21 species from China, India, Southeast Asia, Australia, and Papua New Guinea. The phylogenetic affinities of this group have long been debated because of the unique morphological features they display. The group is divided into three sections: *Disemma, Octandranthus,* and *Hollrungiella.* While limited synapomorphies are apparent at the sectional level and below, the supersection has no clear unifying features due to the high level of morphological diversity observed even among closely related species. Krosnick and Freudenstein (2005) confirmed the monophyly of the group with limited taxon sampling; in that study, NW *P. multiflora* was resolved as sister to *Disemma*. With full sampling of all 21 species and much expanded outgroup sampling, Krosnick (2006) also confirmed the monophyly of *Disemma,* but parsimony analyses could not resolve the position of this clade relative to the rest of subgenus *Decaloba*. In Krosnick (2006), *P. multiflora* was resolved as sister to *P. holosericea* instead of *Disemma.*

The current analysis shows strong support for monophyly of *Disemma* but somewhat weaker support for placement of *P. multiflora* as sister to *Disemma* (clade K). It is notable that the MP analysis does support *P. multiflora* as sister to *Disemma* (75% jackknife support), but does not provide resolution for the position of any supersections distal to supersection *Pterosperma.* Morphological features shared by *P. multiflora* and supersection *Disemma* are difficult to identify because of the lack of apparent synapomorphies in supersection *Disemma* as a whole. *Passiflora multiflora* is unusual in that it has highly branched inflorescences, a feature that is characteristic of supersection *Disemma* section *Octandranthus,* but this trait also occurs elsewhere in the $n = 6$ group.

Within supersection *Disemma,* section *Disemma* (three species, Australia) is strongly supported as monophyletic and is resolved as sister to the remainder of the supersection. All three species in this clade have tube-shaped flowers with a 5-lobed nectar chamber, an erect operculum, sepals with a keel, and lack a limen at the base of the androgynophore. Interestingly, the ML and BI analyses presented here resolve monotypic section *Hollrungiella* (*P. hollrungii*) as sister to the Asian section *Octandranthus,* whereas the MP analysis leaves this species unresolved at the base of supersection *Disemma.* This species is endemic to Papua New Guinea and has a number of features in common with the Australian species, such as the five-lobed nectar chamber, tube-shaped flower, larger fruits that are green at maturity, and absence of a limen at the base of the androgynophore. However, this

species also has some characteristics in common with *Octandranthus,* notably an incurved operculum and smooth, unkeeled sepals. Section *Octandranthus* (17 species, Indochina and SE Asia) is strongly supported as monophyletic in the combined analysis. Species in this clade have truncated midveins, extensively branched inflorescences, and a widened operculum, with reversals of these characters in some species.

Single Loss of rpoC1 Intron—Although weakly supported in the current analysis (Figs. 2, 3 Clade L), an interesting synapomorphy uniting clade L is loss of the *rpoC1* intron, first noted by Hansen et al. (2006) as a potentially informative phylogenetic marker for relationships in subgenus *Decaloba.* In their analysis, the intron was shown to be present in supersections *Multiflora, Auriculata, Disemma, Pterosperma,* and *Hahniopathanthus,* and lost in the remainder of the subgenus. However, the topology obtained from their analysis of the *trnL/trnT* spacer region indicated that it was most parsimonious to hypothesize multiple losses across the subgenus, suggesting that the intron might be too evolutionarily vagile to be useful as a phylogenetic marker. In contrast, the topology obtained in the present analysis supports a single loss of the *rpoC1* intron, an event that distinguishes clade L from the rest of the subgenus. Notably, this character is also congruent with phylogenetic results presented here that show supersection *Multiflora* sensu Feuillet and MacDougal (2003) is not monophyletic: whereas *P. multiflora* has the intron, the remaining species in the supersection (here placed within clade M, see below) do not. The loss of the *rpoC1*intron also supports the basal position of supersections *Pterosperma, Hahniopathanthus,* and *Disemma*, which retain the intron, relative to the rest of the subgenus.

Supersection Auriculata and Former Supersection Multiflora are Paraphyletic (Clade M)—Supersection *Auriculata,* as circumscribed by Feuillet and MacDougal (2003), consists of eight species distinguished by presence of a single pair of auriculate petiolar nectaries, laminar nectaries that are neither strictly submarginal nor in lines between the primary veins, a short androgynophore, and reduced petals. In contrast, supersection *Multiflora,* as circumscribed by Feuillet and MacDougal (2003), includes 22 species with combinations of apomorphic and plesiomorphic characteristics that make their placement challenging. For example, some species display higher order branching within the inflorescences (primitive for tribe Passifloreae) whereas others have abaxial nectaries scattered in diffuse lines between the primary veins (advanced within *Passiflora*). Not surprisingly, the monophyly of supersection *Multiflora* has been questioned for several years (Ulmer and MacDougal 2004; Krosnick and Freudenstein 2005). As noted earlier, *Passiflora multiflora,* the type of supersection *Multiflora*, is shown in this and other recent analyses (Muschner et al. 2003; Krosnick and Freudenstein 2005; Hansen et al. 2006) to be unrelated to the remainder of the supersection. Moreover, the remaining species in supersection *Multiflora* are paraphyletic with respect to supersection *Auriculata* (clade M). To avoid confusion, supersection *Multiflora* (exclusive of *P. multiflora*) + supersection *Auriculata* will be referred to as clade M for the remainder of the discussion.

Within clade M, two lineages are consistent with informal groups proposed previously: the *lobbii* group (Skrabal et al. 2001; Jørgensen and Weigend 2004) and the *apoda* group (MacDougal and Hernández 2014). Interestingly, while these groups were placed in supersection *Multiflora* by Feuillet and MacDougal (2003), some species in the *lobbii* and *apoda* groups have auriculate leaf glands or unusually short androgynophores, features associated with supersection *Auriculata.* The ML and BI analyses agree with regard to relationships within clade M, but the MP topology places *P. holosericea* as unresolved at the base of the *n* = 6 clade.

Passiflora truncata Regel, a poorly understood species with similarities to several supersections in subgenus *Decaloba*, is well supported as part of clade M where its closest relative is "P. intricata," an undescribed species from the Dominican Republic with leaves similar to *P. auriculata*. Muschner et al. (2003) found a similar result, with *P. truncata* resolved as sister to *P. rufa* Feuillet & J. M. MacDougal, a species closely related to *P. auriculata.* Nearly all species in clade M are South American, and it appears that two distinct radiations within this clade occurred in the Andes (*lobbii* group and *apoda* group), with additional lowland species (*auriculata* and relatives). Morphological similarities for clade M include absence of leaf variegation and presence of straight shoot tip growth. *Passiflora sierrae* L. K. Escobar, the one species in Escobar's monotypic subgenus *Porphyropathanthus*, was not sampled in this study for lack of available material. It is somewhat morphologically similar to species in clade M, and future study might place it here. Greater sampling will be necessary to unravel the detailed relationships among the species in clade M, but it is clear that taxonomic revision will be required.

