

## Monophyly and Floral Character Homology of Old World *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*)

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**ABSTRACT.** The circumscription of the Old World *Passiflora* subgenus *Decaloba* supersection *Disemma* has been problematic for more than one hundred years. Supersection *Disemma* consists of 22 Old World species found throughout mainland Asia, Southeast Asia, Australia, and the Pacific. They show great morphological diversity, possessing cup-shaped to tubular flowers and both branched and unbranched inflorescences. These features have been used to link species in *Disemma* to New World members of subgenus *Decaloba*, resulting in the widespread suspicion that *Disemma* is not monophyletic. Plastid (*trnL-F* intron and spacer) and nuclear (ITS) DNA sequences were used to test the monophyly of supersection *Disemma* and reconstruct phylogenetic relationships within this group. Two monotypic genera, *Hollrungia* and *Tetraphathaea*, were included in this analysis based on their strong morphological similarity to *Passiflora*, and the Austral-Pacific distribution they share with many of the Old World species. Supersection *Disemma* is monophyletic, containing two distinct Asian and Australian lineages. *Hollrungia* and *Tetraphathaea* are strongly supported as members of *Passiflora*, though their exact relationships to each other are not resolved. The tubular floral morphology in the Australian species is not homologous with that in the New World species. However, the tubular morphology of *P. hollrungii* from Papua New Guinea is homologous to the New World tubular condition. The architecture of inflorescences in *Disemma* is more similar to that in closely related species of *Passiflora* than to other genera of Passifloraceae.

*Passiflora* provides a remarkable example of floral complexity and diversity. Comprising more than 515 species of vines, lianas, and small trees, the genus is most diverse in Central and South America, with only 22 species native to Southeast Asia, Australia, and the Pacific Islands. *Passiflora* exhibits several unique floral features, including multiple series of brightly colored coronal filaments, diverse operculum morphology, an androgynophore, and elaborate floral nectaries. Vegetatively, *Passiflora* shows a wide range of leaf shapes and great variation in the types of extrafloral nectaries present on leaves, stems, bracts, and petioles.

*Passiflora* was divided into 21 sections by Harms (1925) and the New World species were later divided into 22 subgenera based on floral morphology (Killip 1938). Recently, the infrageneric taxonomy of *Passiflora* has undergone significant revision (Feuillet et al. 1994; Feuillet and MacDougal 1999, 2004). The revision of Feuillet and MacDougal is based on morphological characters and recognizes four subgenera: *Astrophea* (57 New World species), *Deidamioides* (17 New World species), *Passiflora* (234 New World species), and *Decaloba* (204 New and Old World species). The first genus-wide molecular analysis of *Passiflora* (Muschner et al. 2003), using the nuclear ribosomal internal transcribed spacers (ITS-1 and ITS-2) and the plastid *trnL-F* intergenic spacer, generally supported the subgenera proposed by Feuillet and MacDougal. Another recent molecular study using chloroplast-expressed glutamine synthetase (*ncpGS*) supported the monophyly of the four subgenera, but indicated that three additional subgenera, *Polyanthea* (DC.) Killip, *Dysosmia* (DC.) Kil-

lip, and *Tetraphathaea* (DC.) Rchb., should also be recognized (Yockteng and Nadot 2004).

While progress has been made in understanding phylogenetic relationships among the subgenera of *Passiflora*, very little is known about the relationships within them. An example is *Decaloba*, the second largest subgenus in *Passiflora*. *Decaloba* is significant in that it contains all of the Old World species, as well as many New World species. *Decaloba* is currently divided into seven supersections (Feuillet and MacDougal 2004), six of which are found in the New World, while the seventh, supersection *Disemma*, comprises all 22 Old World species. Recent phylogenetic analyses of *Passiflora* have not sampled extensively within subgenus *Decaloba* and have included only the Australian species as representatives of supersection *Disemma* (Muschner et al. 2003; Yockteng and Nadot 2004). Several species in this supersection exhibit unique features, including secondarily-branched inflorescences, unusual leaf morphologies and arrangements, multiplication of floral parts, and tubular floral morphology.

The taxonomic delimitation and position of *Disemma* within *Passiflora* have been uncertain, a situation much complicated by the lack of information about their natural history. Three species of *Disemma* are found in northeastern Australia and Melanesia, one species is endemic to Papua New Guinea, and 18 species occur in China, India, and Southeast Asia. The Asian species typically possess branched inflorescences with 2 to 30 small white flowers. Based on this particular feature, it was suggested that the Southeast Asian species might occupy a basal position in the ge-

nus, perhaps being more closely related to *Adenia* and other genera in Passifloraceae that have similarly branched inflorescences (Cusset 1968; Tillett 1988). Additionally, three Asian species, *P. siamica*, *P. moluccana* var. *glaberrima* (Gagn.) W. J. de Wilde, and *P. tonkinensis* W. J. de Wilde, possess up to eight stamens and five styles, whereas the common condition in *Passiflora* is five stamens and three styles. *Passiflora moluccana* var. *teysmanniana*, a variety that is widespread throughout Southeast Asia, has sub-opposite to completely opposite leaves, a feature unique in Passifloraceae. A further point of confusion is that all of the Australian and New Guinean species have tubular, bird pollinated flowers, a feature that resulted in their historical classification among several New World species exhibiting similar morphology (Masters 1871; Harms 1925).

An additional question regarding Old World *Passiflora* involves two monotypic genera native to the Austral-Pacific, *Tetrapathaea* and *Hollrungia*. These two genera are morphologically very similar to each other and to *Passiflora*. *Hollrungia* has branched cymose inflorescences with a terminal tendril, an androgynophore, operculum, coronal filaments, and glands on the petiole and abaxial surface of the leaves. *Tetrapathaea* also has branched cymose inflorescences, coronal filaments, and an androgynophore. The main reason for the segregation of these species from *Passiflora* is their dioecy. *Hollrungia* flowers retain both the androecium and gynoecium but are functionally unisexual, while in *Tetrapathaea* only rudimentary stamens or carpels are present. The morphological similarity of *Hollrungia* and *Tetrapathaea* to *Passiflora* would be consistent with a close phylogenetic relationship. Given the additional geographical similarity of these species and supersection *Disemma*, they need to be included in any analysis of Old World *Passiflora*.

The objectives of the current study are to use the nuclear ribosomal internal transcribed spacer region (ITS-1, 5.8S, and ITS-2) and the chloroplast *trnL-F* intron and intergenic spacer to evaluate the monophyly of supersection *Disemma* (Taberlet et al. 1991; Baldwin 1992; Baldwin et al. 1995), to examine the position of *Hollrungia* and *Tetrapathaea* within the genus *Passiflora*, and to reexamine the homologies of some of the morphological features that have led to continual uncertainty in the placement of the Old World species of *Passiflora*.

## MATERIALS AND METHODS

**Taxon Sampling and Outgroup Selection.** A broad sampling approach was used, including nine members of supersection *Disemma*, *Tetrapathaea tetrandra*, *Hollrungia* sp. nov., and 25 outgroup taxa including *Adenia*, *Mitostemma*, and *Paropsis* (Passifloraceae) and more distantly, *Malesherbia* (Malesherbiaceae) and *Turnera* (Turneraceae). Because *Malesherbia* has been shown to be closely related to Passifloraceae (Soltis et al. 2000), *M. lanceolata* and *M. weberbaueri* were designated as outgroups. Within *Passiflora*, rep-

resentatives of all four subgenera were included: subgenus *Passiflora* (three species), subgenus *Astropheia* (two species), subgenus *Deidamioides* (one species), and subgenus *Decaloba* (21 species). The nuclear ribosomal internal transcribed spacer region ITS was chosen for this analysis based on the phylogenetic utility demonstrated in other analyses of *Passiflora* and closely related taxa at both the species and subgeneric levels (Gengler-Nowak 2003; Muschner et al. 2003). The chloroplast *trnL-F* intron and intergenic spacer region was chosen because it is more conserved, and has been shown to provide resolution at the subgeneric level and higher in *Passiflora* (Muschner et al. 2003). Besides the 65 new sequences generated for the current study, four outgroup sequences of ITS and three sequences of the *trnL-F* intergenic spacer region were obtained from GenBank. Voucher specimens, their taxonomic placement, and GenBank accession numbers are listed in the Appendix. The datasets have been deposited in TreeBASE (study accession number: S1751, matrix accession numbers: M5547 and M5549).

