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Phylogenetic Relationships and Chromosome Number Evolution in *Passiflora*

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Abstract. The phylogenetic relationships and chromosomal evolution of the diverse tropical genus *Passiflora* (Passifloraceae) are explored using data from two chloroplast markers: the *rpoC1* intron and the *trnL/trnT* spacer region. A survey of the presence or absence of the *rpoC1* intron in 136 species representing 17 of Killip's (1938) 22 subgenera of *Passiflora* and four other genera in the Passifloraceae revealed intron losses in 46 taxa. A minimum of two losses were confirmed by a parametric bootstrap approach on sequence data from the *trnL/trnT* chloroplast non-coding region for 61 taxa. The results of phylogenetic analyses of the *trnL/trnT* sequence data support the reduction of Killip's 22 subgenera to four as proposed in a new classification system by Feuillet and MacDougal (2004). The monophyly of the '*n*=6' and '*n*=9' chromosomal and morphological groups is strongly supported. In addition, these data indicate that *Passiflora biflora*, or closely related species, is the likely continental sister to the red-flowered Caribbean taxa, while *P. auriculata* is weakly supported as the New World sister to the Old World *Passiflora*s. Finally, character optimization of chromosome numbers on the phylogenetic tree supports x=12 as the base chromosome number for *Passiflora*.

Passiflora L. (Passifloraceae) is a genus of more than 530 species of climbing herbs, trees, and woody lianas. They are a conspicuous part of the Neotropical flora and their distribution extends from southern Argentina northward, throughout Central America and Mexico into the southern United States. In addition, 20 species are restricted to the Old World in the tropical and sub-tropical regions of southeast Asia, Australia, and New Zealand. The wide distribution of this species-rich genus affords a variety of life-history strategies, from the weedy colonizers of secondary vegetation to the large canopy lianas of primary forest.

Passiflora is widely cultivated today for its ornamental flowers and edible fruit. The presence of a corona and an androgynophore gives the flowers a striking morphology that has long been a favorite of horticulturists and hobbyists alike. This unique morphology caught the attention of the conquistadors who took it as a symbol of the crucifixion of Christ and consequently a sign that the New World would be converted to Christianity (Uribe 1955; Kugler 2004). This religious symbolism gave the plant their common name, ''Passion Flower,'' referring to the passion of Christ.

Species in *Passiflora* are typically tendril-bearing vines with a non-pedunculate inflorescence and one or two sessile, pentamerous flowers. Although most species are herbaceous vines or woody lianas, members of *Passiflora* subgenus *Astrophea* (DC.) Mast. tend to be shrubs or true trees. The size of the flowers and the degree of complexity in the corona vary widely throughout the genus. The innermost row of the corona, the operculum, interacts with a membrane (limen) at the base of the androgynophore to form a lip or cup over the nectary preventing access by ineffective pollinators. These three characters, corona, operculum, and limen, have historically been heavily relied upon as taxonomic characters for delimiting relationships within *Passiflora*.

The most recent monograph for *Passiflora* (Killip 1938) divided the genus into 22 subgenera. It is problematic for taxonomists for several reasons. First, over 120 new species have been described since. Second, only the New World species were included. Third, many of the ranks below subgenus were invalidly published. Although new insights have been gained into *Passiflora* relationships at the subgeneric level (De Melo et al 2001; Muschner et al. 2003; Yockteng and Nadot 2004), there is still insufficient resolution and support for monophyletic groups below the this level. More information is still needed to ultimately address evolutionary questions at the species level.

The two largest lineages in the genus correspond to Killip's subgenera ''*Plectostemma*'' and ''*Granadilla*'' (*Decaloba* and *Passiflora*, respectively, following ICBN rules). Species of *Decaloba* (220 spp) are mostly herbaceous vines with small flowers and fruit. They occur throughout the entire distribution of the genus including the Old World. *Decaloba* species have an ancestral chromosome number of *n*=6 (Snow and MacDougal 1993) and as such, are informally referred to as the ''*n*=6 group.'' In addition to *Decaloba*, this group includes Killip's subgenera *Astephia*, *Psilanthus*, *Pseudomurucuja*, *Murucuja*, *Chloropathanthus*, and *Apodogyne* (Feuillet and MacDougal 2004). Conversely, species in subgenus *Passiflora* (220 spp) are woody vines with showy flowers and edible fruit. The ancestral chromosome number of *n*=9 has led to the designation of subgenus *Passiflora*, along with subgenera *Adenosepala*,

TABLE 1. Data analyses and tree statistics for the four parsimony analyses of the *trnL/trnT* spacer region. The number of excluded characters refer to the number of base pairs comprising gap regions that were removed in analysis B or the number of positions removed due to homoplasy concerns in analysis D. C. I. values were calculated in PAUP* with uninformative characters excluded.

Analysis	Optimality criterion	# of characters excluded	Gap interleaved characters	Gap treatment	Parsimony informative characters	Number of trees	Tree length	C. I.	R. I.	H. I.
A	Parsimony	None	None	Missing data	88	200,000	265	0.800	0.913	0.200
B	Parsimony	None	75	Missing data	120	200,000	384	0.745	0.883	0.255
C	Parsimony	279	75	Removed	99	200,000	302	0.719	0.880	0.281
D	Parsimony	64	None	Missing data	79	200,000	229	0.795	0.916	0.205

Tacsonia, *Manicata*, *Calopathanthus*, *Dysosmia*, *Dysosmioides*, *Distephana*, *Rathea*, *Tacsonioides* and *Tacsoniopsis* as the ''*n*=9 group'' (Feuillet and MacDougal 2004).

This dichotomy between the ''*n*=6'' and ''*n*=9'' groups has been well known among *Passiflora* researchers for decades (L. Escobar unpubl.; P. M. Jørgensen, J. MacDougal, C. Feuillet, pers. comm.; Presting 1965). However, the first molecular evidence to support the evolutionary integrity of these groups was published by Downie et al. in 1996. In this study, Downie surveyed a wide diversity of angiosperms for the presence or absence of the *rpoC1* intron to assess its phylogenetic utility. Although the marker was homoplastic for higher order relationships, preliminary evidence suggested that it might be useful in studies at the subfamilial level, particularly in *Passiflora*. In their study of 10 species of *Passiflora*, all taxa with a chromosome number of *n*=6 lacked the *rpoC1* intron, while those that had *n*=9 retained it. The sample size was too small in the Downie et al. study to make any conclusive phylogenetic inferences for *Passiflora* as a whole, but indicated that a more intensive sampling strategy might provide additional support for the delineation of these two groups. As part of our work exploring the phylogenetic relationships in *Passiflora*, we expanded the intron survey and combined this character with a chloroplast DNA sequence phylogeny. The combined results are presented here along with discussions of *Passiflora* relationships and taxonomy, chromosome number evolution, and the relative utility of these makers for resolving evolutionary history.

MATERIALS AND METHODS

Chloroplast Phylogeny. Sampling included 61 species, 57 from genus *Passiflora* representing 16 of Killip's subgenera and one species each from genera *Adenia* Forssk., *Dilkea* Mast., *Tetrapathea* DC., and *Tetrastylis* Killip as outgroups (see Appendix). All species included in the phylogenetic analyses were also examined in the intron survey. Total DNA was isolated from single individuals using either fresh leaf material, silica dried field collections, or herbarium specimens (Appendix 1). Protocols for DNA isolation, amplification and sequencing followed those described in Barber et al. (2002). Both strands were sequenced for all taxa.

Sequences were edited using Sequencher v. 3.1 (Gene Codes Corp.). Clustal X (Thomson et al. 1994) was used to perform multiple alignments using pairwise comparisons to obtain an initial alignment. In order to identify potential repeat motifs, we used the Megalign program in DNAstar (DNAstar Madison, WI). This was followed by manual adjustment using two general guidelines: 1) the number of gaps needed to align sequences was minimized (Golenberg 1993) and 2) in areas of tandem repeats, sequences were aligned to maximize percent similarity and minimize the number of substitutions between sequences (Aldrich 1988).

Parsimony as implemented in PAUP* v. 4.0b10 (Swofford 2002) was used for analyses A-D (Table 1). We used the heuristic search method with 100 random addition replications with step-wise addition of taxa, tree bi-section reconnection and MulTrees on. In order to increase the probability that all islands of most-parsimonious trees would be sampled, we ran the analysis on three different computers to find the length of the shortest tree. This was then set as the upper limit for tree length for the final analyses and only 2000 trees equal to this value were saved for each replicate. All subsequent analyses were run on ''phylocluster,'' a NPACI Rocks (http://rockscluster.org) cluster comprised of 24 AMD 1800+ processors. Levels of homoplasy were estimated by calculating the C.I., H.I., and R.I. in PAUP*. Support for monophyletic groups was evaluated by bootstrap analyses using 100 replicates each with a 'MaxTrees' limit of 2000 per replicate. These methods were utilized for all parsimony analyses.