Supersections Cieca Through Decaloba—The next branch resolved in the MP and BI analyses (clade N) consists of three supersections: *Cieca* (clade O, 19 species), *Bryonioides* (clade Q, 22 species) and *Decaloba* (clade R, ca. 130 species). The monophyly of this large clade (Fig. 3 Clades N–R) is well supported in the BI analysis, but only weakly so in the ML topology. Likewise, there is only strong support for relationships among the three supersections only from the BI analysis. No apparent synapomorphies are shared by these three supersections, although geographically they all exhibit species radiations across northern Central America.

Within clade N, support for the monophyly of each of the three supersections is strong. Supersection *Cieca* (clade O) has several clear synapomorphies that unite the clade including loss of petals, peltate leaf shape in seedlings, and floral bract reduction from three to two or fewer. This clade has been examined in depth by Porter-Utley (2003, in press). The present study sampled seven of the 19 species currently recognized in the supersection. The species in this clade are distributed in the southern United States, Mexico, Central America, South America, and the Caribbean. Two species, *P. suberosa* L. and *P. pallida* L., are also naturalized in various regions of the OW. The supersection contains two problematic species complexes, *P. suberosa* and *P. coriacea* Juss. The *P. suberosa* complex exhibits polyploidy suggesting a history of hybridization events (Snow and MacDougal 1993; Porter-Utley 2003, 2007).

Clade P resolves supersection *Bryonioides* as sister to supersection *Decaloba* with strong support only from BI; no morphological synapomorphies are known for the clade. Supersection *Bryonioides* (clade Q) is resolved as monophyletic with the inclusion of *P. gracilis* J. Jacq. ex Link as the basal-most member, as suggested by MacDougal (1994). Several synapomorphies exist between *P. gracilis* and supersection *Bryonioides*: presence of an elongated fruit with irregular dehiscence, bright orange arils, one to several pairs

of teeth at the base of the lamina, a two-ranked corona with the inner series highly reduced to absent, coronal filaments with multiple violet bands, and carinate to shortly horned sepals. However, whereas *Passiflora gracilis* is well supported as basal within this clade, it is quite distinct from the rest of *Bryonioides* in several respects: plants are annuals, flowers are apetalous with a zygomorphic gynoecium, stipules and floral bracts are highly reduced, and petiolar nectaries are somewhat reduced. Notably, *P. gracilis* is completely glabrous, whereas plants of the remainder of species in supersection *Bryonioides* have distinctive hooked trichomes (MacDougal 1994).

***Supersection Decaloba*—**The largest clade (Fig. 3 Clades R–Y) within subgenus *Decaloba,* supersection *Decaloba* (clade R), is the least well known, partly due to its wide geographical distribution, with multiple species radiations in Central America, the Caribbean, and South America. Several poorly understood species complexes exist, including the *P. alnifolia* Kunth and *P. cuneata* Willd. complexes in Colombia and Ecuador, and the *P. misera* Kunth complex in other parts of South America. The supersection contains two sections (Feuillet and MacDougal 2003), *Xerogona* (15 species) and *Decaloba* (ca. 115 species). The phylogenetic analysis presented here incorporates the largest sampling within the supersection to date (64 of 130 currently recognized species). Supersection *Decaloba* is strongly supported as monophyletic in the ML, BI, and MP analyses, and is united by several distinct morphological synapomorphies including cernuous shoot tips, loss of petiolar nectaries, laminar central vein equal to or shorter than the lateral veins, often producing a bilobed leaf, and grooved seeds with rugulose ridges.

Sampling for the present study is dense enough to reveal some resolution among major lineages within the supersection. The ML, BI and MP analyses strongly support monophyly of two major lineages: section *Decaloba* DC. pro parte (p. p.) + section *Xerogona* (clade S) and the remainder of section *Decaloba* (clade W). The smaller of the two clades, S, contains section *Decaloba* p. p. with the informal *sexflora* group (MacDougal 1989a) and *bilobata* group (Killip 1938), and section *Xerogona.* Whereas several species radiations have occurred in South America, clade S is most diverse in Mexico, the Caribbean, and Central America. Clade S is readily distinguished by the complete absence of extrafloral nectaries on the plant. Also characteristic of this lineage is highly reduced coronal banding and, except in the subgroup represented by clade U, absence of floral bracts. A number of the species in this clade appear to have evolved traits associated with wasp pollination (MacDougal 1983, 1994).

Within clade S, the ML and BI trees support a basal clade containing *P. lutea, P. filipes,* and *P. pavonis* as sister to the remaining species, though monophyly of this lineage in weakly supported. Similarities among these three species were first noted by Killip (1938), and it was named as a series by MacDougal (1995). *Passiflora tenella,* an annual species, is then weakly supported as sister to section *Xerogona* (clade T) in the ML and BI topologies. In the MP strict consensus, *P. lutea, P. filipes* + *P. pavonis,* and *P. tenella* are unresolved at the base of supersection *Decaloba.* Boza et al. (in press) formally recognize *P. tenella* as part of section *Xerogona*. That section is characterized by having only slightly cernuous shoot tips, seeds with mostly smooth ridges, a corona often reduced to a single series, and a capsular fruit. *Passiflora tenella* is interesting in this context because the fruits are similar in shape to the rest of section *Xerogona* but do not dehisce and the ridges on the seeds are rugulose. Hummingbird pollination has evolved twice in section *Xerogona* (MacDougal 1989b), and wasp pollination has been noted in *P. capsularis* and *P. costaricensis* (J. MacDougal, pers. obs.).

Sister to *P. tenella* + *Xerogona* is clade U (section *Decaloba* p. p.), a weakly supported lineage comprised of the informal *sexflora* and *bilobata* groups. Shared characteristics for clade U include absence of leaf variegation. Within the *sexflora* group, synapomorphies include presence of higher order branching within the inflorescence and large bracts. Similar to *Xerogona,* shoot tips in this clade are barely if at all cernuous; this trait may be a synapomorphy for *Xerogona* (including *P. tenella*) + clade U, as shoot tips of *P. lutea* and closely related species are more strongly cernuous. Clade V, the Caribbean *bilobata* group, is strongly supported in all three topologies presented, and is distinguished from the s*exflora* group by having small floral bracts and unbranched inflorescences. Interestingly, this clade includes *P. berteroana,* a poorly known species from Puerto Rico and the Dominican Republic with deeply lobed to nearly compound leaves. It is hypothesized that *P. berteroana* will be closely related to *P. insueta* Feuillet & J. M. MacDougal, a Caribbean species with similarly lobed leaves, when the latter species is sampled in future analyses.