**DNA Extraction and Purification.** Fresh or silica gel preserved leaf material was used for DNA extraction. Total genomic DNA was extracted using the CTAB method of Doyle and Doyle (1987). For some species, it was necessary to further purify and clean the DNA with the Elu-Quik Kit (Schleicher & Schuell, Keene, NH).

**Amplification and Sequencing.** The entire ITS region, including ITS-1, the 5.8S gene, and ITS-2, was amplified using primers 5 and 4 of White et al. (1990). The *trnL-F* region of chloroplast DNA was amplified with primers c and f of Taberlet et al. (1991). 50  $\mu$ l amplification reactions for both ITS and *trnL-F* were carried out using standard PCR conditions as summarized by Muschner et al. (2003). Amplifications were purified using 50  $\mu$ l of 20% polyethylene glycol-2.5 M NaCl and sequenced directly. Dideoxy cycle sequencing reactions were performed using BigDye Terminator version 3.1 chemistry (Applied Biosystems, Foster City, CA) scaled down to quarter reaction volume. Reactions were run on an ABI Prism 3100 (PE Biosystems, Foster City, CA) automated sequencer at Ohio State University. Contigs were assembled using Sequencher 3.1.1 (Gene Codes Corporation, Inc., Ann Arbor, MI).

**Phylogenetic Analyses.** All ITS and *trnL-F* sequences were initially aligned using Clustal W (Thompson et al. 1994), and then adjusted manually using Se-Al (Rambaut 2000). Due to the high level of variability in the ITS region, sensitivity analysis sensu Wheeler (1995) was performed on the dataset to examine the effects of the alignment on the trees recovered. Gap opening and extension parameters for both pairwise and multiple alignment functions were set at 9 and 3, 5 and 4, and 2 and 1, respectively. Default values were used for the other parameters. Individual matrices, including the manually adjusted alignment, were then analyzed using PAUP\* version 4.0b 10 (Swofford 2000). The *trnL-F* alignment allowed for straightforward coding of 12 indel characters using both the simple and complex gap coding methods of Simmons and Ochoterena (2000). Regions coded as gaps were then deactivated for phylogenetic analysis in PAUP\*.

An initial heuristic search on the ITS, *trnL-F*, and combined datasets was performed with the following parameters: 2000 random addition sequences, holding two trees at each step during stepwise addition, followed by tree bisection reconnection (TBR) branch swapping with Multrees in effect, each TBR replicate limited to saving two trees, and swapping on best trees only. Trees saved from the initial search were then used as the starting topologies for a second round of extensive branch swapping on the best trees only, with no limit on the number of trees saved.

Datasets were analyzed separately and combined. A total evidence approach (Kluge 1989; Baum 1992; Kluge and Wolf 1993; Nixon and Carpenter 1996) was utilized to permit full interaction of all characters and to allow secondary signal hidden within the datasets to be revealed.

**Branch Support.** Branch support was assessed using 10,000 jackknife replicates in PAUP\*, resampling at 37%, and choosing the "emulate Jac" option. The heuristic searches utilized two random addition sequences per replication, saving only two trees per random addition sequence, with Multrees in effect, and swapping on best trees only. Only clades with a frequency of 50% or higher were retained in the jackknife consensus tree.

**Morphological Investigation.** Fresh floral and inflorescence material of *P. albeilobata* Hemsl., *P. aurantia*, *P. cinnabarina*, *P. herbertiana*, *P. murucuja*, *P. rubra*, and *Hollrungia* sp. nov. were obtained from cultivated or field collections and fixed in FAA (1 part formalin, 1 part glacial acetic acid, 18 parts 70% ethanol). Flowers from herbarium material of *P. hollrungii* and *T. tetrandra* were rehydrated using a 10% Aerosol OT solution, dissected, and examined using a stereo microscope. The tubular floral condition was evaluated for *P. aurantia*, *P. cinnabarina*, *P. herbertiana*, *P. hollrungii*, and *P. murucuja*. *P. rubra* was used as a representative of typical floral morphology in subgenus *Decaloba*. Inflorescence structure was examined in the outgroup taxa *Adenia*, *Paropsia*, and *Mitostemma* using fresh or herbarium material. Cusset (1968) thoroughly described the structure of the inflorescences of the Asian *P. eberhardtii* Gagn., *P. wilsonii*, *P. perakensis* Hallier f., *P. siamica*, and *P. moluccana* var. *teysmanniana*. The descriptions provided by Cusset (1968) were verified with live and herbarium material during the current study and found to be generally accurate; thus, these species were not studied in great detail here. Instead, inflorescences of *P. albeilobata*, *P. aurantia*, *P. biflora*, *P. rubra*, *H. sp. nov.*, and *T. tetrandra* were examined.

## RESULTS

**Sequence Characteristics.** The *trnL-F* intron and intergenic spacer alignment comprised 791 characters, of which 205 (71 within *Disemma*, *Tetraphathaea* and *Hollrungia*) were parsimony informative, including 12 simple gap characters. Sequence divergence was 7.8% (adjusted for missing data) between *Disemma* (using *P. cupiformis* as a representative) and one of the closest outgroup genera, *Mitostemma*. Sequence divergence was 4.1% between *Tetraphathaea* and *Mitostemma*, and 4.5% between *Hollrungia* and *Mitostemma*. There was 1.4% divergence between *Tetraphathaea* and *Hollrungia*. Within *Disemma*, sequence divergence ranged from 2.0–8.1%. The percentage of missing data for the *trnL-F* alignment was 2.9%, due in part to the shorter length of three sequences from Muschner et al. (2003), which comprised only the intergenic spacer region (ranging from 261–313 nucleotides in length).

Direct sequencing of ITS was straightforward and did not suffer from issues of high copy number, as single nucleotide polymorphisms were rarely observed in any particular sequence. The ITS alignment comprised 751 characters, of which 348 (123 within *Disemma*, *Tetraphathaea*, and *Hollrungia*) were parsimony informative. Sequence divergence was 28.6% (adjusted for missing data) between *Disemma* (*P. cupiformis*) and one of the closest outgroup genera, *Mitostemma*. Sequence divergence was 21.1% between *Tetraphathaea* and *Mitostemma*, and 22.3% between *Hollrungia* and *Mitostemma*. Sequence divergence was between 1.2–11.2% within *Disemma*, and 8.2% between *Tetraphathaea* and *Hollrungia*. The percentage of missing data for the ITS alignment was 5.3%, due to the shorter length of the four sequences from the Muschner et al. (2003) study that were missing the 5.8S region.

**Sensitivity Analysis.** The high level of variation in the ITS dataset made alignment difficult. This problem was addressed by performing multiple alignments in

Clustal W with different gap extension and gap opening parameters for both the multiple and pairwise alignment algorithms. In all permutations of the alignment, *Disemma* was resolved as a monophyletic group. The position of *Tetraphathaea* and *Hollrungia* was less consistent; these taxa sometimes separated from one another, but were always placed within *Passiflora*. The positions of *Mitostemma*, *Paropsia*, and *Turnera* also varied depending on the alignment, sometimes appearing within *Passiflora*. The final alignment, chosen based on congruence with published relationships among the outgroup taxa (Soltis et al. 2000; Muschner et al. 2003; Yockteng and Nadot 2004), was obtained with the Clustal W default gap cost parameters of 15 and 6.66, and then manually adjusted using Se-Al.

**Simple versus Complex Gap Coding.** Both simple and complex gap coding methods, as defined by Simmons and Ochoterena (2000), were investigated for the *trnL-F* data matrix. Using complex gap coding, only 48 trees were obtained, while 140 trees were obtained using simple coding. However, the strict consensus trees from both analyses were identical. Because both methods provided the same phylogenetic information, simple gap coding was chosen over the complex method due to the increased tree-search time associated with implementing step matrices using the complex method.

**Relationships.** For the *trnL-F* dataset, tree searches based on 205 informative characters produced 140 equally parsimonious trees of 588 steps (CI = 0.56, RI = 0.67; Fig. 1). *Passiflora* is supported as monophyletic, with a jackknife value of 86%. Subgenus *Passiflora* is also strongly supported as monophyletic (99%), as are subgenera *Astropheia* (93%) and *Decaloba* (86%). The position of the single representative of subgenus *Deidamioides*, *P. arbelaezii*, is not fully resolved.

