PHYLOGENY AND CHARACTER EVOLUTION OF KIELMEYEROIDEAE (CLUSIACEAE) BASED ON MOLECULAR AND MORPHOLOGICAL DATA

By

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Clusiaceae are a family of approximately 1000 species with a pantropical distribution. Based on a morphology-based cladistic analysis, the family has been divided into three subfamilies: Hypericoideae, Clusioideae, and Kielmeyeroideae. Kielmeyeroideae are divided into two tribes: Calophylleae, a large, pantropical tribe (ca. 450 spp), and Endodesmieae, a small, tropical African group (comprising the two monotypic genera *Endodesmia* and *Lebrunia*). Subfamilial assignment of Endodesmieae has been uncertain. Based on fruit characters, Endodesmieae are similar to Calophylleae; however, vegetatively, they are similar to Clusioideae. A previous family-level study based on *rbcL* sequences confirmed the monophyly of the three traditional subfamilies except that *Clusiella*, traditionally a member of Clusioideae, was placed in Kielmeyeroideae. Internal support for relationships within Kielmeyeroidae was weak, leaving it unclear as to which genera *Clusiella* is most closely related. Species of Endodesmieae were not included in the *rbcL* study. The present study determined the

generic relationships within Kielmeyeroideae based on phylogenetic analysis of *rbcL*, *matK*, and ITS sequence data and morphological characters. Parsimony analyses were conducted on each molecular and morphological data set separately and combined. Bayesian and maximum likelihood analyses were performed on separate and combined molecular data sets. Sampling included several species of most genera of Calophylleae, as well as *Clusiella* and the enigmatic *Endodesmia*. The *rbcL* and *matK* data sets support the sister-group relationship of *Endodesmia* to the remaining Kielmeyeroideae and the sister-group relationship of *Mammea* to the rest of Calophylleae. Within the core Calophylleae (all Calophylleae except *Mammea*), the strictly New World genera (*Kielmeyera*, *Caraipa*, *Haploclathra*, *Clusiella*, *Mahurea*, *Neotatea*, and *Marila*) likely form a clade, and the primarily Old World genera (*Kayea*, *Poeciloneuron*, *Mesua*, and *Calophyllum*) constitute a second clade. All morphological characters are mapped onto a total evidence tree in order to infer their evolutionary pattern. Character state reconstructions of several noteworthy morphological characters, such as the presence/absence of latex cavities and canals, leaf arrangement, presence/absence of anther glands, carpel number, fruit type, and seed form are discussed in more detail. Putative morphological synapomorphies for each genus were determined, and a key to the genera of Kielmeyeroideae is provided.

CHAPTER 1
INTRODUCTION

Clusiaceae are a large, pantropical family of approximately 1000 species in the Malpighiales (Stevens, 2001). Within Malpighiales, Clusiaceae are probably most closely related to Bonnetiaceae and Podostemaceae (Gustafsson et al., 2002; Weitzman and Stevens, 1997), but relationships to other families in this order remain uncertain (Soltis et al., 2000). Stevens (1980; in press, and unpubl.) based on morphological and anatomical research, divided Clusiaceae into three subfamilies: Clusioideae, Hypericoideae, and Kielmeyeroideae. Clusioideae are usually glabrous, dioecious plants with latex/resin canals, fasciculate or nonfasciculate stamens, and short or obsolete styles. Members of Hypericoideae are herbs to shrubs of temperate or high-elevation tropical areas with resin cavities, fasciculate stamens, and free styles. Most Kielmeyeroideae have an indumentum of uni- or multicellular hairs, latex/resin cavities and/or canals, nonfasciculate stamens, and fused styles.

Gustafsson et al. (2002) reconstructed the phylogeny of Clusiaceae using the chloroplast gene *rbcL*. The analysis provided support for the monophyly of three clades: Kielmeyeroideae, Clusioideae, and Hypericoideae + Podostemaceae, except that *Clusiella*, traditionally placed in Clusioideae (but questioned by Hammel, 1999), appeared in Kielmeyeroideae. Resolution of the relationships within Kielmeyeroideae was poor, and several genera of Kielmeyeroideae (*Haploclathra*, *Neotatea*, *Poeciloneuron*, and *Endodesmia*) were not included.

Stevens (in press) recognized two tribes within Kielmeyeroideae: Calophylleae, a large, pantropical tribe (11 genera, ca. 450 spp); and Endodesmieae, a small, tropical African group (2 monotypic genera). The placement of Endodesmieae within Kielmeyeroideae was considered tentative because of its similarities to both Clusioideae and Kielmeyeroideae.

Many members of Kielmeyeroideae are economically important. For example, *Mammea americana* is a popular fruit (called the mammey apple) in the Caribbean. Several species of *Calophyllum*, *Kayea*, and *Haploclathra* are used for timber in construction (Stevens, 1980, unpubl.; Vasquez, 1993). *Mesua ferrea* and some species of *Calophyllum* are planted as ornamentals. The fruits of *Calophyllum* yield an oil that is sometimes used medicinally or in lamps (Stevens, 1980). *Calophyllum lanigerum* is being investigated as a possible use for the treatment of AIDS because it contains a nonnucleoside inhibitor of HIV 1 reverse transcriptase (Greer, 2001).

Kielmeyeroideae offer the opportunity to examine the evolution of several noteworthy morphological characters, such as latex system, anther glands, androecial form, and fruit type. The latex system may consist of cavities (more or less spherical latex- or resin-containing structures) and/or canals (elongated latex- or resin-containing structures). In *Calophyllum* and *Neotatea*, canals largely replace intersecondary veins. The mesophyll in the leaves of *Clusiella* and *Endodesmia* contains both cavities and canals; other genera have either cavities or canals. In this study, I assessed the phylogenetic significance of these structures, and attempted to determine the ancestral condition for the subfamily.