Clade W includes the remainder of taxa sampled from subgenus *Decaloba* in this analysis, and is strongly supported as monophyletic. This clade appears to represent a substantial species radiation, comprising ca. 94 (excluding *Decaloba* p. p. in clade S) of the 130 total species in the supersection as currently circumscribed. This clade exhibits high species richness in Central America, Mexico, and the Caribbean, but is most diverse in the Andes and lowland South America. This lineage is characterized by the presence of abaxial laminar nectaries arranged in a V-shaped pattern between the primary veins. These nectaries are often ocellate, and in some species appear as butterfly egg mimics (Gilbert 1982). Pollination syndromes are quite variable, with hummingbird pollination evolving at least four times, and bat pollination evolving in *P. penduliflora* Bertero ex DC. Several species complexes are present in South America, marked by features such as large bracts, flower color, or modifications of the corona. However, most relationships within this lineage are poorly resolved regardless of analytical approach (i.e. ML, BI, MP). Sparse taxon sampling may be partially responsible, but more sequence data may also be necessary to resolve relationships among these species.

Two subclades are worthy of note within clade W because they appear in the ML, BI, and MP trees and are also distinguishable by several morphological characteristics. Clade X includes species recognized in the informal *apetala* group (ca. 13 species). The *apetala* group is distinctive in having highly reduced petals, strongly variegated leaves at maturity, and corona reduced to a single series in several species (MacDougal 2003). Wasp and bee pollination is known in this group (J. MacDougal, pers. obs.). The center of diversity for this lineage is Central America and Mexico. *Passiflora bicornis* Houst. ex Mill. is resolved as sister to the *apetala* group in the ML and BI analyses, but is unresolved at the base of clade W in the MP strict consensus. This species is quite divergent from the others in having numerous rows of coronal filaments, no conspicuous leaf variegation, and large, oily floral bracts. *Passiflora lancearia* Mast. is also resolved within this

clade, but differs markedly in floral, vegetative, and fruit and seed morphology; further investigation is needed to test this placement. Greater sampling in section *Decaloba* will permit testing of the placement of *P. bicornis* and *P. lancearia*.

Clade Y, informally known as the *murucuja* group and formally recognized in the past at various ranks from genus to section (Killip 1938), consists of all the Caribbean red-flowered species and their relatives (Kay 2003). This lineage is distinguished by absence of variegation on leaves in both juveniles and adults. The group is well known for specialized floral morphology associated with hummingbird pollination syndromes. For example, *Passiflora murucuja* L., *P. tulae* Urb., and *P. orbiculata* Cav. have evolved a tube-shaped flower via fusion of the coronal filaments, while other species in clade Y have achieved the same shape through elongation and narrowing of the hypanthium, or floral tube. In the current analysis, *P. helleri,* a bee-pollinated Central American species (J. MacDougal, pers. obs.), is weakly supported as sister to the *murucuja* group, suggesting that the bird pollination syndromes in this clade may have evolved from a bee-pollinated ancestor. *Passiflora talamancensis* Killip is embedded at the middle of an exclusively hummingbird pollinated clade, though support for this placement is weak. This species has white, shallow, diurnal, cup-shaped flowers with thick yellow coronal filaments and a sweet fragrance, features often associated with insect pollination in *Passiflora* (Faegri and Van Der Pijl 1979; MacDougal 1994), suggesting a reversal to bee-pollination. Lability in traits associated with pollination biology has been clearly documented in this group including the emergence of bat-pollination in *P. penduliflora* (Kay 2001). In the present analysis, *P. penduliflora* is also nested within a hummingbird-pollinated clade, suggesting that switches from bee to bird or bird to bat pollination are readily achieved in this group.

Lability of Morphological Character Transformations—The phylogenetic analysis presented here permits examination of patterns of character transformation across the genus toward identifying synapomorphies for both new and well established lineages. This investigation has revealed marked lability in morphological characters in *Passiflora,* with multiple transitions to and from characters present at almost all taxonomic levels examined. For example, higher order branching is scattered across species in subgenus *Astrophea,* subgenus *Tetrapathea,* and in subgenus *Decaloba* supersections *Disemma,* clade M, and supersection *Decaloba.* Floral bracts are highly variable across the genus, ranging from large to small and three to none, with the plesiomorphic condition for Passifloraceae being two small bracts per pedicel. Bracts are further reduced to zero in *Passiflora lutea* and relatives, *Passiflora tenella,* and section *Xerogona.* While the number of vegetative bud scales or prophylls is a character that requires better documentation across the genus as a whole, the number varies from two as the plesiomorphic condition, to one in some lineages within subgenus *Passiflora*, and to one in the $n = 6$ group of subgenus *Decaloba,* with rare reappearances of two prophylls in section *Decaloba.*

Cernuous shoot tip orientation first appears in clade F (Fig. 2), is then lost in *P. obovata* (part of clade I) and in the $n = 6$ group (clade J), regained in supersection *Decaloba* (clade R), and reduced or partially lost again in section *Xerogona* (clade T) and the informal *sexflora* and *bilobata* groups (clades U and V), but is present throughout clade W. Another variable character appears to be the plication, or folding, of the operculum. Nearly all species in subgenus *Decaloba* are characterized by the presence of a strongly plicate operculum, which interacts with the limen at the base of the androgynophore to regulate pollinator access to the nectary. However, subgenus *Deidamioides* section *Tryphostemmatoides* has also been documented as having a slightly plicate to plicate operculum (Holm-Nielsen and Jørgensen 1986). Sister clade subgenus *Astrophea* does not display any plication. In addition, subgenus *Deidamioides* sections *Polyanthea* and *Deidamioides* (clade B) display some degree of folding of the operculum in *P. cirrhiflora* and *P. deidamioides.* Again, no plication is observed in the sister clade, subgenus *Passiflora.* Species in subgenus *Tetrapathea* (clade E) have marked vertical striations along the operculum, but are not folded. Thus, it appears that operculum plication has appeared at least three times independently across the genus. This character is poorly known overall; careful examination of the operculum condition across the genus is needed.