While the exact placement of *Tetraphathaea* and *Hollrungia* within *Passiflora* is unresolved, their inclusion in *Passiflora* is well supported; these genera are united with subgenus *Decaloba* (91%). Among the 140 *trnL-F* trees, there are two common topologies for *Tetraphathaea* and *Hollrungia*: either *Hollrungia* is sister to the rest of *Decaloba* including *Tetraphathaea*, or *Hollrungia* and *Tetraphathaea* are sister taxa. These two conflicting topologies result in the lack of resolution observed in the strict consensus. The *trnL-F* data do not provide strong resolution for relationships within *Disemma*. The Australian *P. cinnabarina* is grouped with *P. multiflora*, a New World species, and the rest of the Australian species are unresolved. *Passiflora henryi* is sister to *P. moluccana* var. *teysmanniana* and *P. wilsonii*.

The ITS dataset, consisting of 348 parsimony-informative characters, produced only one most parsimonious tree of 1564 steps (CI = 0.45, RI = 0.56; Fig. 2). The ITS data provide greater resolution than *trnL-F* at the species level within *Passiflora*. Subgenera *Passiflora*



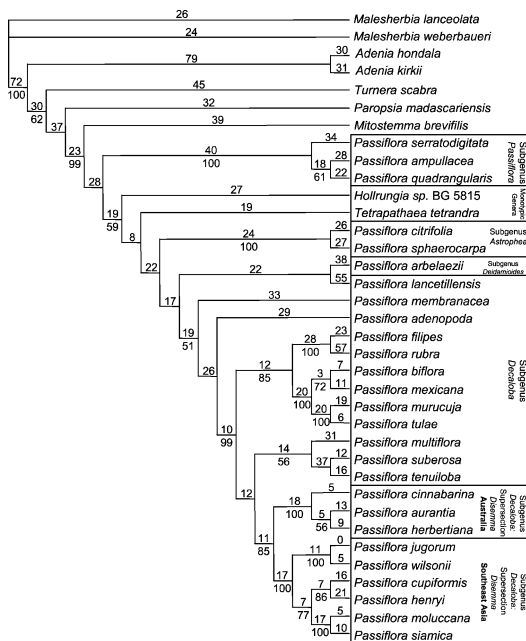


FIG. 2. The single most parsimonious tree produced from the ITS sequence data. Numbers above branches indicate branch lengths and numbers below indicate jackknife support of 50% or higher for 10,000 replicates.

**Re-evaluation of Morphology.** Flowers in subgenus *Decaloba* typically possess two or three series of perianth outgrowths, consisting of one or two series of coronal filaments and a distinct incurved plicate operculum, ca. 2 mm tall at its highest point, as exemplified by *P. rubra* (Fig. 4A). The tubular morphology in the Australian species *P. aurantia*, *P. cinnabarina*, and *P. herbertiana* (Fig. 4B–E) is structurally different from the New World *P. murucuja* (Fig. 4G). While the New World species form a floral tube by fusion of the coronal filaments, the Australian species achieve a superficially similar morphology through modification of the plicate operculum into an erect tubular structure. In *P. cinnabarina*, there is a single series of coronal filaments with an elongate operculum ca. 5 mm in length (Fig. 4B). This operculum is only slightly incurved towards the center of the flower. In *P. herbertiana*, there is a single series of coronal filaments and the plicate operculum is ca. 7.5 mm to 1 cm long (Fig. 4C). In *P. aurantia*, the coronal filaments are greatly reduced, with an elongate operculum, 2 to 3 cm long, that completely surrounds

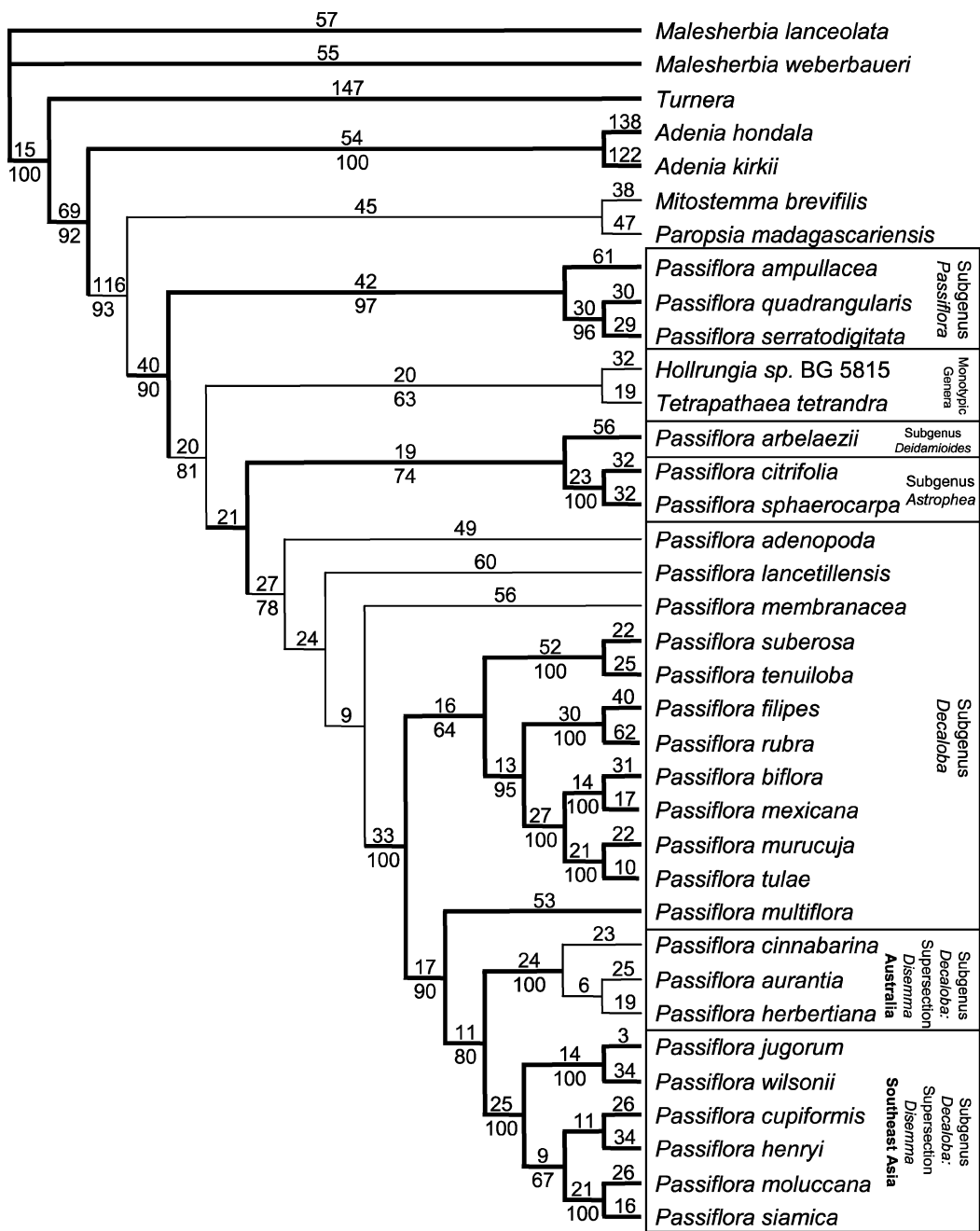
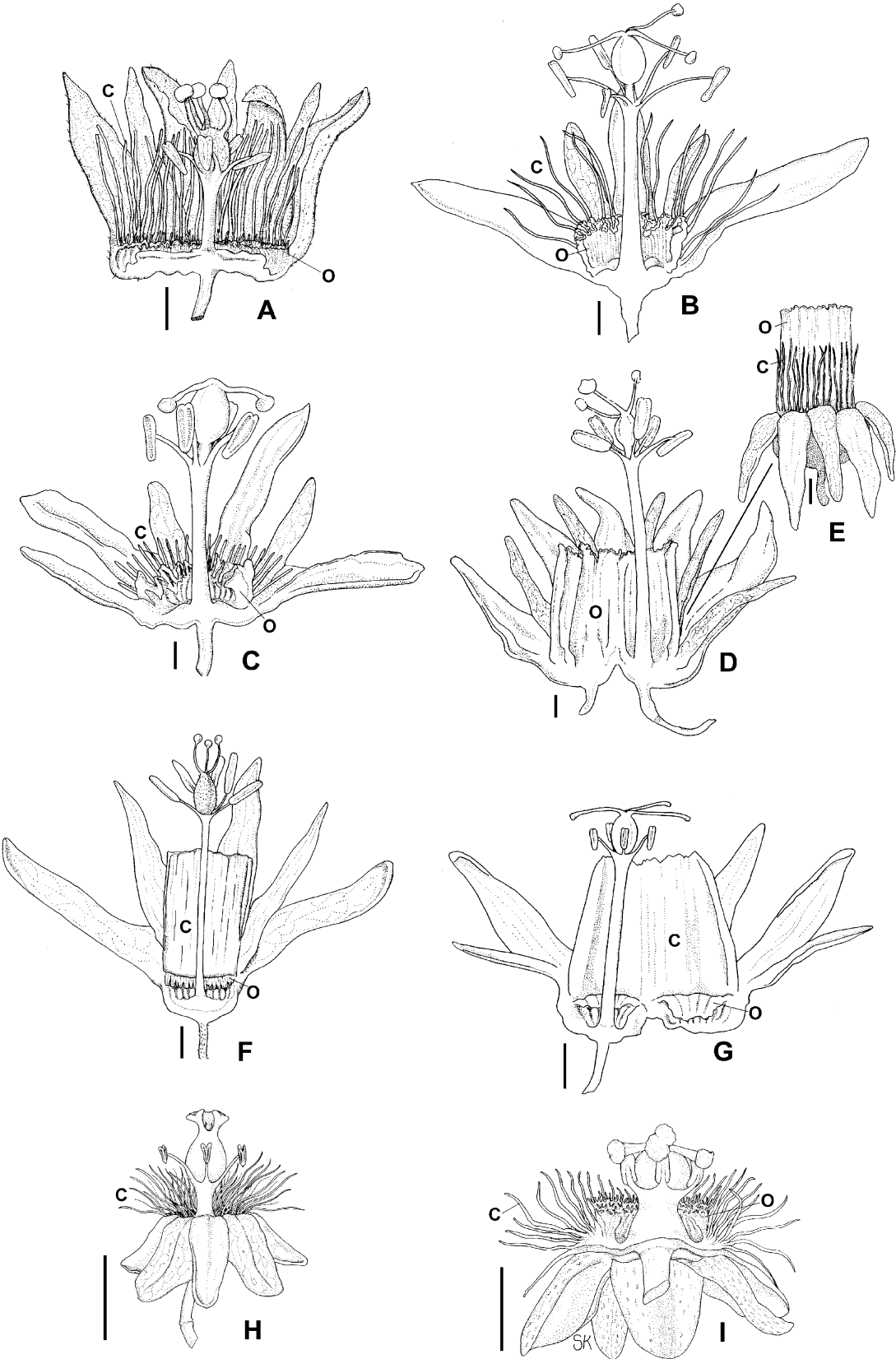