Many members of Kielmeyeroideae have apical "glands" on their anthers, which contain a resinous or oily substance that may serve to attract pollinators (Stevens, unpubl.). The glands range in shape from spherical to elongate or bowl-shaped (crateriform). In addition to having anther glands, the flowers of *Clusiella* also have resin-secreting staminodes, similar to the large genus *Clusia* (Clusioideae: Clusieae), to which *Clusiella* was thought to be related based on its epiphytic habit, sessile, cupuliform stigmas, and non-ascendent ovules (Planchon and Triana, 1860; Engler, 1925). The phylogenetic significance of these characters is assessed in connection with a reconsideration of the phylogenetic placement of *Clusiella*. The taxonomic value of anther glands is considered within a phylogenetic context.

Most members of Kielmeyeroideae produce capsular fruits (septicidal or septifragal); however, several genera produce indehiscent fruits. *Mammea*, *Calophyllum*, and *Endodesmia* produce one-seeded berries; *Clusiella* has a several-seeded berry. A phylogeny of Kielmeyeroideae has allowed me to determine that from a capsular ancestor, fleshy, indehiscent fruits likely have evolved several times within the subfamily. The fruit form within Clusiaceae is quite homoplasious, as in many other families (Kron et al., 2002; Wilson et al., 2001; Judd et al., 2002).

Kielmeyeroideae are distributed throughout the tropics. Within Calophylleae, *Neotatea*, *Marila*, *Mahurea*, *Clusiella*, *Kielmeyera*, *Caraipa*, and *Haploclathra* are found in the New World tropics; *Poeciloneuron*, *Mesua*, and *Kayea* are found only in the Old World tropics; and *Mammea* and *Calophyllum* each have a few species in the New World and a majority of their species in Madagascar to the Pacific (Stevens, 1980, unpubl.). The two genera of Endodesmieae (*Endodesmia* and *Lebrunia*) are found in tropical

Africa. Estimation of a phylogeny for the group allowed analysis of the evolution of taxa in relation to the geographic region in which they occur. Kielmeyeroideae have apparently diversified within both the Paleo- and Neotropics.

The primary goal of this research project was to resolve the generic relationships within Kielmeyeroideae, using molecular and morphological data. Although a previous phylogenetic analysis of Clusiaceae using *rbcL* (Gustafsson et al., 2002) included many members of Kielmeyeroideae and showed support for the monophyly of this subfamily (including *Clusiella*), generic relationships within Kielmeyeroideae were not resolved, and several genera were not included in that analysis (i.e., *Haploclathra*, *Neotatea*, *Poeciloneuron*, and *Endodesmia*). In the present study, *rbcL* and *matK* sequences were used to determine the phylogenetic position of *Endodesmia* (which had never before been included in a molecular cladistic analysis); and *rbcL*, *matK*, ITS sequences and morphology were used to infer generic relationships and circumscription within Kielmeyeroideae. The value of morphology in cladistic analyses was examined by comparing resolution and levels of support in trees resulting from analyses with and without morphology. In addition, all 74 morphological characters were mapped onto the total evidence topology to gain a better understanding of patterns of evolution.

CHAPTER 2
MATERIALS AND METHODS

Taxon Sampling for Molecular Data Sets

Taxa selected and gene regions sequenced are summarized in Appendix A, along with voucher information and GenBank accession numbers. Generally, several species of each genus of Kielmeyeroideae were included in molecular analyses. *Neotatea*, found in the tepuis of northern South America; and *Lebrunia*, an African member of Endodesmieae, are the only genera not represented in the molecular data sets. Several taxa of Kielmeyeroideae not included in the family-level *rbcL* study of Gustafsson et al. (2002) were sequenced and added to the previously published sequences.

DNA Amplification, Sequencing, and Alignment

Total DNA was isolated from 34 species of Kielmeyeroideae according to the methods of Soltis et al. (1991) modified from Doyle and Doyle (1987), scaled down to 1.0-mL extraction volumes. Fifteen to 20 mg of silica-dried leaves or leaf tissue removed from herbarium specimens were ground using ceramic mortars and pestles with liquid nitrogen and a pinch of sand. DNA was incubated overnight at 60°C. After the chloroform extraction, the DNA was precipitated overnight at –20°C with 0.08 volume of 7.5 M ammonium acetate and 0.54 volume of isopropanol. DNA was centrifuged, washed in 70% ethanol, washed again in 95% ethanol, dried, and resuspended in 50 µL of Tris-EDTA buffer and stored at –20°C. Three different gene regions were amplified to resolve phylogenetic relationships within Kielmeyeroideae: *rbcL*, *matK*, and ITS. Amplification of DNA was performed using 25-µL reactions containing 5.0 mmol/L KCl,

2.0 mmol/L Tris pH 8.3, 1.5 mmol/L $MgCl_2$, 0.001% Tween, 0.05% DMSO, 0.2 mmol/L dNTPs, 1.25 units of *Taq* polymerase, and 0.5 μmol/L primer.

The primers used for amplification of *rbcL* were Z-1F, Z-895R, Z-674F, and 3' *rbcL*, designed by G. Zurawski (DNAX Research Institute, Palo Alto, CA, USA). The PCR thermal cycling profile consisted of 35 cycles with an annealing temperature of 55°C. A ca. 500-basepair (bp) region of *matK* was amplified with the primers *trnK*-710F and *matK*-1168R (Johnson and Soltis, 1995). The PCR thermal cycling profile consisted of ten cycles with an annealing temperature of 49°C and 30 cycles with an annealing temperature of 39°C. ITS was amplified using the forward primer N-nc18S10 and reverse primer C26A (Wen and Zimmer 1996). A touchdown thermal cycling program was used; the initial annealing temperature was 53°C, decreasing 1°C per cycle for six cycles, followed by 36 cycles at 48°C.

All PCR products were sequenced in both directions with the PCR primers used as sequencing primers using Beckman-Coulter Dye Terminator Cycle Sequencing Quick-Start kits and a Beckman-Coulter CEQ 2000 or CEQ 8000 automated sequencers following the manufacturer's protocols (Beckman Coulter, Inc., Fullerton, CA, USA), except that half reactions were used.