We evaluated the effects of gap coding by doing four different analyses using the parsimony methods described above (Table 1). The first analysis (A) included all sequence characters and the gap regions were treated as missing data. Gaps were then coded as binary characters utilizing the simple gap coding method of Simmons and Ochoterena (2000) and subsequently appended to the sequence data. Since indel events are frequent occurrences in chloroplast non-coding regions (Kelchner 2000), removing the resulting gap regions from the analysis reduced the number of informative characters by 18%. Due to the loss of such a significant amount of data in an already information-poor region, we analyzed the data with gap regions included and treated as missing data (analysis B) and with gap regions removed (analysis C). Finally, for analysis D, the region corresponding to base pairs 425–489 was removed. This region appears to be a ''hot-spot'' for indel formation and was removed from this analysis to estimate the impact of such regions on levels of homoplasy in our data. Analyses A, B, C, and D correspond to TreeBASE (study accession S1330).

We used Bayesian analyses to evaluate statistically potential monophyletic groups in *Passiflora*. The GTR+G model of sequence evolution was chosen by ModelTest v. 3.06 (Posada and Crandall 1998) as the best fit for these data. MrBayes v. 3.0 (Huelsenbeck and Ronquist 2003) was used for all analyses. Uniform prior distributions were used for 1) alpha shape parameter for the gamma distribution (0.0, 10.0), 2) instantaneous rate matrix (0.0, 100.0), and 3) branch lengths (0.0, 10.0). Base frequencies were estimated and the starting trees were randomly generated. We used a Metropolis-coupled MCMC analysis, where four chains were incrementally heated and run simultaneously. Two separate analyses were done, running for 1–3 $X10^6$ respectively and every 100^{th} tree was sampled. Trees that were generated prior to the plateau of parameter values were deleted. The remaining trees were opened in PAUP* vs. 4.10b (Swofford 2002) and used to generate a 50% Majority Rule tree. Each analysis was subsequently repeated to verify the results.

Intron Survey. Representative taxa from each subgenus, sec-

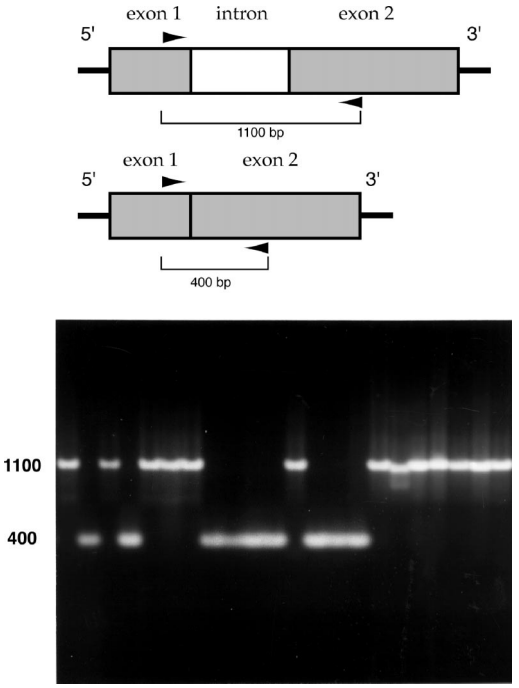

FIG. 1. Top: Diagrammatic representation of *rpoC1* intron. Arrows indicate the relative positions of the two priming sites. Shaded boxes represent coding regions of the *rpo*C1 gene, while the intron disrupting this gene is depicted as white. A fragment size of 1100 bp illustrates the retention of the *rpoC1* intron while a fragment of 400 bp illustrates an intron loss. Bottom: Gel photograph of *rpoC1* intron results.

tion, and series were sampled when available. In total, 136 species from 19 of the Killip's 22 subgenera within *Passiflora* were surveyed for the presence of the *rpoC1* intron. In addition, four other genera in the Passifloraceae were included as outgroups (Appendix 1).

DNA was amplified by polymerase chain reaction (PCR) using the forward 5′ *rpoC1* exon and reverse 3′ *rpoC1* exon primers described in Downie et al. (1996). PCR amplification methods were identical to those described for the *trnL/trnT* sequencing study. Positive and negative intron controls (*Passiflora caerulea* and *P. suberosa*, respectively) that were confirmed by sequencing (Downie et al. 1996) were included in each set of amplification reactions as well as a negative cocktail control (no template). Methods for determining the presence or absence of the *rpoC1* intron followed those described in Downie et al. (1996). Faint bands were not scored. Due to the significant difference in size of fragments with or without the intron, gel interpretation was unambiguous (Fig. 1). These results are listed in Appendix 1.

A parametric bootstrap approach (SOWH test Swofford et al. 1996; Goldman et al. 2000) was utilized to determine whether single or multiple intron loss hypotheses were equally likely given our data. The data was pruned to only include the clade in which intron losses had been detected. This data set was then analyzed using the Parsimony optimality criterion as implemented in PAUP* v. 4.0b10 with 100 random addition replicates, Multrees on and TBR. A second search, where the intron loss was constrained to be monophyletic, followed using the same methods as the unconstrained search. The tree with the best likelihood score from this second analysis was used to simulate 100 data sets using Seq-Gen (Rambaut and Grassly 1997). Each data set was then analyzed in PAUP* with and without the constraint to generate a distribution of tree-length differences. An unconstrained vs. constrained tree length difference greater than 95% of the simulated data sets would allow us to reject the null hypothesis of a single intron loss.

RESULTS

Chloroplast DNA Sequence Phylogeny. There was substantial length variation among taxa in the *trnL/trnT* spacer consistent with other analyses of this region (Kelchner 2000). Unaligned sequences varied in length between 454 bp in *Passiflora deidamioides*, and 577 bp in *Adenia mannii* while the aligned length is 631 bp. Gap coding resulted in 75 characters appended to the sequence data for analyses B and C.

The nucleotide sequence diversity of the *trnL/trnT* spacer is conserved among taxa as illustrated by the low number of parsimony informative variable characters (Table 1). However, the region is clearly heterogeneous in both base composition (A/T rich with 42% A, 12.5% C, 16.7% G and 28.8% T) and the propensity for indels to occur. This is particularly evident within the large poly A–T tract corresponding to bp 425–489 in which numerous indel events of varying lengths appear to have occurred. Determining sequence homology within this and similar areas was problematic. This region, comprising 64 aligned bp, was therefore excluded from analysis D. For all data sets, the number of transversions estimated in MacClade were nearly twice the number of transitions, similar to values reported for other chloroplast non-coding regions (Morton 1995).

The tree statistics for all parsimony analyses are summarized in Table 1. When gaps were treated as missing data (analysis A), all tree statistics were slightly better than analyses when gaps were coded and appended to the sequence data (Analyses B and C). However, bootstrap support for most monophyletic groups was significantly higher when gaps were coded and the gap regions treated as missing data (Analysis B, Table 1). This is likely due to the greater number of parsimony informative characters available in this analysis. When the poly A-T regions were removed (Analysis D), there was no improvement in either number of trees found or tree statistics. In fact, both the C.I. and the H.I. were slightly lower than when that region was included in Analyses A. The drop in the percentage of parsimony informative characters from 14.4% (Analysis A) to 13.7% (Analysis D) probably accounts for this slight difference. There were no differences in topology between any of the analyses for strongly supported groups. Since the tree statistics are not significantly different for any of the analyses, our results do not support the exclusion of poly A-T regions as we anticipated.

ModelTest selected the GTR+G model of evolution for use in our Bayesian analyses. There were no major differences in topology between analyses run for one million and three million generations. These results

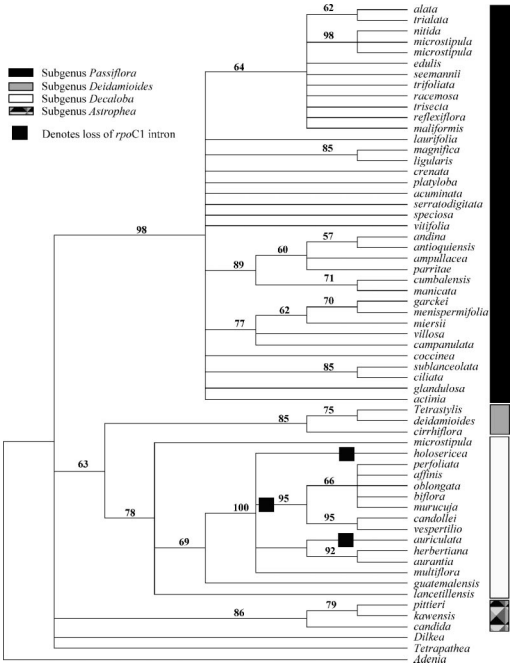

FIG. 2. Strict consensus of 200,000 most parsimonius trees using the *trnL/trnT* chloroplast spacer region. All trees were 265 steps in length. Bootstrap values above 50% are shown in bold. No gap data were included in this analysis (Analysis A; Table 1). The classification used in this figure is that of Feuillet and MacDougal (2004). All specific epithets are from the genus *Passiflora* unless otherwise noted.