Extrafloral nectaries are one of the best examples of morphological lability in *Passiflora.* Subgenera *Astrophea, Passiflora* and *Deidamioides* may have petiolar and/or marginal laminar nectaries, while subgenus *Tetrapathea* has both petiolar nectaries and abaxial laminar nectaries that are restricted along the midvein. Some species in subgenus *Passiflora* have nectaries on the stipules, bracts, and/or sepals. Subgenus *Decaloba* is the most diverse lineage in *Passiflora* with respect to nectaries. Supersection *Pterosperma* has petiolar nectaries in multiple pairs and scattered submarginal abaxial laminar nectaries, while supersection *Hahniopathanthus* has marginal laminar nectaries and several species have no petiolar nectaries. A single pair of petiolar nectaries and submarginal laminar nectaries are present in supersections *Disemma* (clade M), *Cieca* (clade O), and *Bryonioides* (Clade Q). Lastly, the *P. lutea*/*Xerogona*/*sexflora* clade (clade S) within supersection *Decaloba* lacks extrafloral nectaries entirely, while the remainder of the supersection has a unique V-shaped arrangement of ocellate abaxial laminar nectaries and no nectaries on the petiole.

Subgenus *Decaloba* alone provides many examples of gains and losses of notable characters. For example, leaf variegation in juvenile plant tissues first appears in subgenus *Decaloba* (clade F) and is present in supersections *Pterosperma* (clade G) and *Hahniopathanthus* (part of clade I). Variegation is absent in *P. obovata* (part of clade I), *P. multiflora* and parts of supersection *Disemma* (clade K), and clade M (supersection *Auriculata* + remnants of *Multiflora* s. lat.). Variegation is then present in most species of supersection *Cieca* (clade O), absent in supersection *Bryonioides* (clade Q), and again present in sect. *Xerogona* (clade T). It is absent in the *sexflora* and *bilobata* groups (clade U), lost or regained in multiple unrelated lineages in supersection *Decaloba* section *Decaloba* (clade W) and lost in the *murucuja* group (clade Y). The trait is fixed in adult leaves of several unrelated species in supersections *Disemma* and *Decaloba.*

The variation observed in the characters outlined above undoubtedly has been influenced by the numerous ecological associations that *Passiflora* has with both pollinators and herbivores. Pollinators include hummingbirds, bats, bees, and wasps, with specific suites of floral characters associated with each syndrome (Ulmer and MacDougal 2004; note that field observations are lacking for most species). This analysis provides a first step toward examining the

evolutionary transitions between these characters across the genus. Hummingbird-pollinated species display large, solitary, diurnal, brightly colored, tube-shaped flowers with well-developed floral nectaries and little to no floral fragrance (Snow 1982; Bawa 1990; Lindberg and Olesen 2001). Across the genus, hummingbird-pollination has evolved in ca. 125 species in at least 20 lineages. Bat pollination is less common, but has been documented in eight species across the genus and only once in subgenus *Decaloba* (Jørgensen et al. 2012). Bee and wasp pollination appear to be the most common pollination syndromes in *Passiflora* (Kay 2001; Varassin et al. 2001). These flowers are typically smaller compared to hummingbird or bat-pollinated flowers, dish-shaped, emit a strong floral scent, and often have brightly colored coronal filaments (MacDougal 1994; Garcia et al. 2007). While branched inflorescences are relatively infrequent in *Passiflora,* these flowers are typically smaller in size compared to flowers in unbranched inflorescences in related groups, and are often bee or wasp-pollinated (e.g. supersection *Disemma*; Krosnick 2006). In some species, branched inflorescences may be associated with mass-flowering events (Ulmer and MacDougal 2004). Pollinator interactions in *Passiflora* may drive floral diversification through pressures on reproductive traits; conversely, as these relationships become more specialized, they may function as constraints on further evolutionary change.

Beyond pollination, species in *Passiflora* have diverse ecological relationships with many types of insects including Lepidoptera (e.g. tribes Heliconiini, Josiini), ants, beetles, parasitic wasps, and many other specialists and generalists. These interactions have provided some of the best examples of coevolution between plants and animals. For example, Williams and Gilbert (1981) and Gilbert (1982) documented the complex relationship between *Heliconius* butterflies and extrafloral nectaries (EFNs) of *Passiflora*. EFNs occur on floral bracts, external surfaces of sepals, leaf, stipules, and petioles, and serve two important roles in *Passiflora.* First, in some species (e.g. clade W of subgenus *Decaloba*), ocellate laminar nectaries mimic butterfly eggs and have been shown to deter gravid females from laying eggs on the plant (Williams and Gilbert 1981). Second, EFNs attract 'bodyguards' such as ants, predatory wasps, and parasitoids to the plants, which have been shown to reduce herbivory (Apple and Feener 2001; Hossaert-McKey et al. 2001; Wirth and Leal 2001). Another anti-herbivory defense is observed in subgenus *Decaloba* supersection *Bryonioides,* where plants have hooked-trichomes. Gilbert (1971) showed that the sturdy hooked trichomes of *P. adenopoda* puncture the integument of early instar *Heliconius* butterfly larvae, resulting in death. Reduction of host-plant use by gravid butterflies, coupled with the benefits of plant bodyguards and mechanical defenses such as hooked trichomes, likely increase plant fitness. In turn, this increased fitness drives selection for their presence, as well as for traits such as location and appearance, thus increasing morphological diversity.

The examples here highlight a small number of the many plant-animal interactions that involve *Passiflora.* They offer insight into the selective agents that underlie the morphological diversity observed across the genus. Additional studies that document specific plant-animal interactions associated with characters of interest in *Passiflora* are sorely needed, and will be immensely valuable in understanding the true significance of these characters in an ecological as well as phylogenetic context.

***A New Understanding of Subgenus Decaloba*—**The objectives of this analysis were to elucidate the position of subgenus *Decaloba* relative to the rest of the genus, test the infrageneric classification of Feuillet and MacDougal (2003), and examine relationships among the supersections. The combined molecular analysis has provided new insights into the evolution of this subgenus, resulting in the identification of clades in need of revision, as well as those that require additional investigation with regard to morphological characters, chromosome evolution, and/or molecular cloning. First, it is now clear that subgenus *Decaloba* as currently defined is monophyletic and consists of at least 230 species, eight major lineages that can be recognized as supersections, and several additional clades that need to be formally named as sections. As a result of examining the phylogenetic position of subgenus *Decaloba* relative to the other four subgenera, it is clear that subgenus *Deidamioides* is a polyphyletic assemblage that includes at least one species (*P. obovata*) best placed in subgenus *Decaloba.* The OW subgenus *Tetrapathea* is sister to subgenus *Decaloba,* the only other lineage in *Passiflora* that contains OW taxa. Subgenus *Tetrapathea* is sister to subgenus *Decaloba,* yet the basal lineages in subgenus *Decaloba* have a NW distribution. Thus, it would seem that the biogeographical history of these clades is quite complex, with multiple diversification events in both the NW and OW. Subgenus *Decaloba* consists of several clades that can be readily identified by suites of morphological characters (e.g. supersections *Pterosperma, Bryonioides,* and *Cieca*). However, many well-supported clades have no clear morphological synapomorphies (e.g. supersection *Disemma* and clade P [supersection *Bryonioides* with supersection *Decaloba*]). These are likely to be interesting examples of rapid speciation and adaptation, but such hypotheses will require further investigation into the rate and timing of these events.