Sequences in each data set were aligned by eye using SeAl 2.0 (Rambaut, 1996). The alignments were straightforward except for the ITS sequences, where alignment among genera was sometimes difficult. Several alignments were tried, and all alignments resulted in the same topology.

Morphological Characters

Taxa used in the morphological data set were chosen to match those taxa available for the molecular analyses. The morphological data set included all genera of

Kielmeyeroideae except *Lebrunia*, for which herbarium specimens were not available. *Clusia lanceolata* and *Hypericum tetrapetalum*, representatives of Clusioideae and Hypericoideae, respectively, were selected as outgroups based on previous morphological (Stevens, unpubl.) and molecular (Gustafsson et al., 2002) cladistic analyses. When herbarium specimens in reproductive condition were not available for a given species used in the molecular data set, a congeneric species was used as a placeholder. (In combined molecular and morphological analyses, the morphological data of *Mammea subsessifolia* were combined with the DNA data of *Mammea sessiliflora*, the morphological data of *Kielmeyera speciosa* were combined with the DNA data of *Kielmeyera rosea*, the morphological data of *Kielmeyera coriacea* were combined with the DNA data of *Kielmeyera petiolaris*, the morphological data of *Caraipa* sp. were combined with the DNA data of *Caraipa utilis*, the morphological data of *Haploclathra leleanii* were combined with the DNA data of *Haploclathra cordata*, the morphological data of *Kayea borneensis* were combined with the DNA data of *Kayea stylosa*, and the morphological data of *Calophyllum fibrosum* were combined with the DNA data of *Calophyllum soulattri*.) In the characters examined for this analysis, infrageneric variation is minimal, and the analysis focused on intergeneric (not intrageneric) relationships. Therefore, a congeneric placeholder seems justified following the approach of Kron and Judd (1997). Appendix B lists herbarium specimens examined.

Morphological and anatomical characters were examined using herbarium material supplemented by alcohol-preserved flowers collected from cultivated plants at Fairchild Tropical Botanic Garden, in Miami, FL. Dried material was rehydrated overnight in a solution of tap water and a drop of liquid hand soap (Howard, 1974; Judd et al., 2002).

Sectioning for anatomical observations was done by hand with a razor blade, and the sections were stained with 2-3 drops of phloroglucinol and 1 drop of HCl. Temporary slides were created by suspending the sections in glycerol and applying clear nail polish to seal the cover slip.

A data matrix of 74 characters was ultimately constructed (Tables 2-1 and 2-2). Most of the characters examined were easily divisible into discrete states, therefore avoiding arbitrary decisions regarding state delimitation (Stevens, 1991). Several characters, such as bud length to width ratio, bracteole position, and petiole base excavation, were initially observed but excluded from the final matrix because they could not be delimited into clear states. Characters 54-58 in the matrix were taken from Stevens' (unpubl.) morphological analysis of the family. These characters are testa vasculature pattern, exotegmen presence or absence, endosperm presence or absence, embryo length, and cotyledon shape.

The interpretation of stipuliform structures (character 8) was sometimes difficult. When present, most stipuliform structures are attached on the stem adjacent to the region where the basal part of the petiole attaches to the stem (Figure 2-1a); those of *Mahurea exstipulata* are attached towards the upper part of where the petiole attaches to the stem, adjacent to the axillary bud (Figure 2-1b). It is possible that the stipuliform structures of *Mahurea exstipulata* are actually modified colleters (Stevens, unpubl.), but in this analysis they were treated the same as other paired stipuliform structures. The nodes of *Clusiella* have a distinctive interpetiolar stipuliform structure (Figure 2-1c), which was coded as a different state from the petiole-adjacent structures of other taxa.

In most taxa, the distinction between cavities (more or less spherical latex- or resin-containing structures surrounded by veins; character 12) and canals (elongated latex- or resin-containing structures, independent of the course of veins; character 13) is clear. However, the mesophyll of both *Endodesmia* and *Clusiella* contains cavities with a continuous range of sizes from spherical to oblong or elongate cavities that may span almost the entire distance from midvein to margin (Figure 2-2). These taxa were scored as having both cavities and canals.

Inflorescence types (character 17) are defined following Judd et al. (2002). Some inflorescences did not correspond clearly to a specific category. For example, several different types of cymose inflorescences were observed. The *Neotatea*-type cyme can be described as being a congested raceme-like structure with spiraled units associated with large bracts (Figure 2-3a). The *Clusiella*-type cyme has primary and secondary axes that produce several internodes before producing the terminal flower (Figure 2-3b). The other types of inflorescences detected in this study are diagramed in Figure 2-3c-i.

Calyx and corolla aestivation (characters 22 and 26, respectively) were observed at the point of insertion of the sepals or petals, respectively, and were defined according to Endress (1994). A flower with quincuncial aestivation of the calyx or corolla is defined as having two sepals or two petals entirely outside the others; imbricate aestivation refers to having one sepal or one petal entirely outside the others. A contorted corolla is one in which each petal has one side covering the adjacent petal and the other side under the other adjacent petal; no petals are entirely outside the others. When only two sepals are present, aestivation is opposite; aestivation is decussate when there are more than two

sepals present and they are opposite and decussate. Opposite and decussate aestivation patterns were treated as the same state.

A fasciculate androecium (character 30) is a striking feature of many members of Clusioideae and Hypericoideae, but has also been reported in a few members of Kielmeyeroideae. *Endodesmia* has been described as having a fasciculate androecium (Engler, 1925; Stevens, unpubl.), but is scored here as lacking fascicles. Observations of several flowers from different specimens did not indicate that the monadelphous tube of stamens (Figure 2-4a,b) represents a fasciculate androecium. Several species of *Calophyllum* have been described by some authors (Smith, 1920; Robson, 1976) as having fascicles. Stevens (1980) stated that the appearance of fascicles is probably caused by separation of the androecium along lines of weakness due to the removal of the petals during dissection. Following most previous interpretations, *C. inophyllum* and *C. brasiliense* were coded as having fascicles. Developmental studies could clarify the nature of the apparent fasciculation in *Calophyllum*.