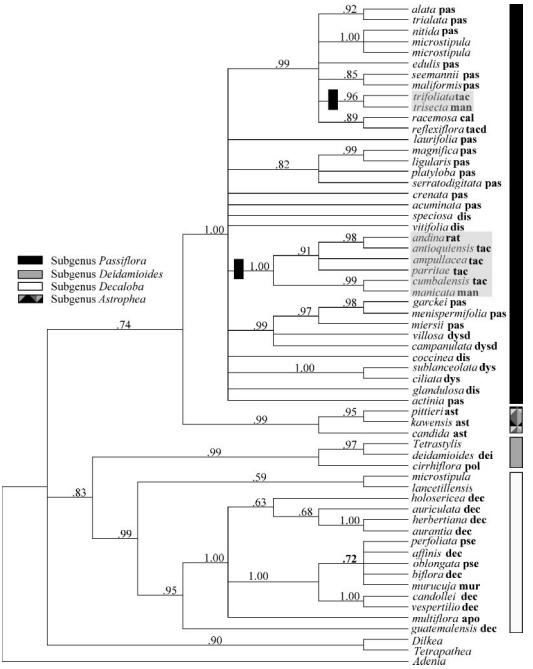

FIG. 3. 50% majority rule tree from a Bayesian analysis of the *trnL/trnT* run for three million generations. Numbers above the branches are posterior probabilities. Bars depict the subgenera of *Passiflora* sensu Feuillet and MacDougal (2004). Highlighted taxa are those that possess the Andean hummingbird pollination syndrome and optimization of this character onto the tree is illustrated by a black box. Killip's (1938) subgenera are in bold and abbreviated after each species name as follows: *Apodogyne* (apo), *Astrophea* (astr), *Calopathanthus* (cal), *Decaloba* (dec), *Deidamioides* (dei), *Distephana* (dis), *Dysosmia* (dys), *Dysosmioides* (dysd), *Manicata* (man), *Murucuja* (mur), *Passiflora* (pas), *Polyanthea* (pol), *Pseudomurucuja* (pse), *Rathea* (rat), *Tacsonia* (tac), *Tacsonioides* (tacd).

were confirmed by running each analysis in duplicate. The likelihood values reached a plateau at −2418.2594 for one million generations and −2416.4027 for three million generations. The numbers of burn-in trees deleted were 60,700 trees for one million generations and 118,700 trees for three million generations. In both cases, the number of generations utilized clearly was in excess of that needed for stabilization of all values. Recent work has suggested that the posterior probabilities of Bayesian analyses can often be misleading, indicating much higher levels of support for a particular clade when compared to bootstrap values (Suzuki et al. 2002; Wilcox et al. 2003). Although the levels of support in the Bayesian analyses were substantially higher than when Parsimony methods were used, the topologies of all of the trees are nearly identical (Figs. 2, 3). A single most parsimonious tree is depicted as a phylogram (fig. 4).

There were no major differences in topology between any of the analyses for groups that had 70% or higher bootstrap support or a posterior probability of 0.70. The only detectable variation between any of the different treatments is on branches that have little support in both parsimony and Bayesian analyses and collapse on the parsimony consensus trees (Figs. 2, 4). Tree statistics for all parsimony analyses are summarized in Table 1. The ''n=9'' and ''n=6'' groups each formed monophyletic groups in all trees regardless of how the data was treated.

Intron Distribution. Interpretation of intron data was straightforward. Visualization of the PCR products on the gels revealed a fragment of 1100 bp when the *rpoC1* intron was present and a fragment of 400 bp when absent (Fig. 1). The presence or absence of the intron is listed by species in the Appendix. The intron was absent in subgenera *Astephia*, *Chloropathanthus*, *Murucuja*, *Pseudomurucuja*, *Psilanthus*, and in all sections of *Decaloba* with the exception of supersections *Hahniopathanthus*, *Disemma*, and *Pterosperma* sensu Feuillet and MacDougal (2004) (Appendix 1). The intron is present in all species surveyed from subgenera *Apodogyne*, *Adenosepala*, *Tryphostemmatoides*, *Deidamioides*, *Tacsonia*, *Manicata*, *Distephana*, *Calopathanthus*, *Passiflora*, *Dysosmia*, *Dysosmioides*, *Polyanthea* and *Astrophea*. Taxa from four additional genera of Passifloraceae, *Dilkea*, *Tetrapathea*, *Adenia*, and *Tetrastylis*, also retained the intron. *Decaloba* is the only subgenus in which there

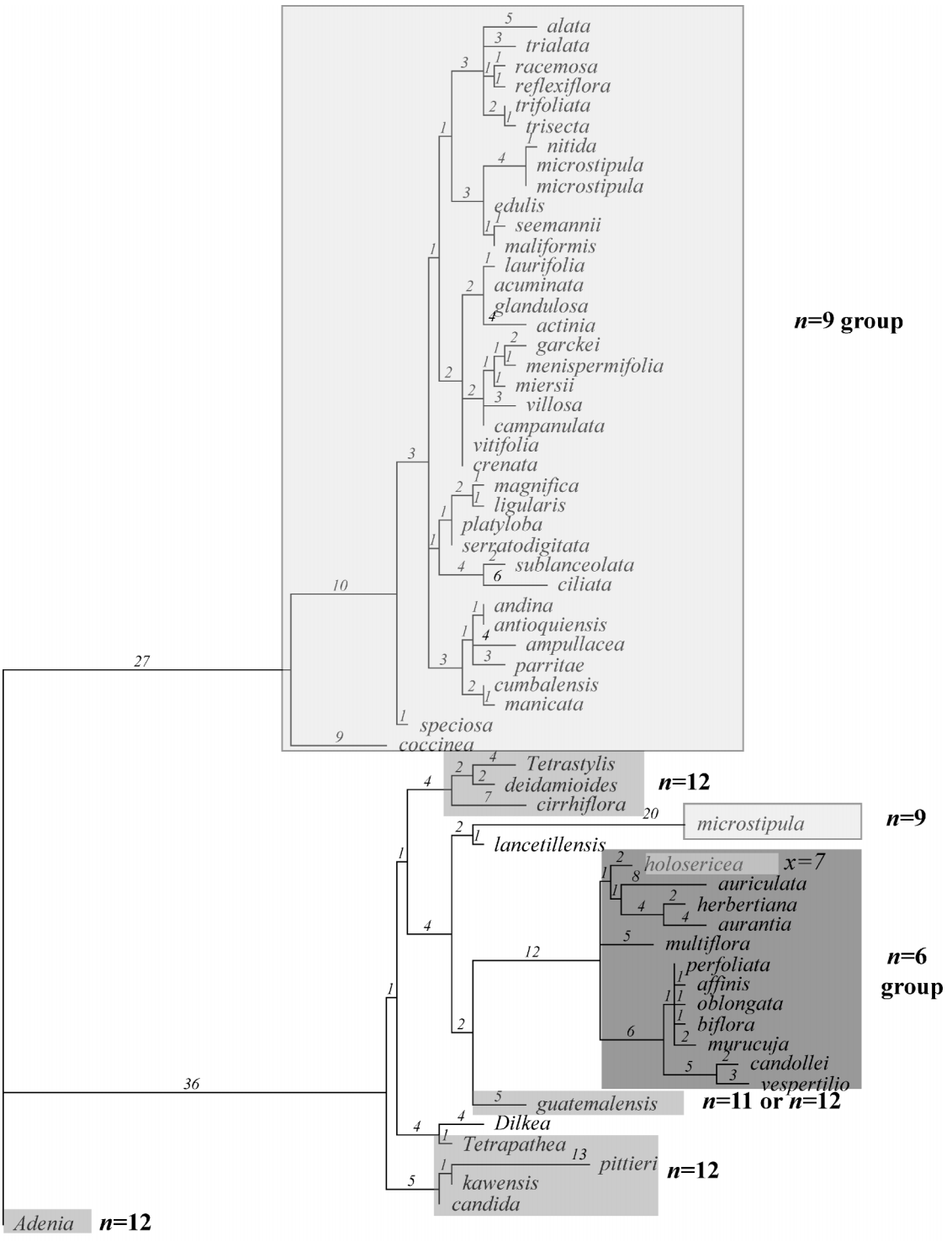

FIG. 4. Phylogram of 1 of 200,000 trees from analysis A of *trnL/trnT* sequence data illustrating the distribution of chromosome numbers in the genus. Not all taxa in each group have been counted. See Appendix for list of taxa with known chromosome numbers. Any known exceptions are highlighted. Branch lengths represent character state changes and the number of changes is listed above each branch.