FIG. 3. One of eight most parsimonious trees based on combined *trnL-F* and ITS sequence data. Numbers above branches indicate branch lengths, and numbers below represent jackknife consensus values of 50% or higher for 10,000 replicates. Branches in bold represent clades retained in the strict consensus.

the base of the androgynophore (Fig. 4 D-E). However, in the Papua New Guinean endemic *P. hollrungii*, the coronal filaments are connate and a distinct plicate operculum is present, located ca. 3 to 4 mm above the base of the coronal filaments (Fig. 4F). The condition in *P. hollrungii* is the same as the tubular corona in *P. murucuja* (Fig. 4G), where an operculum is present and

is similarly attached ca. 4 mm from the base of the tubular coronal filaments. In *Tetrapathaea*, there is no evidence of an operculum in either male or female flowers (Fig. 4H). The coronal filaments are inserted in a single series in both male and female flowers of *Tetrapathaea*. *Hollrungia* flowers retain an operculum; male flowers have an erect operculum ca. 3 mm tall



with a fimbriate margin, while female flowers have an incurved plicate operculum ca. 2 to 3 mm tall and a less pronounced fimbriate margin (Fig. 4I).

The current interpretation of axillary inflorescence structure in Passifloraceae follows Troll (1964) as redrawn in the generalized Passifloraceae inflorescence (Fig. 5). The basic inflorescence type in the family is an axillary compound cyme with various degrees of reduction. The first-order axis may terminate in either a tendril or a flower; the proximal portion of this axis may be referred to as a peduncle. Within the inflorescence, branches are axillary and always subtended by floral bracts. The branches in the inflorescence are assigned higher orders numerically as they diverge successively from the first-order axis.

Most species of *Adenia* and *Mitostemma* retain an elongate peduncle (Fig. 6A, C), while *Paropsia* has a much shorter, but still recognizable, peduncle (Fig. 6B). However, this condition has been largely lost within *Passiflora* (Fig. 6D–G). Most species in *Passiflora* have greatly reduced peduncles; this condition is recognized by the retention of a terminal tendril in the medial position between two second-order side branches. *Passiflora biflora* and *P. rubra*, both New World members of *Decaloba*, illustrate the reduction of the first-order axis into the axil of the leaf (Fig. 6D, E). *Passiflora rubra* provides an example of further reduction of branching, as only one of the two second-order branches is retained.

All species in supersection *Disemma* have clearly reduced first-order axes in the same manner as the rest of *Passiflora*. However, the inflorescences of the Australian species (Fig. 6F) and *P. holrrungii* do not have branching beyond second-order, while most Chinese species possess branched axes through the third-order (Fig. 6G). In *Tetraphathaea*, the peduncle of the inflorescence is elongate, and the apical portion terminates in a flower rather than a tendril (Fig. 6H). Inflorescences of *Holrrungia* also retain an elongate peduncle, and the terminal portion may end in either a flower, as in *Holrrungia aurantioides* K. Schum. (Fig. 6I), or in a tendril, as in *H. sp. nov.* (not shown).



FIG. 5. Hypothetical model inflorescence for Passifloraceae sensu Troll (1964). The first-order stalk (proximal portion = peduncle) may terminate in either a tendril or a flower, followed by successive branching through the fourth order. Vegetative bud is superior to inflorescence bud in the leaf axil (V).

## DISCUSSION

**Robustness of the Phylogenetic Hypothesis.** This is the first study to incorporate a large number of Old World *Passiflora* with extensive sampling across the rest of subgenus *Decaloba*. All four subgenera are represented, although the weakest representation is in subgenus *Deidamioides* with only *P. arbelaezii*; this subgenus contains 17 relatively rare species, all of which were difficult to obtain for analysis. Additionally, this is the only study thus far to evaluate the position of *Holrrungia* relative to *Tetraphathaea* and *Passiflora*.

The *trnL-F* dataset provided the greatest resolution at the subgeneric level and above in *Passiflora* and the outgroups (Fig. 1). While the infrageneric relationships within subgenus *Decaloba* were not well resolved using *trnL-F*, there was strong jackknife support for the inclu-

FIG. 4. Comparison of coronal filaments and operculum in Old and New World *Passiflora*. Coronal filaments (C) and operculum (O) are labeled. All flowers drawn from preserved or herbarium material and sectioned longitudinally. Scale bars = 5 mm. A. *Passiflora rubra* exhibits the typical condition of flowers in subgenus *Decaloba* with two series of coronal filaments and an incurved plicate operculum (Krosnick 27, OS). B. *P. cinnabarina* has a single series of filaments and an erect plicate operculum (Butler 66949, QRS). C. *Passiflora herbertaina* has a reduced series of coronal filaments and a plicate operculum (Krosnick 255, OS). D. *P. aurantia* has an elongate tubular operculum (Krosnick 24, OS) and a single highly reduced series of coronal filaments. E. *P. aurantia* in side view showing a single series of coronal filaments and the elongate operculum, androgynophore is removed (Krosnick 24, OS). F. *P. holrrungii* with a single series of tubular coronal filaments and a plicate operculum at the base of the floral tube (van Royen 11694, LAE). G. *P. murucuja* with a single series of coronal filaments and a plicate operculum at the base of the fused coronal filaments (Krosnick 263, OS). H. *Tetraphathaea tetrandra* with a single series of densely arranged coronal filaments and no operculum (♀ Kirk s.n., WELT). I. *Holrrungia sp. nov.* with a single series of coronal filaments and an erect operculum (♂ Gray 5815, QRS).