Stamen dimorphism (character 31) was observed on perfect and staminate flowers. *Endodesmia calophylloides* has some anthers that produce pollen and others in which pollen is evidently not produced (Figure 2-4c). Both types of anthers produce resin: fertile anthers produce resin in an expanded, elongate connective, while nonfertile anthers secrete resin from most of the anther surface. *Clusiella* also has a dimorphic androecium, but this was coded as a separate state from that of *Endodesmia*. The staminate flowers of *Clusiella* contain both resin-secreting staminodes (at the base of the staminal tube) and fertile stamens, the latter also apparently secrete resin or oil through the connective (Figure 2-5).

In most taxa, the presence or absence of an anther gland (character 34) is an obvious feature (e.g., *Kayea borneensis, Marila laxiflora*, and *Mahurea exstipulata*; Figure 2-4d-f). Some taxa have an expanded connective and it is difficult to determine whether or not this structure is secreting resin. In these cases, the presence of a shiny or oily substance on the structure was considered sufficient evidence to characterize it as an anther gland (e.g., *Endodesmia calophylloides*, Figure 2-4c; *Clusiella axillaris*, Figure 2-5c).

Placentation type (character 38) was generally clear; however, the distinction between axile and intruded parietal placentation is subtle, and it seems probable that one is derived from the other (Figure 2-6a,b). The ovaries of some taxa (e.g., *Mahurea exstipulata*) have both axile and intruded parietal placentation, depending on where the cross section of the ovary is made. My character-state delimitations reflect this by treating these two conditions as the same state (state 0: placentation axile or intruded-parietal). A nonseptate ovary with parietal placentation (e.g., *Hypericum tetrapetalum*, Figure 2-6c), however, was easily distinguishable and considered a distinct state from the axile or deeply intruded parietal (and thus falsely septate) conditions characteristic of most of the other taxa. The ovary of *Clusiella* is distinctive: the placentation is laminar, with the ovules scattered on the partitions (Figure 2-6d).

The mature ovaries of a few taxa have a noteworthy feature of the septa (character 40). In both *Mahurea* and *Neotatea*, the intruded placentae are bordered by the carpel walls that have curled in (Figure 2-6e). In all other taxa with intruded placentae, the carpel walls do not curl in and border the placentae.

Ovule position at anthesis (character 42) may be more or less median, basal, or apical. In a median position, the ovules may be attached throughout the length of the axis or placental region, or may be clumped somewhere in the middle (Figure 2-7a). In a basal position (Figure 2-7b), the ovule(s) are attached only at the base of the ovary or at the base of the axis. An apical position (Figure 2-7c) indicates that the ovule(s) are attached only at the apex of the ovary.

Several taxa of Kielmeyeroideae have winged seeds (character 52). A careful examination of wing morphology and anatomy was needed to hypothesize whether or not wings of these taxa are homologous. A wing may be either two cell layers thick (i.e., *Kielmeyera*) or more than two cell layers thick (i.e., *Caraipa*, *Haploclathra*, *Mahurea*, and *Neotatea*), and each type may extend completely around the seed (i.e., *Kielmeyera*, *Caraipa*, and *Haploclathra*) or only partly around the seed (i.e., *Mahurea* and *Neotatea*). Some of the wings contain vascular tissue (i.e., *Mahurea* and *Neotatea*), while others do not (*Kielmeyera*, *Caraipa*, and *Haploclathra*; Stevens, unpubl.). In this analysis, four different states were delimited: (1) having no wing, (2) having a wing more than two cell layers thick that completely surrounds the seed (Figure 2-8a), (3) having a wing two cell layers thick that completely surrounds the seed (Figure 2-8b), and (4) having a wing more than two cell layers thick that does not completely surround the seed (Figure 2-8c).

Sixteen characters included in the morphological data set were taken from leaf and petiole anatomy (characters 59-74). Most of these characters are diagramed in Figures 2-9 and 2-10.

Table 2-1. Morphological characters used in a cladistic analysis of Kielmeyeroideae (Clusiaceae)

1. Terminal bud present (0); terminal bud not present (1)
2. Terminal bud without scales (0); terminal bud with scales (1)
3. Axillary bud visible but flush with stem (0); axillary bud visible, small to prominent, but not flush with stem (1); axillary bud immersed in stem (2)
4. Indumentum of unbranched unicellular hairs absent (0); indumentum of unbranched unicellular hairs present (1)
5. Indumentum of multicellular hairs absent (0); indumentum of multicellular hairs present (1)
6. Leaf arrangement opposite (0); leaf arrangement alternate (1)
7. Colleters present (0); colleters absent (1)
8. Stipuliform structures absent (0); stipuliform structures present and paired, adjacent to point of attachment of petiole (1); stipuliform structures present and interpetiolar (2)
9. Petiole or leaf bases enclosing terminal bud (0); petiole or leaf bases not enclosing terminal bud (1)
10. Secondary venation eucamptodromous (0); secondary venation brochidodromous (1)
11. Tertiary venation not evident (0); tertiary venation reticulate (1); tertiary venation percurrent (2)
12. Free latex/resin glands not in mesophyll (0); free latex/resin glands present in mesophyll (1)
13. Canals in mesophyll present (0); canals in mesophyll absent (1)
14. Intersecondary veins not replaced by canals (0); intersecondary veins largely replaced by canals (1)
15. Inflorescence/flower position terminalor terminal and axillary (0); inflorescence/flower position axillary (1)
16. Shoot growth monopodial (0); shoot growth sympodial (1)
17. Inflorescence a cyme (0); infloresence a panicle-like cyme (1); inflorescence a *Clusiella*-type cyme (2); inflorescence a *Neotatea*-type cyme (3); inflorescence a raceme (4); inflorescence fasciculate (5)
18. Terminal flower present (0); terminal flower absent (1)
19. Bracteoles present (0); bracteoles absent (1)
20. Bracteoles without abaxial gland (0); bracteoles with abaxial gland (1)
21. Plant dioecious or androdioecious (0); plant monoecious (1)