TABLE 2. Summary of subgeneric classification of *Passiflora* according to Feuillet and MacDougal (2004). The number of species in each of the four subgenera is in parentheses. Killip's (1938) subgenera are listed in parentheses under the subgeneric names used by Feuillet and MacDougal (2004). All ranks are subgeneric except for *Tetrastylis* and *Tetrapathea,* which are generic and denoted with an *.

Subgenus	Defining characters	Distribution
Astrophea (DC.) Mast. (57) (*Astrophea*)	Tendrils present or absent Simple unlobed leaves, margins entire Glands at base of petiole or margins of leaf blade Hypanthium campanulate or tubular Operculum entire, membranous, or tubular Lianas, trees or shrubs	Lowland tropical South America
Decaloba (DC.) Rchb. (220) (*Apodogyne, Astephia, Chloropathanthus, Decaloba, Murucuja, Pseudomurucuja, Psilanthus*)	Tendrils present Palmate venation Glands are laminar Hypanthium flat Plicate operculum Short, fleshy limen Herbaceous vines	Throughout Central and South America and Southern US. 20 species in South East Asia, Australia and New Zealand
Deidamioides (Harms) Killip (13) (*Deidamioides, Polyanthea, Tetrastylis*, Tetrapathea* Tryphostemmatoides*)	Peduncles terminate in tendril Leaves entire or lobed Glands on petiole Hypanthium campanulate or flat Operculum plicate Lianas or herbaceous vines	Throughout Central and South America and Mexico. One species in New Zealand.
Passiflora L. (240) (*Adenosepala, Calopathanthus, Dysosmia, Dysosmioides, Distephana, Manicata, Passiflora, Rathea, Tacsonia, Tacsonioides, Tacsoniopsis*)	Tendrils present Leaves entire or lobed Glands on petioles, stipules, and margins of leaves Hypanthium tubular or campanulate Operculum tubular or filamentous Membranous limen Herbaceous vines or woody lianas	Throughout Central and South America and Southern US

was any variation in intron presence or absence detected with our sampling strategy.

Distribution of Intron on cpDNA Sequence Phylogeny. The loss of the *rpoC1* intron was optimized onto the phylogeny constructed from cpDNA sequence data using the *trnL/trnT* spacer region (Fig. 2). The loss occurred on the branch leading to *Passiflora auriculata* in a weakly supported clade with Old World species *P. herbertiana*, and *P. aurantia*, which retain the intron. *Passiflora holosericea*, whose relationship is unresolved, also has a loss. Another intron loss occurs on the strongly supported branch including seven species traditionally considered to part of the ''$n=6$'' group. Thus, the distribution of the intron loss on the cpDNA sequence phylogeny indicates that this change has occurred a minimum of two times, in the evolution of *Passiflora*.

Multiple losses were confirmed by performing a parametric bootstrap analysis (Swofford et al. 1996; Goldman et al. 2000). Due to computational limitations, the data set was pruned to include only subgenus *Decaloba* and its sister group (*Passiflora deidamioides*, *Tetrastylis ovalis*, and *P. cirrhiflora*; Fig. 2). The unconstrained parsimony analysis of this group found only ten trees and all intron losses occurred within one clade (BS=100). The results of the parametric bootstrap analysis allow us to reject the null hypothesis of a single loss with a p value <0.05. We can say with confidence that there have been a minimum of two losses of the *rpoC1* intron in *Passiflora*.

DISCUSSION

Chloroplast Sequence Phylogeny. Non-coding regions of cpDNA were originally thought to be a potentially rich source of variation for phylogenetic analyses (Curtis and Clegg 1984; Taberlet et al. 1991; Clegg et al. 1994). Recent studies have shown that this is not always the case and in fact, these regions are often surprisingly conserved (Bailey and Doyle 1999; Potter and Luby 2000; Raubeson and Jansen 2004). This is clearly the case for the *trnL-trnT* spacer region in *Passiflora*. Although indels are prevalent in this region, the difficulty in determining their homology limits their phylogenetic utility. The overall tree topology is highly congruent with both morphological data (Table 2) and chromosome number trends (Fig. 4). This is most evident in the strongly supported ''$n=9$'' and ''$n=6$'' clades (Fig. 4). There is, however, little support for the monophyly of most of Killip's (1938) subgenera and sections as has long been suspected by many workers. Although many of the terminal nodes are unresolved in our phylogeny, particularly in subgenus *Passiflora*, species relationships within *Decaloba* are suf-

ficiently resolved to enable us to evaluate the utility of the *rpoC1* intron for identifying monophyletic groups within the genus. Additionally, several strongly supported clades provide valuable insight into questions of historical interest in *Passiflora*, including pollination syndromes and chromosome number evolution, and allow us to evaluate a newly proposed classification system for the genus.

INTRON DISTRIBUTION IN *PASSIFLORA*. Chloroplast structural rearrangements have been shown to be powerful characters for elucidating phylogenetic relationships (Jansen and Palmer 1987; Bruneau et al. 1990; Downie and Palmer 1992; Raubeson and Jansen 1992, 2004; Soltis and Soltis 1998). However, certain types of structural changes are more likely to show homoplasy than others. For example, inversions in the chloroplast genome have been found to be extremely reliable characters with low levels of homoplasy (Raubeson and Jansen 2004) while intron and gene losses are more likely to occur in parallel (Downie et al. 1991, 1996). In *Passiflora*, the distribution of the *rpoC1* intron correlates strongly with chromosomal and morphological data to support subgenera *Astephia*, *Chloropathanthus*, *Murucuja*, *Pseudomurucuja*, *Psilanthus*, and *Decaloba* as a monophyletic group. Indeed, all New World species with a known chromosome number of $n=6$ lack the intron. Only *P. holosericea* and *P. lobata*, with haploid chromosome numbers of $n=7$, have a loss and a chromosome number other than $n=6$. We did not detect any losses outside of the *Decaloba* group sensu Feuillet and MacDougal (2004). This is congruent with the previous study (Downie et al. 1996). Interpretation of the results within subgenus *Decaloba* is more complicated. The intron is present in only seven of the 54 species sampled from this subgenus. Two of these species, *P. aurantia* and *P. herbertiana*, are Old World in distribution. *Passiflora microstipula* and *P. lancetillensis* belong to the newly described supersection *Pterosperma* (MacDougal and Hansen 2003). The remaining three, *P. membranaceae*, *P. hahnii* and *P. guatemalensis*, are members of supersection *Hahniopathanthus* within subgenus *Decaloba*.

In the absence of a phylogenetic tree for the genus, multiple losses would have been an unlikely conclusion with our sampling strategy. However, by mapping the intron loss distribution onto the phylogenetic tree as shown in Fig. 2, it suggests that multiple losses have in fact occurred. This hypothesis is further confirmed by the parametric bootstrap analysis that allowed us to reject a single loss hypothesis at the <0.05 level and to conclude that the *rpoC1* intron loss is not a reliable predictor of relationships within *Passiflora* below the subgeneric level.

''$n=6$ GROUP''. The ''$n=6$ group,'' corresponding to subgenera *Decaloba*, *Astephia*, *Murucuja*, *Pseudomurucuja*, *Apodogyne*, and *Psilanthus* in Killip (1938), forms a monophyletic group with 100% bootstrap support in the parsimony analysis and a posterior probability of 1.00 in the Bayesian analysis (Figs. 2, 3). This group of species is less showy than the ''granadillas'' of subgenus *Passiflora*, with much smaller flowers and a typically herbaceous habit. The leaves are palmately veined, and usually with laminar nectaries. The floral tube/hypanthium is flat while the operculum is plicate and the limen, when present, is short and fleshy. The pollen is distinct from the ''$n=9$ group'' in that it has six pairs of colpi that anastamose toward the poles, while the ''$n=9$ group'' has only three pairs of colpi (Amela et al. 2002; Spirlet 1965; Presting 1965).