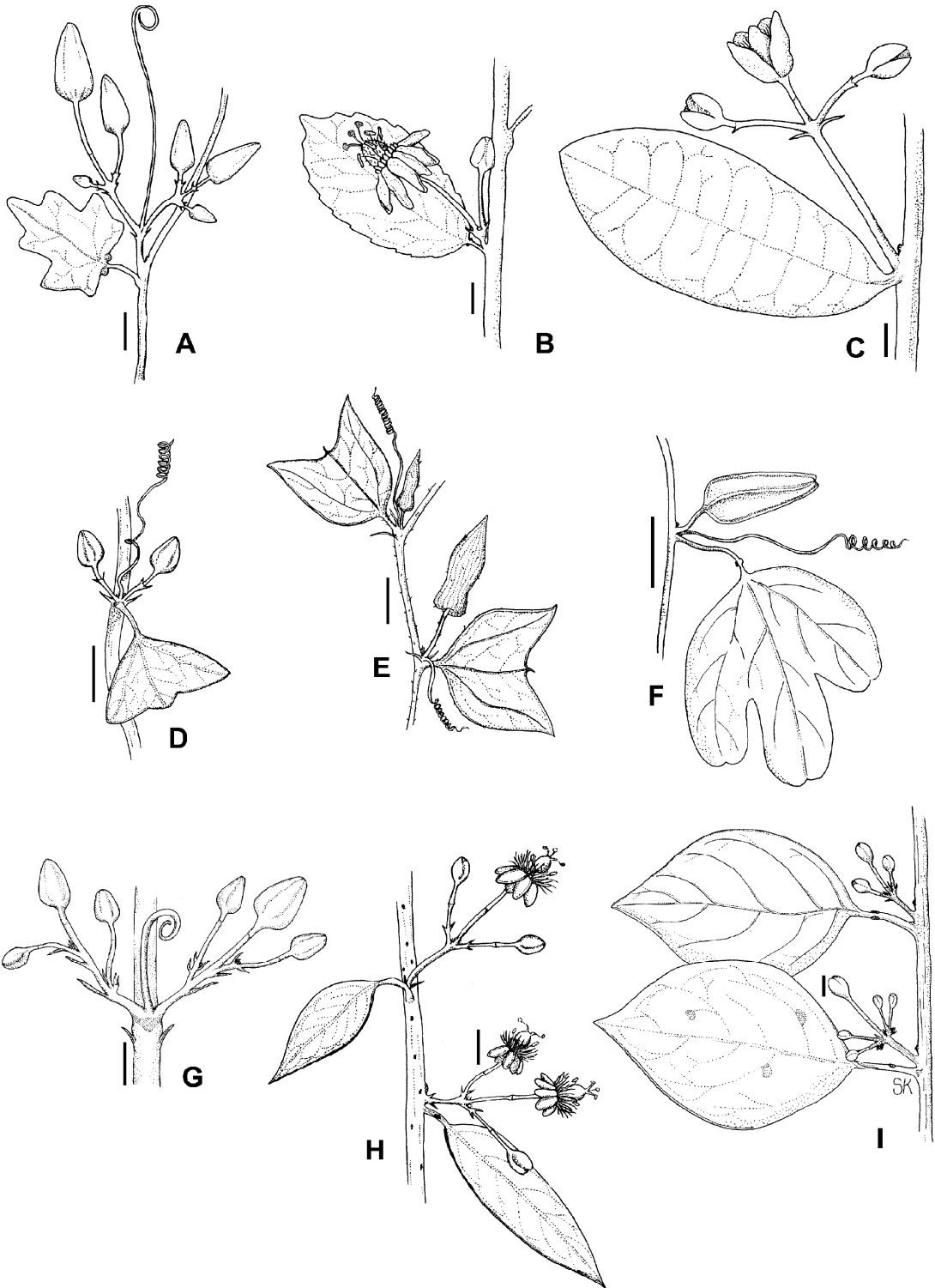


FIG. 6. Inflorescences in Passifloraceae. All inflorescences drawn from preserved or herbarium material. Scale bars = 1 cm. A. *Adenia lobata* exhibits an elongate peduncle and branching through the third-order (Krosnick 267, OS). B. *Paropsia greviioides* retains a short peduncle, but the distal portion of the first-order stalk aborts leaving only second-order branches (collector unknown 9197, US). C. *Mitostemma brevifilis* demonstrates an elongate peduncle with second-order branching (Reidel s.n., NY). D. *P. biflora* has a reduced primary stalk, a terminal tendrill and two second-order branches (Krosnick 260, OS) E. *P. rubra* retains only one second-order side branch and a terminal tendrill (Krosnick 27, OS). F. *P. aurantia* has only one second-order branch and



sion of *Hollrungia* and *Tetraphathaea* within *Passiflora*. In contrast, the ITS pattern was well supported at the infrageneric and species level for subgenus *Decaloba* and outgroups (Fig. 2). There was strong jackknife support for the monophyly of supersection *Disemma*, and the species-level relationships within *Disemma* were well resolved. While the ITS data did not provide strong resolution for the position of *Hollrungia* relative to *Tetraphathaea*, they did provide strong jackknife support for the inclusion of these two genera in *Passiflora*. The combined molecular dataset provides strong support for previously unelucidated phylogenetic relationships and yields a well-resolved strict consensus tree.

**Monophyly of *Disemma*.** The high level of morphological diversity found within this group has historically made its taxonomic delimitation difficult. Both Masters (1871) and Harms (1925) suggested that *Disemma* might not be a natural group and instead hypothesized that the Australian and New Guinean species were more closely related to New World members of subgenus *Decaloba* than to the Southeast Asian species. Harms (1925) accommodated the Old World species by dividing them among four sections: the Australian species and most Asian species were placed in section *Decaloba* with New World species, section *Hollrungiella* was created for *Passiflora hollrungii*, section *Octandranthus* for *P. siamica*, and section *Anomopanthus* for *P. moluccana*. Later, De Wilde (1972a) suggested that the Old World species formed a monophyletic section and divided them into three informal subsections: subsection one contained the Southeast Asian species, subsection two contained the Australian species, and subsection three consisted of *P. hollrungii* K. Schum. The current classification of the genus recognizes all of the Old World species in subgenus *Decaloba* supersection *Disemma* with three sections: *Octandranthus* Harms, containing all the Asian species, *Disemma* (Labill.) J. M. MacDougal and Feuillet, with the Australian species, and *Hollrungiella* Harms with *P. hollrungii* (Feuillet and MacDougal 2004).

The combined dataset strongly supports the monophyly of supersection *Disemma*. At present, it is possible to define two main clades within *Disemma*: an Australian clade and a mainland Asian clade. Although the relationships among the Australian species are unresolved here, this group is strongly supported as monophyletic based on the combined dataset. This differs from Yockteng and Nadot (2004), in which all Australian species fall within subgenus *Decaloba*, but are not monophyletic. In their analysis, *P. cinnabarin*

and *P. aurantia* fall together as basal members of *Decaloba*, but *P. herbertiana* is near the New World *P. helleri* Peyr. and *P. trifasciata* Lem. The differing results of Yockteng and Nadot (2004) are most likely due to a lack of adequate sampling within subgenus *Decaloba*, specifically in supersection *Disemma*. The Australian species share several characteristics: an inflorescence with a reduced primary stalk and second-order branching only, a plicate, erect operculum, a single series of coronal filaments, a general reduction in the prominence of the corona, trilobed leaves, and short-cylindric petiolar nectaries.

The Asian species in section *Octandranthus* sampled for this analysis are from three southern provinces in China: *P. siamica*, *P. henryi*, *P. wilsonii*, and *P. jugorum* are all endemic to Yunnan, *P. cupiformis* is found in Guangxi, and *P. moluccana* var. *teysmanniana* is native to Hainan Island in Guangdong. These species have never been included in any phylogenetic analyses of *Passiflora*, and are well supported here as monophyletic within supersection *Disemma*. There are several unique features found among the Asian species, including sub-opposite to opposite leaf arrangement in *P. moluccana* var. *teysmanniana*, and extra stamens and carpels in *P. siamica*, *P. moluccana* var. *glaberrima*, and *P. tonkinensis*. The Asian species may be recognized by a combination of features: an inflorescence with a reduced first-order stalk, higher order branching, and two series of coronal filaments. Other important features such as leaf shape, nectary location, and nectary type are too variable in the Asian species to be useful in the recognition of section *Octandranthus* in its entirety.