Table 2-1. Continued

22. Calyx aestivation decussate and/or opposite (0); calyx aestivation quincuncial (1); calyx aestivation imbricate (2)
23. Sepals eight (0); sepals five (1); sepals four (2); sepals two (3)
24. Sepals enclosing petals before anthesis (0); sepals not enclosing petals before anthesis (1)
25. Sepals free (0); sepals fully or partly connate, at least part way (1); outer whorl of sepals connate and inner whorl of sepals free (2)
26. Corolla aestivation imbricate (0); corolla aestivation contorted (1); corolla aestivation decussate (2)
27. Petals five (0); petals four (1); petals two (2)
28. Each petal bilaterally symmetric (0); each petal asymmetric (1)
29. Stamens many (0); stamens fifteen (1)
30. Stamens not in fascicles (0); stamens in fascicles (1)
31. Stamens monomorphic (0); stamens with *Clusiella*-type dimorphism (1); stamens with *Endodesmia*-type dimorphism (2)
32. Filaments free (0); filaments connate only at base (1); filaments monadelphous, *Clusiella*-type (2); filaments monadelphous, *Endodesmia*-type (3)
33. Anthers less than or equal to three mm long (0); anthers four to five mm long (1); anthers greater than or equal to six mm long (2)
34. Anther glands absent (0); anther glands present (1)
35. Anther glands spherical to elongate (0); anther glands bowl-shaped (1)
36. Anther dehiscence by slits (0); anther dehiscence by terminal pores (1)
37. Carpels four to eight (0); carpels one (1); carpels two (2); carpels three (3); carpels four (4); carpels ten to thirty (5)
38. Placentation axile or intruded-parietal (0); placentation non-intruded-parietal (1); placentation laminar, with ovules scattered on the partitions (2); placentation basal (3); placentation apical (4)
39. U-shaped structure absent in ovary (0); U-shaped structure emerging from base of ovary forming a pseudopartition (1)
40. Placentation not intruded-axile bordered by mesocarp tissue that has curled in (0); placentation intruded-axile bordered by mesocarp tissue that has curled in (1)
41. Ovules one to eight per carpel (0); ovules fifteen or more per carpel (1); ovule one per gynoecium (2)
42. Ovule position at anthesis +/- median (0); ovule position basal at anthesis (1); ovule position apical at anthsis (2)

Table 2-1. Continued

43. Style length to ovary length ratio less than or equal to four (0); style length to ovary length ratio greater than or equal to six (1)
44. Styles +/- free (0); styles +/- fused (1)
45. Stigma shape expanded (0); stigma shape narrow (1)
46. Calyx persistent (0); calyx not persistent (1)
47. Calyx not accrescent (0); calyx accrescent (1)
48. Pedicel not swollen in fruit (0); pedicel swollen in fruit (1)
49. Endocarp not bony (0); endocarp bony (1)
50. Fruit dehiscence septicidal (0); fruit dehiscence septifragal (1); fruit indehiscent (2)
51. Seeds not winged (0); seeds with wing several cells thick, going completely around seed, with no vascular tissue (1); seeds with wing two cells thick, going completely around seed, with no vascular tissue (2); seeds with elongate wing, several cells thick, not going completely around seed, with a peripheral vascular bundle (3)
52. Seeds not plumose (0); seeds plumose (1)
53. Seeds not arillate (0); seeds arillate (1)
54. Testa with unbranched or braided raphal bundle (0); testa with bundles throughout (1)
55. Exotegmen present (0); extotegmen absent (1)
56. Endosperm present in ripe seeds (0); endosperm absent in ripe seeds (1)
57. Embryo less than four mm long (0); embryo more than four mm long (1)
58. Cotyledon not cordate in shape (0); cotyledon cordate in shape (1)
59. Abaxial phloem not partitioned by fibers/lignified cells (0); abaxial phloem partitioned by fibers/lignified cells (1); abaxial phloem doubly partitioned by fibers/lignified cells (2)
60. Group of fibers adaxial to midrib bundle absent (0); patch of fibers adaxial to midrib bundle present (1)
61. Fibers adjacent to abaxial epidermis absent (0); fibers adjacent to abaxial epidermins present, scattered only (1); fibers adjacent to abaxial epidermis present, in continuous band (2)
62. Fiber strand not associated with adaxial side of abaxial-most xylem (0); fiber strand associated with adaxial side of abaxial-most xylem (1)

Table 2-1. Continued

63. Lateral bundles not transcurrent (0); lateral bundles transcurrent (1)
64. Canals not immediately adaxially and abaxially positioned in relation to secondary veins (0); canals immediately adaxially and abaxially positioned in relation to secondary veins (1)
65. Marginal lignification absent (0); marginal lignification present (1)
66. Marginal canal present (0); marginal canal absent (1)
67. One layer of xylem in midrib (0); two layers of xylem in midrib (1); three to six layers of xylem in midrib (2); midrib xylem in bundles (3)
68. Lignified spongy mesophyll absent (0); lignified spongy mesophyll present (1)
69. Leaf blade without abaxial palisade mesophyll (0); leaf blade with abaxial palisade mesophyll
70. Hypodermis absent (0); hypodermis present (1)
71. Epidermis not lignified (0); epidermis lignified (1)
72. Abaxial epidermis not papillose (0); abaxial epidermis papillose (1)
73. Druse crystals in petiole absent (0); druse crystals in petiole present (1)
74. Petiole bundle arched to circular, solid or fragmented (0); petiole bundle with three layers (1); petiole bundle an arch with dorsal groupings of xylem and phloem (2)

Characters 54-58 are taken from Stevens (unpubl.). State zero (0) is used to represent the state found in the taxa, *Clusia lanceolata* and *Hypericum tetrapetalum*. When outgroups differed, the "0" state was arbitrarily assigned to one of the outgroup genera.