This study has provided the first insights into relationships within subgenus *Decaloba* supersection *Decaloba,* the largest and least well-understood clade in the subgenus. Molecular data have elucidated two monophyletic lineages: a smaller lineage completely lacking extrafloral nectaries (clade S), and a larger one where plants display elaborate nectaries (clade W). The present study has also highlighted the need for additional research on chromosome numbers across the genus. The $n = 6$ group is well established, but more work is needed on the basal lineages of subgenus *Decaloba* (e.g. supersections *Pterosperma, Hahniopathanthus,* and *P. obovata*). Additional chromosome counts for subgenus *Tetrapathea* are also needed in order to shed light on the genome-level changes that occurred in the evolution of subgenus *Decaloba.*

This study has also emphasized the need for careful consideration of the specific evolutionary factors that may be influencing each locus used in phylogenetic reconstruction. *Passiflora* has a complex genetic history due in part to polyploidization and hybridization events in some clades. Subgenus *Decaloba* has its own suite of challenges including suspected cases of heteroplasmy, gene copy number evolution, and polyploidy. This study has provided many new insights into the evolution of subgenus *Decaloba,* but reveals as many new challenges that need to be addressed. Notably, strengthening support for the backbone of relationships

across the subgenus warrants focused effort, as does achieving greater resolution of relationships in supersection *Decaloba*. Such improvements will provide a solid basis for taxonomic revisions of the group, clarify evolutionary patterns and processes, and allow for more detailed studies of morphological character transformation and biogeography in this charismatic lineage.

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Appendix 1. Voucher information for DNA sources used in phylogenetic analyses. Each specimen is listed with collector name, number, and voucher location. Four taxa were unvouchered (indicated with a "*"); in these cases, species identifications were independently verified by sequencing vouchered specimens for nrITS, vouchers listed following the asterisk. Four voucher specimens collected by A. Hernández were lost during fieldwork in Colombia, and are indicated with a "§." Sequence used from Ossowski listed as unvouchered in GenBank is indicated with a "¥." Loci not sequenced for a particular taxon are indicated as "-." GenBank accession numbers listed in the following order: nrITS, ncpGS, ndhF and trnL-F.

Outgroup Genera—Adenia heterophylla (Blume) Koord. *S. Krosnick 4* (OS), JX470766, JX847204, JX679725, JX470860. ***Basananthe triloba*** (Bolus ex Schinz) W. J. de Wilde. *L. A. McDade & K. Balkwill 1254* (J, MO), DQ521293, JX847205, JX679726, JX470937. ***Malesherbia lanceolata*** Ricardi. *K. Gengler 54* (OS), JX470765, JX847206, JX679727, JX470861. ***Malesherbia weberbaueri*** var. ***weberbaueri*** Gilg. *K. Gengler 288* (OS), AY632697, -, JX679728, AY632722. ***Paropsia madagascariensis*** (Mast.) H. Perrier. *M. Zyhra 949* (WIS), -, -, AY757164, AY636105; *D. Hearn Mad037* (ARIZ), DQ521377, -, -, -. ***Turnera ulmifolia*** L. *S. Krosnick 296* (OS), -, -, JX679858, JX470917; *D. Hearn Mad009* (ARIZ), DQ521284, -, -, -.

Ingroup—Passiflora adenopoda DC. *S. Krosnick 258* (OS), AY632702, DQ458122, JX679729, AY632727. ***Passiflora allantophylla*** Mast. *S. Krosnick 25* (OS), DQ458069, DQ458123, JX679730, DQ458114. ***Passiflora alnifolia*** Kunth. *L. A. McDade 1339* (MO), -, JX847207, JX679731, JX470862. ***Passiflora altebilobata*** Hemsl. *S. Krosnick 3* (OS), DQ458078, DQ458124, JX679732, DQ458105. ***Passiflora ampullacea*** (Mast.) Harms. *S. Krosnick 262* (**P. M. Jørgensen 61434*), AY632720, -, JX679733, AY632745. ***Passiflora anadenia*** Urb. *S. Krosnick 621* (MO), JX470833, JX847208, JX679734, JX470863. ***Passiflora apetala*** Killip. *E. E. Kay 194* (MO), JX470822, JX847209, JX679735, JX470918. ***Passiflora apoda*** Harms. *A. Hernández 235* (MO), JX470779, JX847210, JX679736, JX470864. ***Passiflora arbelaezii*** L. Uribe. *S. Krosnick 259* (OS), AY632703, DQ463766, JX679737, AY632728. ***Passiflora arborea*** Spreng. *A. Hernández 234* (MO), JX470767, JX847211, JX679738, JX470865. ***Passiflora aurantia*** G. Forst. *S. Krosnick 24* (OS), AY632704, DQ458125, JX679739, AY632729. ***Passiflora aurantioides*** (K. Schum.) Krosnick. *B. Gray 2035IV* (OS), DQ458057, DQ463767, JX679740, DQ458085. ***Passiflora auriculata*** Kunth. *S. Krosnick 350* (OS, RSA-POM), DQ284532, DQ458126, JX679741, DQ284534. ***Passiflora berteroana*** Balb. ex DC. *S. Krosnick 610* (MO), JX470780, JX847212, JX679742, -. ***Passiflora bicornis*** Houston ex Miller. *K. Porter-Utley 418* (KESC), JX470836, JX847213, JX679743, JX470866. ***Passiflora bicrura*** Urb. *S. Krosnick 581* (MO), JX470834, JX847214, JX679744, -. ***Passiflora biflora*** Lam. *E. E. Kay 197* (MO), -, JX847215, JX679745, -; *K. Porter-Utley & D. Mondragon-Chaparro 327* (CICY), JX470837, -, -, JX470867. ***Passiflora boenderi*** J. M. MacDougal. *K. Porter-Utley 416* (KESC), JX470823, JX847259, JX679746, JX470868. ***Passiflora bryonioides*** Kunth. *D. H. Goldman 2266* (BH), JX470796, JX847217, JX679829, JX470869. ***Passiflora calcicola*** Proctor. *E. E. Kay 131* (MO), JX470813, JX847218, JX679748, JX470944. ***Passiflora capsularis*** L. *A. Hernández 262* (§), JX470806, JX847219, JX679749, -; *A. P. Lorenz-Lemke s. n.* (ICN), -, -, -, DQ123029. ***Passiflora chelidonea*** Mast. *A. Hernández 175* (MO), JX470838, JX847220, JX679750, JX470870; *T. Croat 93100* (MO), -, JX847221, JX679751, JX470871. ***Passiflora chrysosepala*** Schwerdtfeger. *P. M. Jørgensen & S. Chimbolema 2479* (MO), JX470839, JX847222, JX679793, JX470872. ***Passiflora cinnabarina*** Lindl. *G. Butler 66949* (CBG), AY632706, DQ458129, JX679753, AY632731. ***Passiflora cirrhiflora*** Juss. *H. Wouters s. n.* (MO), DQ458063, DQ463762, JX679754, DQ458093. ***Passiflora citrina*** J. M. MacDougal. *S. Krosnick 23* (OS), DQ458083, DQ458130, JX679755, DQ458101. ***Passiflora cobanensis*** Killip. *K. Porter-Utley, N. Martínez M. & M. A. Pérez F. 461* (KESC, HEM), JX470807, -, JX679756, JX470873. ***Passiflora cochinchinensis*** Spreng. *S. Krosnick 326* (OS),