The Old World species of *Passiflora*, represented here by *Passiflora aurantia* and *P. herbertiana*, both native to Australia, come out nested within subgenus *Decaloba* with very strong bootstrap support of 87–92% and a posterior probability of 1.0 (Figs. 2, 3). Although their New World sister group has long been debated, they have historically been given their own section within *Decaloba* (DeWilde 1972; Feuillet and MacDougal 2004). Morphologically they possess several characters typical of *Decaloba*, including plicate opercula, laminar nectaries, and a chromosome number of $n=6$ (Snow and MacDougal 1993). Our Bayesian phylogeny indicates that *P. auriculata*, or close relative, is the likely New World sister to this group (Fig. 3). However, an expanded sampling strategy including southeast Asian *Passiflora* will be needed to verify this placement. A phylogenetic study already underway (S. Krosnick pers. comm.) including all the Old World taxa is expected to clarify the biogeographic relationships between New and Old World *Passiflora*.

The most surprising outcome in this clade was the placement of *Passiflora microstipula* and *P. lancetillensis* within subgenus *Decaloba*. They belong to a recently discovered group that occurs in Mexico and Central America (Gilbert and MacDougal 2001) and has a unique morphology. These large lianas bear flowers from the tendril, an uncommon, but not unknown character state in *Passiflora*. The seeds are unusually large and bear conspicuous wings on the edges, a character unique in *Passiflora*. This combination of characters indicated that this group represented a very basal lineage within the genus and was placed within subgenus *Deidamioides* by Feuillet and MacDougal (1999). However, our data place this group in a basal position within subgenus *Decaloba* with strong support. Morphological evidence such as variegated juveniles, cernuous new tip growth and a plicate operculum also support this placement (J. MacDougal per. comm.). *Passiflora microstipula* and *P. lancetillensis*, along with three Central American species are placed in the new supersection *Pterosperma* of subgenus *Decaloba* (MacDougal and Hansen 2003; Feuillet and MacDougal 2004).

Passiflora microstipula deserves some added discussion as it comes out in both subgenera *Passiflora* and *Decaloba* in the chloroplast tree. We can find no biological evidence for its placement within the showy granadillas of subgenus *Passiflora*. Although *P. microstipula* does have a haploid chromosome number of $n=9$, the karyotype is much more similar to members of subgenus *Decaloba* than those of subgenus *Passiflora* (Snow and MacDougal 1993). In order to rule out contamination of DNA or PCR products, DNAs from two different accessions of *P. microstipula*, one from Veracruz and one from Oaxaca, were re-isolated from living material. These PCR products were subsequently cloned and sequenced. All clones of the Oaxaca population came out within subgenus *Decaloba*, while clones from the Veracruz population were split between the *Passiflora* and *Decaloba* subgenera. The strong statistical support for the sequence similarities between *P. nitida* (subgenus *Passiflora*) and *P. microstipula* is surprising, but experimentally repeatable. *Passiflora nitida* is occasionally cultivated in Central and South America for its fruit, but it is not known to occur in Mexico. Fieldwork in Veracruz and herbarium studies conducted by John MacDougal (pers. comm.) found no evidence that *P. nitida* ever co-occurred with *P. microstipula*. In addition, several crosses between both populations of *P. microstipula* and various species of subgenus *Passiflora* were carried out in the greenhouses at The University of Texas over a span of several years, without any evidence of fruit set or other indication of compatibility (L. Gilbert unpubl.). However, our work on plastid inheritance in *Passiflora* has clearly demonstrated that heteroplasmy does occur in this genus (K. Hansen et al. in prep.). So, although we can find no historical evidence at this time to support a chloroplast capture scenario between these taxa, we must consider the idea that the relationship between these two chloroplast types is the result of a past biological phenomenon. We do not feel that this evidence should have any bearing on *P. microstipula*'s taxonomic placement due to the multitude of morphological and molecular evidence supporting its strong relationship with other species in subgenus *Decaloba* (Fig. 2; Muchner et al. 2003; Yockteng and Nadot 2004).

Most of the Caribbean members of *Passiflora* have traditionally been placed in their own subgenera due to their highly modified flowers suggestive of various pollinator syndromes. The taxa that were placed in Killip's subgenera *Murucuja*, and *Pseudomurucuja* are pollinated primarily by hummingbirds while bat pollination has been documented in *P. penduliflora* of subgenus *Astephia* (Kay 2001). Feuillet and MacDougal (1999 and 2004) recognized that these flowers were probably highly modified representatives of subgenus *Decaloba*. Our data clearly support this placement and identify the *P. biflora* lineage as the likely continental sister group.

''$n=9$ Group''. The large flowered granadillas form a monophyletic group with very strong bootstrap (99–100%) and posterior probability (1.0) support. These correspond to Killip's subgenera *Passiflora*, *Tacsonia*, *Manicata*, *Calopathanthus*, *Dysosmia*, *Dysosmioides*, and *Distephana* and are all placed in subgenus *Passiflora* in the new classification by Feuillet and MacDougal (2004). In addition to a chromosome number of $n=9$ (Snow and MacDougal 1993), this group is held together morphologically by having a tubular or campanulate hypanthium, tubular or filamentous operculum, a membranous limen, foliaceous bracts, and highly reticulate pollen with three pairs of colpi (Presting 1965; Spirlet 1965; Amela 2002). The showy flowers and edible fruit of this group have led to their cultivation and introduction throughout the Old and New World. This group appears to have undergone a relatively rapid radiation, as many of the relationships within subgenus *Passiflora* are unresolved in our data as well as in two other recent *Passiflora* phylogenies (Muchner et al. 2003; Yockteng and Nadot 2004).

One particularly interesting revelation is that species that exhibit the Andean hummingbird pollination syndrome do not form a clade (shaded taxa in Fig. 3). Endemic to the high elevations of the Andes in South America, taxa in this group have long, tubular red, pink or orange flowers and are visited by hummingbirds. Called ''tacsos,'' these plants produce a sweet, banana-shaped fruit that is cultivated throughout the Andes and is prevalent in local markets. This is a difficult group taxonomically and they have historically been split into three closely allied subgenera (*Tacsonia*, *Manicata*, and *Rathea*) based primarily on coronal shape, degree of bractal fusion and the point of petal insertion in the floral tube (Escobar 1988). Rather than forming a monophyletic assemblage as we expected, the tacsos are split into two very strongly supported groups (shaded taxa in Fig. 3). In the Bayesian phylogeny, *P. antioquiensis*, *P. ampullacea*, *P. parritae*, *P. cumbalensis*, *P. manicata*, and *P. andina*, form a monophyletic group with a posterior probability of 1.00. *Passiflora trifoliata*, along with the bat-pollinated *P. trisecta*, (Ulmer and MacDougal 2004) is nested within a group of tropical lowland, bee-pollinated species with a posterior probability of 0.99. That the tacsos do not form a monophyletic group in our data suggests that the Andean hummingbird pollination syndrome had at least two independent origins. Only Masters, in his 1871 monograph, indicated that this syndrome might have originated more than once in this group.

Chromosome Number Evolution. Chromosome number is a very useful predictor of relationships in *Passiflora*. There are 83 published chromosome numbers, which account for roughly 17% of the genus (re-

viewed by Snow and MacDougal [1993] and De Melo et al. [2001]). The two largest subgenera, *Passiflora* and *Decaloba*, have ancestral haploid numbers of $n=9$ and $n=6$, respectively. Each of these groups is monophyletic with 90% or greater bootstrap support (Figs. 2, 4). Conversely, subgenera *Astrophea* and *Deidamioides* have haploid numbers of $n=12$. Polyploidy in the genus is infrequent with only six reports to date.

Several numbers have been proposed as the base number for *Passiflora*, most commonly $x=3$, $x=6$, $x=9$ or $x=12$ (Storey 1950; Raven 1975; Morawetz 1986; Snow and MacDougal 1993; De Melo et al. 2001; De Melo and Guerra 2003). Insightful discussions of this topic have been published in recent years (Snow and MacDougal 1993; De Melo et al. 2001). In the Passifloraceae, chromosome numbers of $n=12$ (*Tetrapathea* in Hair and Beuzenberg 1959 and *Adenia* in De Rocher et al. 1990) and probable aneuploid derivatives in $n=11$ (*Deidamia* Noronha ex Thouarsand *Crossostemma* Planchon ex Hook. in Gadella 1969, 1970) are the only counts available for other genera in the family. Although chromosome numbers are known for only five of the 17 genera in the Passifloraceae, these numbers would suggest a base chromosome number of $x=12$ for the genus *Passiflora* and the Passifloraceae. However, De Melo and Guerra (2003) examined the number of 5S and 4.5S sites revealed by chromosomal staining in the major karyotype groups within the genus and found compelling, but inconclusive, evidence that supports a base chromosome number of $x=6$ for *Passiflora*.