Phylogenetic Analyses

Maximum parsimony, Bayesian inference, and maximum likelihood were used to estimate phylogeny. Maximum parsimony analyses were conducted using PAUP* 4.0 b10 (Swofford, 2000), with all characters weighted equally and gaps treated as missing data. Analyses were conducted using heuristic searches with 100 random addition replicates, TBR (tree-bisection-reconnection) branch swapping, and the MulTrees option in effect. Support for clades was estimated using 500 bootstrap replicates with 10 random addition replicates, and TBR branch swapping; 1000 trees were saved per replicate (with MulTrees option in effect).

MrBayes 3.0 (Huelsenbeck, 2000) was used for Bayesian inference of phylogeny. The GTR+I+Γ substitution model was employed in each data set (*rbcL*, *matK*, ITS, *rbcL* + *matK* + ITS unpruned, and *rbcL* + *matK* + ITS pruned) based on the results of a likelihood ratio test done for each data set using Modeltest 3.06 (Posada and Crandall, 1998). The GTR+I+Γ model consists of separate, time-reversible parameters for all of the possible base substitutions (GTR; Yang, 1994), and two parameters to account for substitution rate heterogeneity across sites, a fixed proportion of invariant sites (I; Hasegawa et al., 1985), with the sites free to vary fitted to a discrete approximation (four categories) of a gamma distribution (Γ; Yang, 1994). Each Bayesian analysis was run with four chains of two million generations. Results were printed to the screen every 100 generations and one tree was saved to the file every 10 generations. Log likelihood values were graphed in Microsoft Excel and burn-in was determined when likelihoods became stationary. Posterior probabilities of clades were calculated from the frequency at which each clade appeared among the trees visited by creating a majority-rule

consensus (excluding trees produced during burn-in phase) using PAUP* 4.0 b10 (Swofford, 2000)

Maximum likelihood analyses were completed using PAUP* 4.0 b10 (Swofford, 2000). For each molecular data set, base frequencies were calculated and a model of substitution was determined by Modeltest 3.06 (Posada and Crandall, 1998). For each data set (*rbcL*, *matK*, ITS, *rbcL* + *matK* + ITS unpruned, and *rbcL* + *matK* + ITS pruned), it was determined that a GTR+I+Γ model of substitution best fit the data. Analyses were conducted using heuristic searches with 100 random addition replicates, TBR (tree-bisection-reconnection) branch swapping, and the MulTrees option in effect. Support for clades was estimated using 100 bootstrap replicates, with starting trees obtained by neighbor joining. Bootstrap analyses were performed on all molecular separate and combined data sets except for *rbcL* of Clusiaceae and because of the extremely long computational time to complete this analysis.

Preliminary analyses of each separate data set (using maximum parsimony for molecular and morphological data sets, and Bayesian inference and maximum likelihood for the molecular data sets) showed they differed only in their amount of resolution. Several analyses were performed, each with its own objectives. Analysis 1 included *rbcL* sequences from throughout Clusiaceae taken from a previous study (Gustafsson et al., 2002), and also included several new sequences from Kielmeyeroideae (i.e., *Calophyllum goniocarpum*, *Calophyllum inophyllum*, *Calophyllum leleanii*, *Calophyllum soulattri*, *Calophyllum vexans, Caraipa densifolia*, *Caraipa savannarum*, *Caraipa utilis*, *Caraipa valioli*, *Clusiella isthmensis*, *Endodesmia calophylloides, Haploclathra cordata*, *Kielmeyera petiolaris*, *Kielmeyera rosea*, *Mammea sessiliflora*, *Mammea siamensis*,

Mammea usambarensis, *Marila laxiflora*, *Marila tomentosa*, *Mesua ferrea*, and *Poeciloneuron indicum*). *Bonnetia roraimae* (Bonnetiaceae), *Viola sororia* (Violaceae), and *Chrysobalanus icaco* (Chrysobalanaceae), representing three different families of Malpighiales, were used as outgroups in this analysis based on the multi-gene angiosperm topology by Soltis et al. (2000) and the *rbcL* phylogeny of Gustafsson et al. (2002). The *rbcL* family-level analysis was done to determine the phylogenetic placement of *Endodesmia*, a genus not included in the study of Gustafsson et al. (2002). Ascertaining the placement of *Endodesmia* (see Results) then allowed its use as a functional outgroup (Watrous and Wheeler, 1981) in subsequent analyses. The *rbcL* analysis was also done to test the possible sister-group relationship of *Mammea* and the rest of Calophylleae. This relationship appeared in the strict consensus of the analysis of Gustafsson et al. (2002), but did not have bootstrap support.

Analysis 2 combined *rbcL* and *matK* sequences, which increased the number of variable sites. This analysis included members of Kielmeyeroideae and was performed in an effort to resolve major clades within this subfamily. In Analysis 2, two members of Clusioideae, *Clusia lanceolata* and *Garcinia spicata*, served as outgroups. (DNA was isolated from five different species of *Hypericum* and one species of *Triadenum* in an attempt to include an outgroup from Hypericoideae, but none of the nine different primer combinations tried resulted in the successful amplification of the *matK* region.)

Analysis 3 used only ITS sequence data. Due to the difficulty in aligning some portions of ITS among phylogenetically distant genera, the ITS analysis was performed primarily to resolve relationships between phylogenetically close genera. *Endodesmia*, based on its position in analyses 1 and 2 (see Results), was used as a functional outgroup

in this analysis because of alignment problems with sequences from taxa outside of Kielmeyeroideae.