DQ087421, DQ458150, JX679757, DQ087430. ***Passiflora colimensis*** Mast. & Rose. *K. Porter-Utley 490* (KESC, HEM), JX470797, JX847223, JX679758, -. ***Passiflora complanata*** J. M. MacDougal. *MacDougal 555GR* (MO), JX470827, JX847224, JX679759, JX470874. ***Passiflora coriacea*** Juss. *K. Porter-Utley P-68* (FLAS), JX470790, JX847225, JX679760, JX470919. ***Passiflora cubensis*** Urban. *E. E. Kay 233* (MO), JX470814, JX847226, JX679761, JX470875. ***Passiflora cuneata*** Willd. *J. M. MacDougal 431* (MO), JX470840, JX847227, JX679762, JX470920. ***Passiflora cupiformis*** Mast. *S. Krosnick 253* (OS), AY632708, DQ458132, JX679763, AY632733. ***Passiflora cupraea*** L. *E. E. Kay 227* (MO), JX470815, JX847228, JX679764, JX470876. ***Passiflora deidamioides*** Harms. *G. Mader s. n.* (ICN), EU907257, -, -, -; *R. Marquete 3080* (RB), -, -, -, DQ445920. ***Passiflora discophora*** Jørgensen & Lawesson. *S. Krosnick 352* (MO), JX470772, DQ463761, JX679765, DQ458092. ***Passiflora dolichocarpa*** Killip. *D. E. Breedlove 58312* (MO), JX470798, JX847229, JX679766, JX470921. ***Passiflora eberhardtii*** Gagn. *S. Krosnick 292* (OS), JX470778, JX847230, JX679767, JX470877. ***Passiflora edulis*** Sims. *R. Yockteng 46* (P), -, AY261575, -, -; *S. Krosnick 365* (RSA-POM), JX470774, -, JX679769, JX470878. ***Passiflora escobariana*** J. M. MacDougal. *J. M. MacDougal 3823* (MO), JX470808, JX847232, -, JX470879. ***Passiflora exsudans*** Zucc. *J. M. MacDougal 3015* (MO), JX470799, JX847233, -, JX470880. ***Passiflora filipes*** Benth. *D. H. Goldman 2153* (BH), AY632709, DQ458134, JX679853, AY632734. ***Passiflora foetida*** L. *S. Krosnick 351* (RSA-POM), DQ458053, DQ463760, -, DQ458094. ***Passiflora gilbertiana*** J. M. MacDougal. *B. E. Hammel 20544* (MO), JX470824, JX847234, JX679771, JX470881. ***Passiflora gracilis*** Jacq. ex Link. *S. Krosnick 413* (**J. M. MacDougal 1519GR87*), JX470800, JX847235, JX679772, -; ***Passiflora*** aff. ***gracillima*** Killip. *S. Krosnick 357* (MO), -, DQ463778, JX679773, -. ***Passiflora gracillima*** Killip. *P. M. Jørgensen & S. Chimbolema 2466* (MO), JX470773, -, -, JX470882. ***Passiflora guatemalensis*** S. Watson. *S. Krosnick 347* (**J. M. MacDougal 6297*), DQ087419, DQ458137, JX679774, JX470883. ***Passiflora hahnii*** (E. Fourn.) Mast. *R. Yockteng 65* (P), -, AY261591, -, -; *K. Porter-Utley, N. Martínez M. & R. Martínez C. 436* (KESC, HEM), JX470777, -, JX679775, JX470884. ***Passiflora helleri*** Peyr. *S. Krosnick 356* (RSA-POM), DQ458082, DQ458138, JX679776, DQ458106. ***Passiflora henryi*** Hemsl. *S. Krosnick 8* (OS), AY632710, DQ458128, JX679777, AY632735. ***Passiflora herbertiana*** Lindl. *S. Krosnick 255* (OS), AY632711, DQ458139, JX679778, AY632736. ***Passiflora hirtiflora*** Jørgensen & Holm-Nielsen. *R. Yockteng 67* (P), JX470841, JX847236, JX679779, JX470861. ***Passiflora hollrungii*** K. Schum. *R. Banka & S. Krosnick 2051* (LAE), DQ458081, DQ458140, JX679731, DQ458110. ***Passiflora holosericea*** L. *S. Krosnick 328* (OS), DQ087417, -, JX679781, DQ087426; *S. Krosnick 719* (**Porter-Utley 487*), JX470781, -, -, -. ***Passiflora ichthyura*** Mast. *M. H. Nee 36203* (MO), JX470842, JX847237, JX679782, JX470886. "**Passiflora ilamo**" J. M. MacDougal & MacVean sp. nov. ined. *K. Porter-Utley, N. Martínez M. & M. A. Pérez F. 434* (KESC, HEM), JX470825, -, -, -; *J. M. MacDougal 6203* (MO), -, JX847238, JX679783, -. ***Passiflora indecora*** Kunth. *R. Yockteng 70* (P), -, AY261596, -, -; *S. Krosnick 508* (MO), JX470843, -, JX679784, JX470938. "**Passiflora intricata**" J. M. MacDougal sp. nov. ined. *S. Krosnick 631* (MO), JX470844, JX847239, JX679785, -. ***Passiflora jatunsachensis*** Schwerdtfeger. *J. M. MacDougal 4983* (MO), -, JX847240, JX679786, JX470923. ***Passiflora jugorum*** W. W. Smith. *S. Krosnick 15* (OS), AY632712, DQ458143, JX679787, AY632737. ***Passiflora juliana*** J. M. MacDougal. *K. Porter-Utley & D. Mondragon-Chaparro 357* (CICY), JX470791, -, -, -; *K. Porter-Utley & D. Mondragon-Chaparro 359* (CICY, FLAS), -, JX847241, -, -; *K. Porter-Utley & D. Mondragon-Chaparro P-4* (FLAS), -, -, -, JX470924. ***Passiflora jussieui*** Feuillet. *S. Krosnick 261* (MO), JX470768, JX847242, -, JX470943. ***Passiflora karwinskii*** Mast. *K. Porter-Utley & C. Fernández Ríos 425* (KESC, HEM), JX470801, JX847243, JX679788, JX470887. ***Passiflora kuranda*** Krosnick & A. J. Ford. *S. Krosnick 334* (OS), DQ458060, DQ463773, JX679789, DQ458090. ***Passiflora lancearia*** Mast. *E. E. Kay 222* (MO), JX470845, -, JX679790, JX470888. ***Passiflora lancetillensis*** J. M. MacDougal & Meerman. *S. Krosnick 447* (MO), -, -, JX679791, JX470925; *S. Krosnick 564* (RSA-POM), JX470775, JX847244, -, -. ***Passiflora lancifolia*** Ham. *K. Porter-Utley & A. Paul P-51* (FLAS), JX470792, JX847245, JX679792, JX470926. ***Passiflora leptoclada*** Harms. *L. A. McDade 1368* (MO), JX470846, JX847246, JX679793, JX470889. ***Passiflora lobata*** (Killip) Hutch. ex J. M. MacDougal. *S. Krosnick 486* (MO), JX470802, JX847247, JX679794, JX470927. ***Passiflora lobbii*** Mast. *subsp.