Using a different approach, we examined the base number for the genus by optimizing chromosome numbers on the most parsimonious trees using MacClade v. 4.0 (Madison and Madison 2000). An unpublished count of $n=11$ or $n=12$ for *P. guatemalensis* (J. MacDougal pers. comm.), part of a basal clade in subgenus *Decaloba*, is critical for understanding chromosome evolution in *Passiflora* and was included in our analysis (Fig. 2). An ancestral base chromosome number of $x=12$ required five steps while $x=6$ and $x=9$ each required six steps.

A difference of a single step is too slight to designate a base chromosome number of $x=12$ for *Passiflora* with confidence, especially when the convincing evidence presented in favor of $x=6$ (De Melo and Guerra 2003) is considered. However, since $n=6$ is not found outside of subgenus *Decaloba* in either the family or genus, it seems likely that $n=6$ had a single origin in the ancestor to this monophyletic group. In our opinion, this evidence, combined with the fact that $n=12$ and $n=11$ are the only known numbers in the Passifloraceae supports $x=12$ as the base chromosome number for *Passiflora*. Clearly, more information is needed to understand chromosome number evolution in *Passiflora*. Of particular importance, would be more chromosome counts for other genera in the Passifloraceae and for the poorly known subgenera *Deidamioides* and *Astrophea*, within the genus *Passiflora*.

Taxonomic Implications. Killip's monograph of the New World Passifloraceae has been the classification system most widely used since its publication in 1938. However, the rapidity with which new species have been discovered in recent years and the omission of the Old World species has limited its utility. A new, innovative classification system by Feuillet and MacDougal (2004) has built on the solid foundation established by Killip by including all known species of *Passiflora* and correcting many nomenclatural errors. By reducing Killip's 22 subgenera to four, this treatment more accurately represents the evolutionary relationships between species (Figs. 2, 3). Without exception, Feuillet and MacDougal have successfully identified monophyletic species assemblages at this level. Some of the supersections and series do not form monophyletic groups in the cpDNA phylogeny, but the low resolution in our phylogeny precludes a detailed evaluation of their system at this level.

A brief comparison of Feuillet and MacDougal's classification system with that of Killip's is shown in Table 2. Our data unequivocally support their species groupings at the subgeneric level. This is illustrated in Fig. 2 where subgenera *Passiflora*, *Astrophea*, *Decaloba*, and *Deidamioides* are all monophyletic with strong statistical support.

The relationship of *Tetrapathea*, a monotypic New Zealand genus with dioecious plants and four-merous flowers, to the rest of the subgenera is unresolved in our data. With the exception of Killip (1938), most *Passiflora* systematists beginning with Masters (1870) have considered *Tetrapathea* to be a member of the genus *Passiflora*. However, there has been little consensus as to which subgenera it is most closely related to or whether it deserves its own monotypic rank. Based on morphological characters, Feuillet and MacDougal (2004) included *Tetrapathea* within subgenus *Deidamioides* while Green (1972) gave *Tetrapathea* its own subgenus. Most recently, Yockteng and Nadot (2004), using the nuclear encoded chloroplast gutamate synthase gene (*ncpGS*), found a highly supported relationship between *Tetrapathea* and subgenus *Decaloba*. Interestingly, our Bayesian analyses show a strong relationship between *Tetrapathea* and *Dilkea* with a posterior probability of 0.90. *Dilkea* (South America, 9 spp.) was described by Masters (1870) and excluded from *Passiflora* based on its lack of an androgynophore and four-merous flowers. Although the relationship between *Tetrapathea* and *Dilkea* was not evident in parsimony analyses, the common character of four-merous flowers suggests that it may not be an artifact of our data. A more variable marker may be able to clarify *Tetrapathea*'s placement within *Passiflora* and its relationship to *Dilkea*.

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APPENDIX 1

Species, in alphabetical order, sampled for *rpoC1* intron survey and chloroplast *trnL/trnT* spacer sequence phylogeny. Subgeneric placement (Killip 1938 followed by Feuillet and MacDougal 2004) is provided after species names (''NA'' if not treated). Presence of absence of *rpoC1* intron is denoted by a (+) or (-) respectively. Chromosome numbers, if known, and references are listed. Plant material was field collected by A. L. Escobar, L. Gilbert, K. Hansen, J. MacDougal or José Panero or cultivated at the University of Texas by L. E. Gilbert or at Butterfly World by Ron Boender. All vouchers are held at HUA, DUKE, TEX, or UPR. Sequences are deposited in GenBank under accession numbers DQ096733–DQ096793.

Adenia mannii Engl., NA, NA, +, Africa; *L. Escobar 92–38* (TEX), DQ96755, $n=12$ (de Rocher et al. 1990)

Tetrapathea tetranda Cheeseman, NA, NA, +, Brazil; *L. Escobar 71* (TEX), DQ96754, $n=12$ (Hair & Beuzenberg 1959)

Tetrastylis ovalis Vell. Ex M. Roem.) Killip, NA, Deidamioides, +, Brazil; *K. Hansen 152* (TEX), DQ96752

Passiflora actinia Hook., *Passiflora*, *Passiflora*, +, Brazil; *K. Hansen 36* (TEX), DQ96792, $n=9$ (De Melo et al. 2001); *P. acuminata* DC., *Passiflora*, *Passiflora*, +, French Guiana; *L. Escobar s.n.* (UPR), DQ96740; *P. adenopoda* DC., *Decaloba*, *Decaloba*, -, J. MacDougal 2012 *(MO),* NA; *P. adulterina* L., *Tacsonia*, *Passiflora*, +, Colombia; *L. Escobar s.n.* (HUA), NA; *P. affinis* Englem., *Decaloba*, *Decaloba*, -, Texas; *K. Hansen 251* (TEX), DQ96764; *P. alata* Curtis, *Passiflora*, *Passiflora*, +, Brazil; *K. Hansen 248* (TEX), DQ096733, $n=9$ Guerra 1999; *P. allantophylla* Mast., *Decaloba*, *Decaloba*, -, Guatemala; *J. MacDougal 638* (DUKE), NA; *P. alnifolia* Kunth, *Decaloba*, *Decaloba*, -, Colombia; *K. Hansen 141* (TEX), NA; *P. ambigua* Hemsl., *Passiflora*, *Passiflora*, +, Costa Rica; *L. Escobar 88-22* (TEX), NA; *P. amethystina* J.C. Mikan, *Passiflora*, *Passiflora*, +, Brazil; *K. Hansen 40* (TEX), NA, $n=9$ (De Melo et al. 2001); *P. amoena* L.K. Escobar, NA, Astrophea, +, French Guiana; *L. Escobar 9869* (UPR), NA; *P. ampullacea* (Mast.) Harms, *Tacsonia*, *Passiflora*, +, Colombia; *L. Escobar 88-53* (TEX), DQ96776; *P. andina* Killip, *Rathea*, *Passiflora*, +, Ecuador; *K. Hansen 50* (TEX), DQ96751; *P. anfracta* Mast. & André, *Decaloba*, *Decaloba*, -, Ecuador; *K. Hansen s.n.* (TEX), NA; *P. antioquiensis* H. Karst., *Tacsonia*, *Passiflora*, +, L. Escobar 88–51 *(TEX),* DQ96779, $n=9$ (Snow & MacDougal 1993); *P. apetala* Killip, *Decaloba*, *Decaloba*, -, Costa Rica; *K. Hansen 147* (TEX), NA; *P. arbaelezii* L. Uribe, NA, *Deidamioides*, Cultivated UT; *L. Escobar 92–8* (TEX), NA; *P. arborea* Spreng., *Astrophea*, *Astrophea*, +, Ecuador; *L. Escobar 8581* (TEX), NA; *P. aurantia* G. Forester, NA, *Decaloba*, +, Australia; *K. Hansen 201* (TEX), DQ96784, $n=6$ (Beal 1971); *P. auriculata* Kunth, *Decaloba*, *Decaloba*, -, *Costa Rica; James P. Folsom 9078* (TEX), DQ96765

P. biflora Lam., *Decaloba*, *Decaloba*, -, Costa Rica; *K. Hansen s.n.* (TEX), DQ96787, $n=6$ (Beal 1971); *P. boenderii* J.M. MacDougal, NA, *Decaloba*, -, Costa Rica; *K. Hansen 210* (TEX), NA; *P. buchtienii* Killip, *Distephana*, *Passiflora*, +, L. Escobar 92–60 *(TEX),* NA