Analysis 4 combined all three gene regions to maximize character evidence. This combined molecular data matrix included taxa that lacked sequences from one or more of the three gene regions employed because of their inability to amplify. Of 37 taxa included in this multigene analysis, 16 were missing sequence data from one or more of the gene regions (ca. 22% missing data). *Clusia lanceolata* was used as the outgroup in this analysis.

To minimize the effect of missing data, Analysis 5 combined ITS, *matK*, and *rbcL* sequences, but included only those taxa for which all three sequences were available. This pruning resulted in a data set of 21 taxa. *Endodesmia* was used as a functional outgroup. (*Clusia lanceolata* was not used as an outgroup in this pruned analysis because it was missing sequence data for ITS as a result of the problematic alignment of sequences from outside Kielmeyeroideae.)

Analysis 6 included only morphological characters for genera of Kielmeyeroideae. Representatives of the other two subfamilies, *Clusia lanceolata* and *Hypericum tetrapetalum*, were used as outgroups.

Analysis 7 combined the 74 morphological characters with all three molecular data sets (Analysis 4) and included all taxa except *Neotatea*, for which molecular data were lacking. *Clusia lanceolata* was used as the outgroup in this analysis.

Analysis 8 combined the morphological characters with the molecular characters, but only included those taxa common to all four data sets. *Endodesmia* was used as a functional outgroup based on its position in analyses 1 and 2 (see results).

Additional analyses were performed that included *Neotatea*, for which only morphological characters were used. In Analysis 9, *Neotatea* was added to the unpruned total evidence data set (DNA + morphology), and Analysis 10 added *Neotatea* to the pruned total evidence data set.

Using MacClade 4.05 (Maddison and Maddison, 2001), morphological character-state transformations were mapped onto one of the most parsimonious trees from the unpruned total evidence analysis (Analysis 7), following the approach of Kron et al. (2002). In the case of equivocal tracing of characters, MacClade was set to show all most parsimonious states at each node. In addition, morphological characters were mapped onto a most parsimonious tree from the pruned total evidence analysis that included *Neotatea* (Analysis 10). In the unpruned total evidence analysis (Analysis 9), the position of *Neotatea* was unresolved.

Table 2-2. Morphological character-state coding used in phylogenetic analysis of Kielmeyeroideae (Clusiaceae)

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
Clusia lanceolata	0	0	0	0	0	0	0	0	0	A	0	0	0	0	0	0	0	0
Hypericum tetrapetalum	0	0	1	0	0	0	1	0	1	?	0	1	1	0	0	0	0	0
Endodesmia calophylloides	1	?	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
Mammea subsessifolia	0	1	1	0	0	0	0	0	1	A	1	1	1	0	1	0	5	1
Mammea siamensis	0	1	1	0	0	0	0	0	1	A	1	1	1	0	1	0	5	1
Mammea americana	0	1	1	0	0	0	0	0	1	A	1	1	1	0	1	0	5	1
Neotatea duidae	0	0	1	1	0	1	1	0	1	1	0	0	0	1	0	0	3	0
Neotatea longifolia	0	0	1	1	0	1	1	0	1	1	0	0	0	1	0	0	3	0
Neotatea neblinae	0	0	1	1	0	1	1	0	1	1	0	0	0	1	0	0	3	0
Marila racemosa	0	0	1	0	1	0	0	0	1	1	2	1	1	0	1	0	4	1
Marila laxiflora	0	0	1	1	0	0	1	0	1	1	2	1	1	0	1	0	4	1
Marila plumbaginea	0	0	1	1	0	0	0	0	1	1	2	1	1	0	1	0	4	1
Mahurea exstipulata	0	1	1	1	0	1	0	1	1	0	2	1	1	0	0	0	1	0
Kielmeyera speciosa	0	1	1	0	1	1	0	0	1	1	1	1	1	0	0	0	1	0
Kielmeyera coriacea	0	1	1	1	1	1	0	0	1	1	1	1	1	0	0	0	1	0
Caraipa densifolia	0	0	1	0	1	1	0	0	1	A	B	1	1	0	0	0	1	0
Caraipa sp	0	0	1	0	1	1	0	0	1	A	B	1	1	0	0	0	1	0
Caraipa savanarum	0	0	1	0	1	1	0	0	1	1	1	1	1	0	0	0	1	0
Haploclathra paniculata	0	0	1	0	1	0	0	0	1	A	B	1	1	0	0	0	1	0
Haploclathra leiantha	0	0	1	0	1	0	0	0	1	A	1	1	1	0	0	0	1	0
Clusiella isthmensis	0	1	1	0	0	0	0	2	1	1	1	1	0	0	0	1	2	0
Clusiella axillaris	0	1	1	0	0	0	0	2	1	0	1	1	0	0	0	1	2	0
Kayea kunstleri	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
Kayea elmeri	0	1	1	0	0	0	0	0	1	A	1	1	1	0	0	0	0	0
Kayea borneensis	0	1	1	0	0	0	0	0	1	A	1	1	1	0	0	0	1	0
Mesua ferrea	1	?	2	0	0	0	1	0	1	1	1	1	1	0	1	0	0	0
Poeciloneuron indicum	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	1	0
Calophyllum inophyllum	0	0	1	0	1	0	1	0	1	0	0	0	0	1	1	0	1	0
Calophyllum brasiliense	0	0	1	0	1	0	1	0	1	0	0	0	0	1	1	0	1	0
Calophyllum fibrosum	0	0	1	0	1	0	1	0	1	0	0	0	0	1	1	0	1	0