#### **Phylogenetic Position of *Hollrungia* and *Tetraphathaea*.**

These two genera are unique in their geographical distribution relative to the rest of Passifloraceae. While other genera in the family are also distributed in the Old World, their primary distribution is on the African continent and Madagascar (*Adenia* Forssk., *Ancistrothyrsus* Harms, *Barteria* Hook. f., *Basananthe* Peyr., *Crosostemma* Hook., *Deidamia* Thouars, *Paropsia* Thouars, *Smeathmannia* R. Br., and *Viridivia* J.H. Hemsl. & Verdc.). *Hollrungia* is native to Melanesia and is also widespread throughout northeastern Australia in rainforest habitats. *Tetraphathaea* is found only in New Zealand and has a range extending from North Island through the Banks Peninsula on South Island.

*Hollrungia* flowers have six to eight stamens and three to five carpels, and are functionally dioecious (De Wilde 1972b). *Tetraphathaea* flowers possess four to

←

a terminal tendril (Krosnick 24, OS). G. *P. altilobata* exhibits a reduced peduncle with branching through the third-order (Krosnick 03, OS). H. *Tetraphathaea tetrandra* has an elongate peduncle terminating in a flower (♀ Krosnick 266; OS). I. *Hollrungia* sp. nov. has an elongate peduncle branching through the third-order (♂ Hoogland and Craven 10210, BRI).

five stamens and two to three carpels, and are completely dioecious (De Wilde 1971). While *Hollrungia* has historically retained generic status, *Tetrapathaea* has been less stable taxonomically. *Tetrapathaea* was originally published as a section of *Passiflora* by DeCandolle (1822) to accommodate *P. tetrandra* DC. However, the dioecy observed in this species led Reichenbach (1828) to segregate *Tetrapathaea* as a distinct genus. Masters (1871) placed *Tetrapathaea* back in *Passiflora* in subgenus *Plecostemma*, and assigned it to a monotypic section. Harms (1925) then re-elevated *Tetrapathaea* to generic status within Passifloraceae. More recently, Green (1972) returned *Tetrapathaea* to *Passiflora* and designated a new subgenus to accommodate it. Yockteng and Naudot (2004) included *Tetrapathaea* in their analysis of *Passiflora* using *ncpGS* data, where it occupied a basal position within subgenus *Decaloba*. They suggested that subgenus *Tetrapathaea* (DC.) P. S. Green be retained for *Tetrapathaea tetrandra* (= *Passiflora tetrandra* DC.). The similar results obtained here using different plant material sources, different gene regions, and more extensive outgroup sampling lend support to their conclusion that *Tetrapathaea* belongs in *Passiflora*. Based on the high level of jackknife support in both the individual and combined analyses here, it seems reasonable that both *Hollrungia* and *Tetrapathaea* should be recognized as part of *Passiflora*.

It is currently unclear whether *Hollrungia* and *Tetrapathaea* are sister taxa or if *Hollrungia* is sister to *Tetrapathaea* plus the rest of the genus (Fig. 3). In the eight most parsimonious trees produced by the combined dataset, six trees suggest that *Hollrungia* is sister to *Tetrapathaea* + *Astropheia*, *Deidamioides*, and *Decaloba*, while two trees place *Hollrungia* and *Tetrapathaea* as sisters. While the latter clade is not retained in the strict consensus, the jackknife tree for the combined dataset placed both taxa together as sisters, though with low support (63%). The retention of an elongate primary stalk in *Hollrungia* and *Tetrapathaea*, also exhibited in subgenus *Deidamioides*, suggests that greater sampling must be conducted within this subgenus before any definitive taxonomic revision can be completed. Until greater resolution is obtained for the relationship between *Hollrungia* and *Tetrapathaea*, it is not yet possible to recommend the placement of *Hollrungia* or *Tetrapathaea* in *Passiflora* subgenus *Tetrapathaea*, nor is it possible to definitively support the recognition of *Tetrapathaea* as a subgenus in *Passiflora*.

**Tubular Floral Morphology.** While *Passiflora* exhibits highly complex floral morphology, relatively few developmental and anatomical studies have been performed that have addressed their unique features across a wide range of species. Of particular relevance to the Old World species is the nature of the coronal filaments and operculum, as these structures are highly modified in the Austral-Pacific species. Payer (1857),

in his examination of the New World hybrid *P. loudonii* W. H. Baxter & G. Don, concluded that coronal and opercular outgrowths were petaloid in nature. Masters (1871) suggested that the corona, based on its late ontogenetic formation, was an organ *sui generis*, developed for use as part of the pollination mechanism associated with the flowers. Later, Weberling (1989) determined the coronal filaments to be outgrowths of receptacular tissue, while Endress (1994) suggested that the corona may represent staminodes from a once polyandrous androecium. Most recently, Bernhard (1999) concluded that the coronal filaments and operculum could not be decisively linked to a particular floral whorl, but was able to demonstrate clearly their close ontogenetic connection in *P. racemosa* Brot. He showed that the corona develops as a flat bulge, the upper edge becoming filamentous and the inner edge forming a ring-like protrusion that later forms the operculum. While the coronal filaments and operculum appear to be derived from the same initial tissue (Bernhard 1999), both features are highly conserved and distinct from one another in structure and function across the entire genus. The operculum serves as the main regulator of pollinator access to the nectar chamber; almost all species in *Passiflora* possess an operculum, though its shape and arrangement may be highly diverse (Tillett 1988). The coronal filaments serve as a landing platform, and function to position the pollinators to achieve efficient pollination (Endress 1994). Unfortunately, however, the developmental changes leading to the observed diversity in these structures have not been examined in any species with tubular flowers in either the New World or the Old World.

Among the Old World species, the Australian *Passiflora* are unique in possessing a tubular, erect operculum and reduced coronal filaments within their flowers, which are typically brightly colored red, orange, or yellow. *Passiflora hollrungii* is also distinct in its blue flowers and tubular floral morphology. Little is known about what types of animals pollinate the flowers of the Austral-Pacific species of *Passiflora*, though they are generally believed to be bird pollinated based on their bright coloration and tubular floral morphology. New World hummingbird-pollinated species in section *Decaloba*, such as *P. murucuja*, *P. tulae*, *P. orbiculata* Cav., *P. schaeferi* Britton, and *P. maestrensis* Duharte, have brightly colored red or pink flowers with connate coronal filaments (Kay 2003), suggesting that an equivalent avian pollinator may be associated with the Old World species. Based on the outward similarity between the tubular morphology observed in the Old and New World species, it is understandable that the Australian species have been thought to be related to the hummingbird pollinated species in the New World. However, while the floral morphology in