P. caerulea L., *Passiflora*, *Passiflora*, +, Paraguay; *K. Hansen 16* (TEX), NA, $n=9$ (Heitz 1926); *P. campanulata* Mast., *Dysosmioides*, *Passiflora*, +, Brazil; *K. Hansen 46* (TEX), DQ96775; *P. candida* (Poepp. & Endl.) Mast., *Astrophea*, *Astrophea*, +, French Guiana; *L. Escobar 9901* (TEX), DQ96786; *P. candollei* Triana. & Planch., NA, Decaloba, -, Colombia; *K. Hansen 186* (TEX), DQ96770, $n=6$ (Snow and MacDougal 1993); *P. capsularis* L., *Decaloba*, *Decaloba*, -, L. Escobar s.n. *(TEX),* NA, $n=6$ (Beal 1971); *P. ciliata* Aiton, *Dysosmia*, *Passiflora*, +, L. Escobar 92–71 *(TEX),* DQ96790; *P. cincinnata* Mast., *Passiflora*, *Passifora*, +, Argentina; *K. Hansen 175* (TEX), NA; *P. cirrhiflora* Juss., *Polyanthea*, *Deidamioides*, +, French Guiana; *K. Hansen 222* (TEX), DQ96756, $n=9$ (Beal 1971); *P. citrifolia* (Juss.) Mast., *Astrophea*, *Astrophea*, +, L. Escobar 9921 *(TEX),* NA; *P. coactilis* (Mast.) Killip, *Tacsonia*, *Passiflora*, +, *Ecuador*;Jose Panero *(TEX),* NA; *P. coccinea* Aubl., *Distephana*, *Passiflora*, +, Brazil; *K. Hansen 145* (TEX), DQ96777; *P. complanata* J.M MacDougal inedit., NA, Decaloba, -, Mexico; *K. Hansen 171* (TEX), NA, $n=9$ (Beal 1971); *P. conzattianna* Killip, *Decaloba*, *Decaloba*, -, Mexico; *K. Hansen 143* (TEX), NA; *P. coriaceae* Juss., *Decaloba*, *Decaloba*, -, Belize; *K. Hansen 246* (TEX), NA, $n=6$ (Snow and MacDougal 1993); *P. costaricensis* Killip, *Decaloba*, *Decaloba*, -, Costa Rica; *K. Hansen 146* (TEX), NA, $n=6$ (Beal 1971); *P. crenata* Feuillet & Cremers, NA, Passiflora, +, French Guiana; *K. Hansen 216* (TEX), DQ96737, $n=6$ (Snow and MacDougal 1993); *P. crispolanata* L. Uribe, *Tacsonia*, *Passiflora*, +, L. Escobar 92–58 *(TEX),* NA; P. *cumbalensis* (H. Karst.) Harms, *Tacsonia*, *Passiflora*, +, Ecuador; *L. Escobar 88–46* (HUA), DQ96773

P. deidamioides Harms, *Deidamioides*, *Deidamioides*, +, Brazil; *K. Hansen 152* (TEX), DQ96757; *P. discophora* P. Jørg. & Lawesson, NA, Deidamioides, +, Ecuador; *K. Hansen s.n.* (TEX), NA

P. edulis Sims, *Passiflora*, *Passiflora*, +, Colombia; *L. Escobar 88–14* (TEX), DQ96743; *P. eichleriana* Mast., *Passiflora*, *Passiflora*, +, Brazil; Cultivated UT, NA, $n=9$ (Janaki Ammal 1945); *P. escobariana* J.M. MacDougal, NA, Decaloba, -, Colombia; *L. Escobar s.n.* (TEX), NA; *P. ernestii* Harms, *Adenosepala*, *Passiflora*, +, GH, NA, $n=6$ (Snow and MacDougal 1993); *P. exura* Feuillet, NA, Passiflora, +, French Guiana; *L. Escobar s.n.* (UPR), NA

P. foetida L., *Dysosmia*, *Passiflora*, +, Mexico; *L. Escobar 88–16* (TEX), NA; *P. ferruginea* Mast., *Decaloba*, *Decaloba*, -, French Guiana; *L. Escobar 92–15* (TEX), NA, $n=9$ Guerra 1986, $n=10$ Storey 1950, $n=11$ (Harvey 1966)

P. garkei Mast., *Passiflora*, *Passiflora*, +, French Guiana; *K. Hansen 177* (TEX), DQ96763; *P. gilbertiana* J.M. MacDougal, NA, Decaloba, -, *L. Escobar s.n.* (TEX), NA; *P. glandulosa* Cav., *Distephana*, *Passiflora*, +, *L. Escobar 9881* (TEX), DQ96791, $n=6$ (Snow and MacDougal 1993); *P. guatemalensis* S. Watson, *Decaloba*, *Decaloba*, +, Guatemala; *K. Hansen 201* (TEX), DQ96762, $n=9$ (De Melo et al. 2001)

P. hahnii (E. Fourn.) Mast., *Decaloba*, *Decaloba*, +, Cultivated UT, NA, $n=11/n=12$ John Macdougal*; *P. herbertiana* Ker Gawl, NA, Decaloba, +, Australia; *L. Escobar 88–48* (TEX), DQ96783; *P. holosericea* L., *Decaloba*, *Decaloba*, -, Guatemala; *K. Hansen 182* (TEX), DQ96759, $n=6$ (Beal 1969)

P. incarnata L., *Passiflora*, *Passiflora*, +, Texas; *L. Escobar 9264* (TEX), NA, $n=7$ (Snow and MacDougal 1993)

P. jamesonii (Mast.) Bailey, *Tacsonia*, *Passiflora*, +, Ecuador; *K. Hansen 60* (TEX), NA, $n=9$ (Heitz 1926)

P. karwinskii Mast., *Decaloba*, *Decaloba*, -, Mexico; *K. Hansen 227* (TEX), NA; *P. kawensis* Feuillet, NA, Astrophea, +, French Guiana; *L. Escobar 9910* (TEX), DQ96769, $n=6$ (MacDougal 1983)

P. lancearia Mast., *Decaloba*, *Decaloba*, -, Costa Rica; *K. Hansen 172* (TEX), NA; *P. lancetillensis* J.M. MacDougal & Meerman, NA, Decaloba, +, *L. Gilbert s.n.* (TEX), DQ96760; *P. laurifolia* L., Passiflora, *Passiflora*, +, French Guiana; *L. Escobar 88–46* (TEX), DQ096734; *P. ligularis* Juss., Passiflora, Passiflora, +, Ecuador; *K. Hansen 81* (TEX), DQ96736, $n=9$ (Storey 1950); *P. lindeniana* Planch. Ex Triana & Planch, Astrophea, *Astrophea*, +, Venezuela; *L. Gilbert s.n.* (TEX), NA, $n=9$ (Storey 1950); *P. lobata* (Killip) Hutch ex J.M. MacDougal, *Tetrastylis* (genus), *Decaloba*, -, Costa Rica; *K. Hansen 258* (TEX), NA, $n=12$ (Berry 1987); *P. lutea* L., *Decaloba*, *Decaloba*, -, *L. Escobar s.n.* (TEX), NA, $n=7$ (MacDougal 1983)

P. macrophylla Spruce ex Mast., *Astrophea*, *Astrophea*, +, Ecuador; *L. Gilbert s.n.* (TEX), NA, $2n=24$ (Baldwin 1949), $2n=84$ (Bowden 1940), $n=6$ (De Melo et al. 2001); *P. magnifica* L.K. Escobar, NA, Passiflora, +, Colombia; *K. Hansen 154* (TEX), DQ96735; *P. maliformis* L., *Passiflora*, *Passiflora*, +, Colombia; *K. Hansen 156* (TEX), DQ967, $n=9$ (Snow and MacDougal 1993); *P. manicata* (Juss.) Pers., *Tacsonia*, *Passiflora*, +, Ecuador; *K. Hansen 51 TEX),* DQ96778, $n=9$ (Storey 1950); *P. mathewsii* (Mast.) Killip, *Tacsonia*, *Passiflora*, +, Ecuador; *J. MacDougal* (TEX), NA, $n=9$ (Storey 1950); *P. mayarum* J.M. MacDougal, NA, Passiflora, +, Guatemala; *K. Hansen 117* (TEX),