* ***ayacuchoensis*** Skrabal & Weigend. *M. Weigend 2000/385* (MO), JX470782, JX847248, -, JX470928. ***Passiflora lutea*** L. *J. M. MacDougal 224* (MO), -, -, JX679795, JX470890*B. W. Wells 4417* (US), DQ006022, -, -, -. ***Passiflora macrophylla*** Spruce ex Mast. *S. Krosnick 353* (MO), DQ458062, DQ463776, JX679796, DQ458097. ***Passiflora maestrensis*** Duharte. *E. E. Kay 231* (MO), JX470816, JX847249, JX679797, JX470891. ***Passiflora membranacea*** Benth. *S. Krosnick 19* (OS), AY632701, DQ458146, JX679798, AY632726. ***Passiflora mexicana*** Juss. *D. H. Goldman 1774* (BH), AY632713, DQ458147, JX679840, AY632738. ***Passiflora micropetala*** Mast. *J. M. MacDougal and Lalumondier 4982* (MO), JX470847, JX847250, JX679800, -. ***Passiflora microstipula*** L. Gilbert & J. M. MacDougal. *J. M. MacDougal 3012* (MO), DQ458066, DQ463769, JX679801, -. ***Passiflora misera*** Kunth. *S. Krosnick 371* (RSA-POM), JX470848, JX847251, JX679802, JX470892. ***Passiflora monadelpha*** Jørgensen & Holm-Nielsen. *A. Hernández 256* (§), JX470783, JX847252, JX679803, *P. Jørgensen, C. Ulloa, E. Narvaez & M. Lara 1774* (MO), -, -, -, DQ087427. ***Passiflora morifolia*** Mast. *S. Krosnick 311* (OS), DQ284535, DQ458155, JX679804, DQ284535. ***Passiflora multiflora*** L. *D. H. Goldman 2164* (BH), AY632715, DQ458152, JX679810, AY632740. "**Passiflora munchiquensis**" A. Hernández sp. nov. ined. *A. Hernández 252* (§), JX470784, JX847253, JX679806, JX470893. ***Passiflora murucuja*** L. *E. E. Kay 217* (MO), JX470817, -, -, JX470894*S. Krosnick 263* (OS), -, DQ458153, JX679807, -. ***Passiflora oblongata*** Sw. *E. E. Kay 177* (MO), JX470818, JX847254, JX679808, JX470895. ***Passiflora obovata*** Killip. *S. Krosnick 355* (MO), DQ458064, DQ463779, JX679809, DQ458098. ***Passiflora obtusifolia*** Sessé & Mociño. *L. A. McDade 1348* (MO), JX470793, JX847255, JX679810, JX470896. "**Passiflora occidentalis**" J. M. MacDougal & Hernández sp. nov. ined. *A. Hernández 257* (§), JX470849, JX847256, JX679811, JX470897. ***Passiflora orbiculata*** Cav. *S. Krosnick 601* (MO), JX470819, JX847257, JX679812, -. ***Passiflora ornithoura*** Mast. *J. M. MacDougal 6205* (MO), JX470826, JX847258, JX679813, -. ***Passiflora ovalis*** Vell. *G. Mader s.n.* (ICN), EU258359, -, -, -; *T. S. Nunes, J. G. Jardim, M. V. Moraes & B. M. Silva 745* (HUEFS), -, -, -, DQ123122. ***Passiflora pardifolia*** Vanderplank. *Vanderplank s.n.* (MO), JX470850, JX847259, JX679814, -. ***Passiflora pavonis*** Mast. *K. Porter-Utley & N. Martinez M. & M. Perez 467* (KESC, HEM), JX470831, JX847260, JX679815, -. ***Passiflora pedicellaris*** J. M. MacDougal. *J. M. MacDougal 6215* (MO), JX470776, JX847261, JX679816, -. ***Passiflora pendens*** J. M. MacDougal. *J. M. MacDougal 571* (MO), JX470803, JX847262, JX679817, JX470929. ***Passiflora penduliflora*** Bert. ex DC. *E. E. Kay 230* (MO), JX470820, JX847263, JX679818, JX470898. ***Passiflora perakensis*** Hall. f. *S. Krosnick 314* (OS), DQ087422, DQ458158, JX679819, DQ087431. ***Passiflora perfoliata*** L. *K. Porter-Utley & A. Paul P-55* (FLAS), JX470821, JX847264, JX679820, JX470899. ***Passiflora pilosa*** Ruiz & Pav. ex DC. subsp. ***dimidiata*** J. M. MacDougal. *K. Porter-Utley & D. Mondragon-Chaparro 341* (CICY, FLAS), -, JX847265, JX679821, -. ***Passiflora pilosa*** Ruiz & Pav. ex DC. subsp. ***pilosa***. *J. M. MacDougal 528GR* (MO), JX470804, -, -, JX470930. ***Passiflora pittieri*** Mast. *R. Boender s.n.* (MO), DQ995476, DQ995474, JX679822, DQ995475. ***Passiflora porphyretica*** Mast. var. ***angustata*** Killip. *J. M. MacDougal 2027* (MO), -, JX847266, JX679726, JX470939. ***Passiflora punctata*** L. *S. Krosnick 363* (RSA-POM), JX470851, JX847267, JX679824, -. ***Passiflora pusilla*** J. M. MacDougal. *K. Porter-Utley 420* (KESC), JX470809, -, JX679825, JX470900. ***Passiflora pyrrhantha*** Harms. *S. Krosnick 391* (MO), JX470771, -, JX679826, JX470901. ***Passiflora quadrangularis*** L. *S. Krosnick 1* (OS), AY636106, DQ463780, JX679827, AY636106. ***Passiflora rovirosae*** Killip *K. Porter-Utley & D. Mondragon-Chaparro 309* (CICY, FLAS), JX470810, JX847268, JX679828, JX470931. ***Passiflora rubra*** L. *L. A. McDade 1358* (MO), JX470811, JX847269, JX679829, -. ***Passiflora rufa*** Feuillet & J. M. MacDougal. *J. M. MacDougal 6019* (MO), JX470789, JX847270, JX679830, JX470902. ***Passiflora rugosissima*** Killip. *K. Porter-Utley, N. Martínez M. & R. Martínez C. 428* (KESC, HEM), JX470828, JX847271, JX679831, JX470903; *R. R. Santos 520* (MO), -, JX847272, JX679832, JX470932. ***Passiflora sagasteguii*** Skrabal & Weigend. *T. Henning 3* (MO), JX470785, JX847273, JX679833, JX470904. ***Passiflora sandrae*** J. M. MacDougal. *R. Yockteng 112* (P), -, AY261641, -, -; *J. M. MacDougal 6036* (MO), JX470852, -, JX679834, JX470940. ***Passiflora sanguinolenta*** Mast. *S. Krosnick 28* (OS), JX470812, -, JX679835, JX470905; *C. Morse 199800014* (CONN), -, AY261643, -, -. ***Passiflora serratodigitata*** L. *S. Krosnick 264* (OS), AY636108, AY261645, -, AY636109. ***Passiflora sexflora*** Juss. *S. Krosnick 626* (MO), JX470830, JX847275, JX679837, JX470906; *K. Porter-Utley & A. Paul P-48* (FLAS), JX470829, JX847274, JX679836, -. ***Passiflora siamica*** W. G. Craib. *S. Krosnick 346* (OS), DQ087423, DQ458162, JX679838, DQ087432. ***Passiflora sicyoides*** Schlecht. & Cham. *K. Porter-Utley & D. Mondragon-Chaparro 338* (CICY, FLAS), JX470805, -, -, JX470933; *R. H. Magana 6414* (MO), -, JX847276, JX679839, -. ***Passiflora sodiroi*** Harms. *J. M. MacDougal 1941* (MO), JX470786, JX847277, -, JX470907. ***Passiflora solomonii*** L. K. Escobar. *J. L. Carretero 1031* (MO), JX470787, -, JX679840, JX470908. ***Passiflora sp. nov.*** aff. ***eckmanii*** Liogier. *S. Krosnick 595* (MO), JX470835, JX847231, JX679768, JX470922. ***Passiflora sphaerocarpa*** Tr. & Planch. *S. Krosnick 418* (MO), JX470769, JX847278, JX679841, JX470909. ***Passiflora tacanensis*** Port.-Utl. *K. Porter-Utley & N. Martinez M. & R. Martinez C. 435* (KESC, HEM), JX470794, JX847279, JX679842, JX470910. ***Passiflora talamancensis*** Killip. *R. Yockteng 125* (P), -, AY261653, -, -; *J. M. MacDougal 410* (MO), -, -, JX679843, -; *J. Vanderplank 1243/07a* (NCP), AF454809, -, -, AF454793. ***Passiflora tatei*** Killip & Rusby. *S. Krosnick 387* (RSA-POM), JX470853, JX847280, JX679844, JX470941. ***Passiflora telesiphe*** Knapp & Mallet. *R. Yockteng 127* (P), JX470854, JX847281, JX679845, -. ***Passiflora tenella*** Killip. *B. B. Klitgaard, B. Merino, P. Lozano*