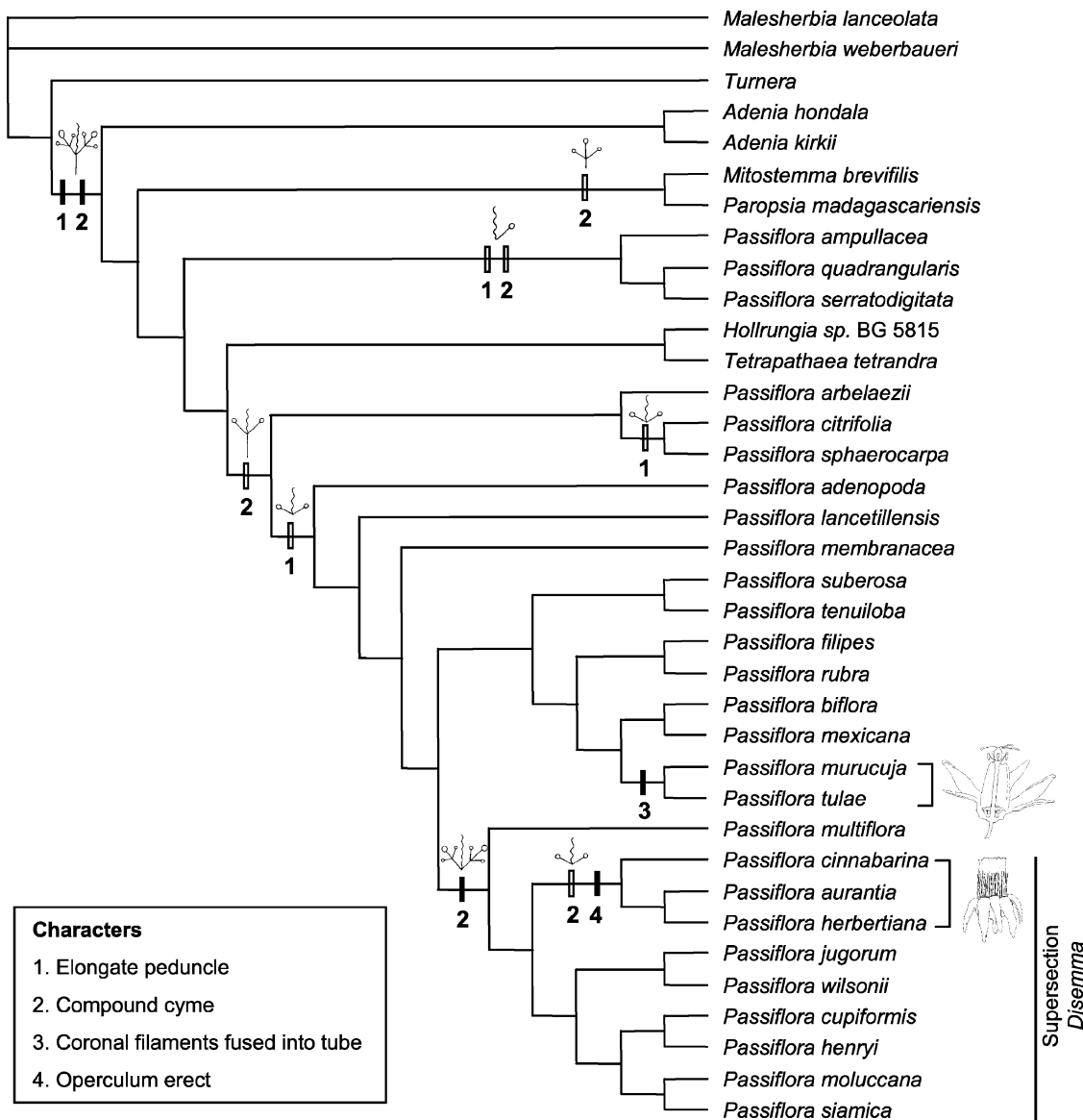


FIG. 7. Character evolution of inflorescence structure and tubular floral morphology in Passifloraceae based on combined *trnL-F* and ITS data. Solid tick marks indicate synapomorphies, hollow tick marks indicate reversals. Character 1, presence of an elongate first-order stalk (= peduncle); character 2, presence of a compound cyme with branching beyond the second-order; character 3, tubular floral morphology resulting from fusion of coronal filaments; character 4, tubular floral morphology created by an erect, plicate operculum.

the Australian section *Disemma* appears similar to that in the New World tubular species, it is shown here to be structurally different (Fig. 4). The operculum in Australian species has been modified into a floral tube, whereas it is the coronal filaments that have been modified into a tube in both the New World species and *P. hollrungii*. In the phylogenetic analysis, *Passiflora murucuja* and *P. tulae*, both New World hummingbird-pollinated species, form a highly supported monophyletic clade (100%) that is distinct from supersection *Disemma*.

*ma*. Therefore, molecular data support the idea that tubular flowers have been derived in parallel in both the New and Old World (Figs. 3, 7). In the Australian species, the operculum extends upwards into a tubular structure and there is no longer any physical protection of the nectar ring. All of the Australian species exhibit the same floral condition to varying degrees. The least tubular morphology is found in *P. cinnabarina*, which retains a short operculum and rather elongate coronal filaments. *Passiflora herbertiana* may be

considered intermediate and *P. aurantia* the most tubular, with its highly reduced filaments and operculum that extends two to three centimeters beyond the petals. It would appear that the Old World and New World species have reduced the number of coronal series from two to one in parallel, but have exaggerated their tubular morphology by modifying different parts of the perianth.

The similarity observed between the Australian and South American species may help to explain the classification of the Old World species by Masters (1871) and Harms (1925). Masters put most of these species into two groups within subgenus *Plecostemma*, section *Decaloba*. The Australian species were placed with New World members (§2, *inflorescentia simplex*), while the Asian species were placed with other New World species (§1, *inflorescentia cymoso-paniculata*). He also likened the “flattened corona” observed in *P. murucuja* and *P. tulae* to that found in the Australian species. Similarly, Harms (1925) placed the Australian species in his section *Decaloba* due to their greater morphological similarity to other New World members than to the Asian or Pacific species.

*Passiflora hollrungii*, endemic to Papua New Guinea, has a tubular condition that appears to be structurally equivalent to that in the New World species. This poorly known rainforest species has blue petals and a single series of purple coronal filaments fused into a tube. A plicate operculum is retained at the base of the coronal filaments in the same manner as the New World hummingbird pollinated species. The phylogenetic position of this species has not yet been investigated, although it is currently placed in *Disemma* (MacDougal and Feuillet 2004). Interestingly, Harms (1925) acknowledged the similarity of this species to the New World members of subsection *Murucuja*; in his classification, he listed section *Hollrungeiella* directly between sections *Murucuja* and *Pseudomurucuja*, two sections that contain most of the New World tubular-flowered species.

The floral condition observed in *Hollrungia* and *Tetrapathaea* is unique because they would represent the only two dioecious taxa known in *Passiflora*. The flowers of *Hollrungia* are white and small, being only 2–3 cm wide, with two short series of coronal filaments. *Tetrapathaea* has smaller white flowers that are 1–2 cm across and have a single series of densely arranged coronal filaments. While *Hollrungia* retains an operculum and a nectar ring at the base of the flower, *Tetrapathaea* has lost these structures entirely. In *Tetrapathaea*, female flowers have a stipitate ovary and barren rudimentary anthers at anthesis. In mature male flowers, the ovary is highly reduced while the anthers are large and prominent. The flowers are strongly scented and clustered in axillary cymes along the stem. Little is known about pollination in either of these species.

**Inflorescence Structure.** The nature of the inflorescence is poorly understood in Passifloraceae. Cusset (1968) provided the most detailed account of inflorescence structure for the family, though his study dealt only with *Adenia* and *Passiflora*. Inflorescence development in the economically important *P. edulis* Sims and *P. quadrangularis* L. was investigated by Moncur (1988), but these species have highly reduced inflorescences, making it difficult to compare them to other species in the genus. Based on specimens examined for this analysis, the inflorescences of *Adenia*, *Paropsia*, and *Mitostemma* generally follow a basic model (as illustrated in Fig. 5) consisting of two features: an elongate first-order axis that often terminates in a tendril, and higher order branching resulting in a compound cyme (Fig. 6 A–C).

*Passiflora*, the most species rich genus in the family, exhibits a wide range of modifications on the basic compound cyme model presented here (Fig. 5). The elongate first-order axis is almost completely absent except in subgenus *Deidamioides*. In many species of *Passiflora*, the second-order branches may also be greatly reduced, leaving only a terminal tendril and one lateral branch (Fig. 6 D–F). Compound cymose inflorescences and the presence of a peduncle have traditionally been regarded as primitive features for the family, and this is confirmed by the basal positions of genera with both elongate peduncles and higher order branching (*Adenia*, *Mitostemma*, and *Paropsia*) in the molecular analyses presented here (Figs. 3, 7). Based on the inferred phylogenetic significance of these features, cases of branched inflorescences occurring within *Passiflora* have raised questions about the phylogenetic position of those species exhibiting these features (Tillet 1988).

There has been much confusion regarding inflorescence structure in the Old World species of *Passiflora*. Much of this uncertainty may be explained by Cusset's (1968) paper on the tendrils of Passifloraceae. Cusset's work was the first to thoroughly investigate the inflorescences in *Disemma* and provided the foundation for our current understanding of the inflorescence morphology in *Passiflora*. However, the illustration of an apparently anomalous specimen of *Passiflora moluccana* var. *teysmanniana* by Cusset (1968, Plate III, Fig. 6, as *P. horsfieldii* Blume) suggests that the Old World species exhibit both an elongate peduncle and higher order branching in their inflorescences. Cusset's illustration was redrawn from one provided by Blume (1837) in the original type description for *P. horsfieldii*. The original illustration of this species clearly shows a reduced peduncle with branching through the fourth order. In Cusset's diagrammatic representations of Blume's original illustration (1968, Plate III, Fig. 6, and Plate V, Fig. 19), he incorrectly portrayed the peduncle as elongate, thus creating the confusion about peduncle presence



in *Disemma*. There is no current evidence that any of the Old World species retain an elongate peduncle.