NA; *P. membranacea* Benth., *Decaloba*, *Decaloba*, +, Mexico; *L. Escobar 88–50* (TEX), NA; *P. menispermifolia* Kunth., *Passiflora*, *Passiflora*, +, Costa Rica; *L. Escobar 88–13* (TEX), DQ96785; *P. microstipula* L.E. Gilbert & J.M. MacDougal, NA, Decaloba, +, Vera Cruz, Mexico; *L. Gilbert s.n.* (TEX), DQ96782, DQ96789; *P. microstipula* L.E. Gilbert & J.M. MacDougal, NA, Decaloba, +, Oaxaca, Mexico; *L. Gilbert s.n.* (TEX), DQ96758, *n*=9 (Snow and MacDougal 1993); *P. miersii* Mast., *Passiflora*, *Passiflora*, +, Brazil; *K. Hansen 153* (TEX), DQ96766, *n*=9 (Snow and MacDougal 1993); *P. misera* Kunth., *Decaloba*, *Decaloba*, -, Ecuador; *L. Escobar 88–26* (TEX), NA; *P. mixta* L. f., *Tacsonia*, *Passiflora*, +, Ecuador; *L. Escobar 88–45* (TEX), NA, *n*=6 (De Melo et al. 2001); *P. mucronata* Lam., *Passiflora*, *Passiflora*, +, Cultivated UT, NA, *n*=9 (La Cour 1952); *P. multiflora* L., *Apodogyne*, *Decaloba*, +, Florida; *K. Hansen 189* (TEX), DQ96793, *n*=9 (De Melo et al. 2001); *P. murucuja* L., *Murucuja*, *Decaloba*, -, Puerto Rico; *K. Hansen 219* (TEX), DQ96788

P. nelsonii Mast. & Rose, *Passiflora*, *Passiflora*, +, Costa Rica; *L. Escobar 88–24* (TEX), NA; *P. nephrodes* Mast., *Passiflora*, *Passiflora*, +, Brazil; *K. Hansen 211* (TEX), NA; *P. nitida* Kunth, *Passiflora*, *Passiflora*, +, French Guiana; *K. Hansen 217* (TEX), DQ96739

P. oblongata Sw., *Pseudomurucuja*, *Decaloba*, -, Jamaica; *L. Escobar s.n.* (UPR), DQ96772, *n*=9 (De Melo et al. 2001); *P. oerstedii* Mast., *Passiflora*, *Passiflora*, +, Costa Rica; *L. Gilbert s.n.* (TEX), NA; *P.* aff. *Oerstedii* Mast., *Passiflora*, *Passiflora*, +, Ecuador; *K. Hansen 242* (TEX), NA; *P. organensis* Gardner., *Decaloba*, *Decaloba*, -, Brazil; *K. Hansen 195* (TEX), NA

P. pallens Poepp. ex Mast., *Passiflora*, *Passiflora*, +, Florida; *L. Escobar s.n.* (TEX), NA; *P. palmeri* Rose, *Dysosmia*, *Passiflora*, +, Cultivated UT, NA; *P. parritae* (Mast.) L.H. Bailey, *Tacsonia*, *Passiflora*, +, *Colombia; L. Escobar s.n.* (HUA), DQ96780; *P. penduliflora* Bertero ex DC., *Astephia*, *Decaloba*, -, Jamaica; *K. Hansen 241* (TEX), NA; *P. perfoliata* L., *Pseudomurucuja*, *Decaloba*, -, L. Escobar 88–36 *(TEX),* DQ96761, *n*=6 (Beal 1971); *P. pitteri* Mast., *Astrophea*, *Astrophea*, +, Costa Rica; *K. Hansen 155* (TEX), DQ96768, *n*=6 (Beal 1971); *P. platyloba* Killip, *Passiflora*, *Passiflora*, +, Cultivated UT, DQ96738; *P. porphyretica* Mast., *Pseudogranadilla*, *Decaloba*, -, *L. Escobar s.n.* (HUA), NA

P. quadrangularis L., *Passiflora*, *Passiflora*, +, Costa Rica; *K. Hansen 183* (TEX), NA, *n*=6 (Snow and MacDougal 1993); *P. quindiensis* Killip, *Tacsonia*, *Passiflora*, +, Colombia; *L. Escobar s.n.* (HUA), NA, *n*=9 (Janaki Ammal 1945); *P. quinquangularis* J. MacDougal, *Decaloba*, *Decaloba*, -, Guatemala; *K. Hansen 146* (TEX), NA

P. racemosa Brot., *Calopathanthus*, *Passiflora*, +, Brazil; *K. Hansen 157* (TEX), DQ96746, *n*=6 (Snow and MacDougal 1993); *P. reflexiflora* Cav., *Tacsonioides*, *Passiflora*, +, Ecuador; *K. Hansen 72* (TEX), DQ96750, *n*=9 (Bowden 1945); *P. resticulata* Mast. & André, *Passiflora*, *Passiflora*, +, Cultivated UT, NA; *P. retipetala* Mast., *Passiflora*, *Passiflora*, +, Trinidad; *L. Escobar s.n.* (HUA), NA, *n*=9 (Escobar 1986); *P. rovirosae* Killip, *Decaloba*, *Decaloba*, -, Mexico; *L. Gilbert 8056* (TEX), NA; *P. rubra* L., *Decaloba*, *Decaloba*, -, Ecuador; *K. Hansen 245* (TEX), NA, *n*=6 (Snow and MacDougal 1993)

P. sanguinolenta Mast. & Linden, *Decaloba*, *Decaloba*, -, *Ecuador; L. Escobar s.n.* (HUA), NA, *n*=6 (Snow and MacDougal 1993); *P. seemannii* Griseb., *Passiflora*, *Passiflora*, +, Colombia; *L. Escobar 92–83* (TEX), DQ96744, *n*=6 (Snow and MacDougal 1993); *P. serratifolia* L., *Passiflora*, *Passiflora*, +, Belize; *K. Hansen 159* (TEX), NA, *n*=9 (Storey 1950); *P. serratodigitata* L., *Passiflora*, *Passiflora*, +, Trinidad; *K. Hansen 158* (TEX), DQ96742; *P. serrulata* Jacq., *Passiflora*, *Passiflora*, +, Colombia; *L. Escobar s.n.* (HUA), NA; *P. sexflora* Juss., *Decaloba*, *Decaloba*, -, *L. Escobar s.n.* (HUA), NA; *P. speciosa* Gardner, *Distephana*, *Passiflora*, +, Brazil; *L. Escobar s.n.* (TEX), DQ96747; *P. suberosa* L., *Decaloba*, *Decaloba*, -, Mexico; *K. Hansen 142* (TEX), NA; *P. sublanceolata* (Killip) MacDougal, *Dysosmia*, *Dysosmia*, Cultivated UT, DQ96781; *P. subpeltata* Ortega, *Passiflora*, *Passiflora*, +, Cultivated UT, NA, *n*=6 Diers 1961, *n*=12 (Storey 1950)

P. tacsonioides Griseb., *Pseudomurucuja*, *Decaloba*, -, Jamaica; *K. Hansen 223* (TEX), NA, *n*=9 (Storey 1950); *P. talamancensis* Killip, *Decaloba*, *Decaloba*, -, Cultivated UT, NA; *P. tiliifolia* L., *Passiflora*, *Passiflora*, +, Cultivated UT, NA; *P. trialata* Feuillet and J.M. MacDougal, NA, Passiflora, +, French Guiana; *K. Hansen 230* (TEX), DQ96741; *P. tricuspis* Mast., *Decaloba*, *Decaloba*, -, Bolivia; *K. Hansen 208* (TEX), NA; *P. trifasciata* Lem., *Decaloba*, *Decaloba*, -, Ecuador; *L. Escobar s.n.* (HUA), NA, *n*=6 (De Melo et al. 2001); *P. trifoliata* Cav., *Tacsonia*, *Passiflora*, +, Colombia; *L. Escobar s.n.* (HUA), DQ96745; *P. tripartita* (Juss.) Poir., *Tacsonia*, *Passiflora*, +, Peru; *L. Escobar 88–44* (TEX), NA; *P. trisecta* Mast., *Tacsonia*, *Passiflora*, +, Colombia; *L. Escobar s.n.* (HUA), DQ96749, *n*=9 (Heiser 1963); *P. tuberosa* Jacq., *Decaloba*, *Decaloba*, -, Trinidad; *L. Escobar 88–6* (TEX), NA; *P. tulae* Urban, *Decaloba*, *Decaloba*, -, Puerto Rico; *K. Hansen 167* (TEX), NA

P. vespertilio L., *Decaloba*, *Decaloba*, -, Brazil; *K. Hansen 192* (TEX), DQ96771; *P. villosa* Vell., *Dysosmioides*, *Passiflora*, +, Brazil; *K. Hansen 38* (TEX), DQ96767; *P. viridiflora* Cav., *Decaloba*, *Decaloba*, -, Mexico; *L. Escobar s.n.* (HUA), NA; *P. vitifolia* Kunth, *Distephana*, *Passiflora*, +, Brazil; *K. Hansen 174* (TEX), DQ96748

P. xiikzodz J.M. MacDougal, NA, Decaloba, -, Guatemala; *L. Escobar s.n.* (TEX), NA, *n*=9 (Storey 1950)

P. yucatanensis Killip, *Decaloba*, *Decaloba*, -, Mexico; *K. Hansen 150* (TEX), NA