& T. Delgado 426 (MO), JX470832, JX847282, JX679846, JX470934. ***Passiflora tenuiloba*** Engelm. *D. H. Goldman 1770* (BH), AY632719, DQ458154, -, AY632744. ***Passiflora tetrandra*** Banks & Sol. ex DC. *S. Krosnick 266* (OS), AY632721, DQ463764, JX679847, AY632746. ***Passiflora tina*** Boender & Ulmer. *S. Krosnick 569* (RSA-POM), JX470770, JX847283, JX679848, JX470911. ***Passiflora tricuspis*** Mast. *S. Krosnick 385* (RSA-POM), JX470855, JX847284, JX679849, JX470935. ***Passiflora truncata*** Regel. *S. Krosnick 465* (MO), JX470788, JX847285, JX679850, JX470936. ***Passiflora tuberosa*** Jacq. *L. A. McDade 1360* (MO), JX470856, JX847286, JX679851, -. ***Passiflora tulae*** Urban. *S. Krosnick 345* (OS), -, DQ458164, JX679852, JX470912. ***Passiflora urnifolia*** Rusby. *L. A. McDade 1344* (MO), JX470857, JX847287, JX679853, JX470942. ***Passiflora vespertilio*** L. *R. Yockteng 137* (P), -, AY261668, -, *J. M. MacDougal 6022* (MO), JX470858, -, -, JX470913. ***Passiflora*** cf. ***viridescens*** L. K. Escobar. *P. M. Jørgensen & S. Chimbolema 2468* (MO), JX470859, JX847288, JX679851, JX470914. ***Passiflora vitifolia*** Kunth *K. Porter-Utley 98–1* (FLAS), -, -, JX679855, JX470915; *Tolima Botanical Garden JBAVH 3320* (P), -, AY261670, -, -; *A. M. Ossowski s.n.* (¥), AF454796, -, -, -; ***Passiflora wilsonii*** Hemsl. *J. Wen 5973* (F), DQ087425, DQ458165, JX679856, DQ087434. ***Passiflora xiikzodz*** J. M. MacDougal. *K. Porter-Utley & D. Mondragon-Chaparro 387* (CICY), JX470795, -, JX679857, JX470916.