The second presumed feature of the primitive inflorescence of Passifloraceae is the presence of higher order branching resulting in a compound cyme. The presence of higher order branching observed in the Old World members of *Passiflora* is not unique to supersection *Disemma*. In fact, a small number of species within subgenera *Astrophea* and *Decaloba* also have higher order branching, such as *P. callistemma* L. K. Escobar, and *P. sexflora* Juss. It should be noted that the branched inflorescences referred to here are truly compound cymes, distinct from the pseudo-inflorescence type observed in certain parts of Passifloraceae, as in *Adenia racemosa*, *A. globosa*, *Passiflora coriacea* Juss., *P. racemosa*, and *P. ovalis* M. Roem. (De Wilde 1971). The type of inflorescence observed in these species actually represents growth of the superior vegetative bud out of the leaf axil, followed by profuse axillary flowering at each node along this shortened vegetative axis. Interestingly, subgenus *Decaloba* supersection *Multiflora*, the group shown in this analysis to be most closely related to supersection *Disemma* (Fig. 7), contains most of the species outside of the Old World that exhibit higher order branching. In fact, Masters (1871) placed the Asian species in the same section and series as *P. multiflora* and *P. sexflora* based on their branched inflorescences. Several Ecuadorian members of supersection *Multiflora*, including *P. sodiroi* Harms, *P. ursina* Killip & Cuatrec., *P. monadelpha* Jørgensen & Holm-Nielsen, and *P. apoda* Harms, also have reduced peduncles with higher order branching in their inflorescences. Therefore, it appears that the inflorescences in *Disemma* are more similar to others found within the same subgenus than they are to any other genera in the family.

The inflorescences of *Hollrungia* and *Tetraphathaea*, as mentioned earlier, appear to exhibit a plesiomorphic retention of an elongate peduncle. *Hollrungia* has definite higher order branching in the inflorescence, and the terminal tendril is retained, though it may rarely partially abort. *Tetraphathaea* has a regular three-flowered cyme, though rarely up to six flowers were observed per inflorescence. Both species are similar to subgenus *Deidamioides* in their retention of an elongate peduncle. The higher order branching observed in *Hollrungia*, and more rarely in *Tetraphathaea*, may represent an independent reversal to the primitive condition that is also sometimes observed in subgenera *Astrophea* and *Decaloba* of *Passiflora*.

While some of the problems of morphological homology in *Disemma* have been addressed here, there are still many unresolved issues. The homology of inflorescence structure in supersections *Disemma* and *Multiflora* needs further clarification. Many species in supersection *Multiflora* appear to have higher order branches that form true cymes, while the Asian species

of supersection *Disemma* often demonstrate what might be considered recaulescence (sensu Cusset 1968), and thus have sub-opposite to alternate cymose branching patterns. Before this can be confirmed for the Asian species, detailed ontogenetic studies are required. Examples of delayed initiation of branching in the immature inflorescence would affect the placement of branches and bracts associated with these branches, and might confirm the hypotheses presented by Cusset. Additionally, greater efforts must be directed at resolving the position of *Hollrungia* and *Tetraphathaea* within *Passiflora*. Taxonomic placement of these species cannot be resolved until the phylogenetic relationships between these two species are resolved with a high level of support.

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## APPENDIX

List of species of *Passiflora* and outgroups used for molecular analysis, including subgeneric classification, collector, voucher information, location and GenBank accession numbers for regions sequenced.

*Malesherbia lanceolata* Ricardi—K. Gengler-Nowak 56 (OS), *trnL-F* AY636104, ITS 1–2 AY636103. *M. weberbaueri* Gilg—K. Gengler-Nowak 288 (OS), *trnL-F* AY632722, ITS 1–2 AY632697. *Turnera scabra* Millsp.—Gengler-Nowak (2003): ITS-1 AF276316, ITS-2 AF276369. *T. ulmifolia* L.—M. Alford 3039 (BH), *trnL-F* AY636110. *Adenia hondala* (Gaertn.) W.J. De Wilde—S. Krosnick 256 (OS) *trnL-F* AY632724, ITS 1–2 AY632699. *A. kirkii* (Mast.) Engl.—S. Krosnick 257 (OS), *trnL-F* AY632723, ITS 1–2 AY632698. *Mitostemma brevifiliis* Gontsch.—Muschner et al. (2003): *trnL-F* intergenic spacer AY102386, ITS-1 AY102359, ITS-2 AY102379. *Paropsia madascariensis* (Mast.) H. Perrier—M. Zyltra 949 (WIS), *trnL-F* AY636105, Muschner et al. 2003: ITS-1 AY102365, ITS-2 AY102385. *Hollrungia* K. Schum. *H. sp. nov.*—B. Gray 5815 (QRS), *trnL-F* AY632725, ITS 1–2 AY632700. *Passiflora* L. subgenus *Astropheae* (DC.) Mast.—*P. citrifolia* Salisb.—S. Krosnick 261 (OS), *trnL-F* AY632732, ITS 1–2 AY632707. *Passiflora* subgenus *Decaloba* (DC.) Rchb.—*P. adenopoda* DC.—S. Krosnick 258 (OS), *trnL-F* AY632727, ITS 1–2 AY632702. *P. aurantia* G. Forst.—S. Krosnick 24 (OS), *trnL-F* AY632729, ITS 1–2 AY632704. *P. biflora* Lam.—S. Krosnick 260 (OS), *trnL-F* AY632730, ITS 1–2 AY632705. *P. cinnabarina* Lindl.—G. Butler 66949 (CBG), *trnL-F* AY632731, ITS 1–2 AY632706. *P. cupiformis* Mast.—S. Krosnick 253 (OS), *trnL-F* AY632733, ITS 1–2 AY632708. *P. filipes* Benth.—D. Goldman 2153 (BH), *trnL-F* AY632734, ITS 1–2 AY632709. *P. henryi* Hemsl.—S. Krosnick 08 (OS), *trnL-F* AY632735, ITS 1–2 AY632710. *P. herbertiana* Ker Gawl.—S. Krosnick 255 (OS), *trnL-F* AY632736, ITS 1–2 AY632711. *P. jugorum* W. W. Sm.—S. Krosnick 15 (OS), *trnL-F* AY632737, ITS 1–2 AY632712. *P. lancetillensis* J. M. MacDougal & J. Meerman—Muschner et al. (2003): *trnL-F* intergenic spacer AY210963, ITS-1 AY210943, ITS-2 AY210924. *P. membranacea* Benth.—S. Krosnick 19 (OS), *trnL-F* AY632726, ITS 1–2 AY632701. *P. mexicana* Juss.—D. Goldman 1774 (BH), *trnL-F* AY632738, ITS 1–2 AY632713. *P. moluccana* var. *teysmanniana* (Miq.) W. J. De Wilde—S. Krosnick 198 (OS), *trnL-F* AY632739, ITS 1–2 AY632714. *P. multiflora* L.—D. Goldman 2164 (BH), *trnL-F* AY632740, ITS 1–2 AY632715. *P. murucuja* L.—S. Krosnick 263 (OS), *trnL-F* AY632747, E. Kay 217 (MO), ITS AY648559. *P. rubra* L.—S. Krosnick 27 (OS), *trnL-F* AY632741, ITS 1–2 AY632716. *P. siamica* Craib—S. Krosnick 07 (OS) *trnL-F* AY632742, ITS 1–2 AY632717. *P. suberosa* L.—S. Krosnick 265 (OS), *trnL-F* AY632743, ITS 1–2 AY632718. *P. temuloba* Engelm.—D. Goldman 1770 (BH), *trnL-F* AY632744, ITS 1–2 AY632719. *P. tulae* Urb.—Muschner et al. (2003): *trnL-F* intergenic spacer AY102392, ITS-1 AY102352, ITS-2 AY102372. *Passiflora* subgenus *Deidamoides* (Harms) Killip—*P. arbelaezii* L. Uribe—S. Krosnick 259 (OS), *trnL-F* AY632728, ITS 1–2 AY632703. *Passiflora* subgenus *Passiflora*—*P. ampullacea* (Mast.) Harms—S. Krosnick 262 (OS), *trnL-F* AY632745, ITS 1–2 AY632720. *P. quadrangularis* L.—S. Krosnick 01 (OS), *trnL-F* AY636106, ITS 1–2 AY636107. *P. serratodigitata* L.—S. Krosnick 264 (OS), *trnL-F* AY636109, ITS 1–2 AY636108. *Tetraphathea* (DC.) Rchb. *T. tetrandra* (Banks and Sol. ex DC.) Cheeseman—S. Krosnick 266 (OS), *trnL-F* AY632746, ITS 1–2 AY632721.