Table 2-2. Continued

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Clusia lanceolata	0	0	0	0	0	0	0	0	1	0	0	0	0	0	?	0	?	0
Hypericum tetrapetalum	1	0	1	0	2	0	0	1	1	1	0	?	0	0	0	0	?	0
Endodesmia calophylloides	0	0	1	1	1	1	0	1	0	1	0	0	2	3	0	1	0	0
Mammea subsessifolia	0	0	0	0	3	0	1	2	1	0	0	0	0	1	0	0	?	0
Mammea siamensis	0	0	0	0	3	0	1	2	1	0	0	0	0	1	0	1	0	0
Mammea americana	0	0	0	0	3	0	1	2	1	0	0	0	0	0	0	1	0	0
Neotatea duidae	1	0	1	2	1	0	0	1	0	0	0	0	0	0	?	1	0	0
Neotatea longifolia	1	0	1	2	1	0	0	1	0	0	0	0	0	0	2	1	0	0
Neotatea neblinae	1	0	1	2	1	0	0	1	0	0	0	0	0	0	2	1	0	0
Marila racemosa	0	0	1	1	1	0	1	A	0	0	0	0	0	1	0	1	1	0
Marila laxiflora	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	0
Marila plumbaginea	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	1	0	0
Mahurea exstipulata	0	0	1	1	1	A	0	1	0	0	0	0	0	0	0	1	1	0
Kielmeyera speciosa	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	?	0
Kielmeyera coriacea	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0	0
Caraipa densifolia	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0
Caraipa sp	0	0	1	1	1	1	0	1	0	0	0	0	0	1	0	1	1	0
Caraipa savanarum	0	0	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	0
Haploclathra paniculata	0	0	1	1	1	1	0	1	0	0	0	0	0	1	1	0	?	0
Haploclathra leiantha	0	0	1	1	1	1	0	1	0	0	0	0	0	1	0	0	?	0
Clusiella isthmensis	0	1	0	2	1	1	0	1	0	0	0	0	1	2	0	1	0	0
Clusiella axillaris	0	1	0	2	1	1	0	1	0	0	0	0	1	2	0	1	0	0
Kayea kunstleri	0	0	1	0	2	0	2	0	1	0	0	0	0	1	0	0	?	0
Kayea elmeri	0	0	1	0	2	0	2	0	1	0	0	0	0	1	0	1	0	0
Kayea borneensis	0	0	1	0	2	0	2	0	1	0	0	0	0	0	0	1	0	0
Mesua ferrea	1	0	1	0	2	A	0	2	1	0	0	0	0	1	0	0	?	0
Poeciloneuron indicum	0	0	1	1	1	1	0	1	0	0	1	0	0	0	0	0	?	1
Calophyllum inophyllum	1	0	1	0	3	0	0	1	1	0	0	1	0	1	0	0	?	0
Calophyllum brasiliense	1	0	1	0	3	1	?	2	2	0	0	1	0	1	0	0	?	0
Calophyllum fibrosum	1	0	1	0	2	A	0	2	1	0	0	0	0	1	0	0	?	0

Table 2-2. Continued

	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Clusia lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hypericum tetrapetalum	3	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Endodesmia calophylloides	1	4	0	0	0	2	0	?	1	0	0	1	0	2	0	0	0	1
Mammea subsessifolia	2	3	0	0	0	1	0	1	0	1	0	0	0	2	0	0	0	1
Mammea siamensis	2	3	0	0	0	1	0	1	0	1	0	0	0	2	0	0	0	1
Mammea americana	2	3	0	0	0	1	0	1	0	1	0	0	0	2	0	0	0	1
Neotatea duidae	3	0	0	1	1	0	?	1	0	0	0	0	0	0	3	0	0	0
Neotatea longifolia	3	0	0	1	1	0	0	1	0	0	0	0	0	0	3	0	0	0
Neotatea neblinae	3	0	0	1	1	0	0	1	0	0	0	0	0	0	3	0	0	0
Marila racemosa	C	0	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0
Marila laxiflora	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0
Marila plumbaginea	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0
Mahurea exstipulata	3	0	0	1	1	0	0	1	0	0	0	0	0	0	3	0	0	0
Kielmeyera speciosa	3	0	0	0	1	0	0	1	0	1	0	0	0	0	2	0	0	0
Kielmeyera coriacea	3	0	0	0	1	0	0	1	0	1	0	0	0	0	2	0	0	0
Caraipa densifolia	3	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1
Caraipa sp	3	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1
Caraipa savanarum	3	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1
Haploclathra paniculata	3	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1
Haploclathra leiantha	3	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1
Clusiella isthmensis	5	2	0	0	1	0	0	?	0	0	0	0	0	2	0	0	0	0
Clusiella axillaris	5	2	0	0	1	0	0	?	0	0	0	0	0	2	0	0	0	0
Kayea kunstleri	4	3	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	1
Kayea elmeri	4	3	0	0	0	1	0	1	1	0	0	0	0	2	0	0	0	1
Kayea borneensis	4	3	0	0	0	1	1	1	1	0	1	0	0	?	0	0	0	1
Mesua ferrea	2	3	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1
Poeciloneuron indicum	2	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Calophyllum inophyllum	3	3	0	0	2	1	0	1	0	1	0	0	1	2	0	0	0	1
Calophyllum brasiliense	2	3	0	0	2	1	0	1	0	1	0	0	1	2	0	0	0	1
Calophyllum fibrosum	2	3	0	0	2	1	0	1	0	1	0	0	1	2	0	0	0	1

Table 2-2. Continued

	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
Clusia lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0
Hypericum tetrapetalum	0	0	1	0	0	0	0	?	0	0	0	1	0	0	1	0	0	0
Endodesmia calophylloides	1	1	0	?	0	0	0	?	0	0	0	0	0	0	0	0	0	0
Mammea subsessifolia	1	1	0	0	0	0	1	?	1	0	1	0	0	0	0	0	0	0
Mammea siamensis	1	1	0	0	0	0	0	?	1	0	1	0	0	0	0	0	0	0
Mammea americana	1	1	0	0	0	0	0	?	1	0	1	0	0	0	0	0	0	0
Neotatea duidae	0	0	1	0	2	0	2	?	A	1	1	0	0	0	0	1	0	1
Neotatea longifolia	0	0	1	0	2	0	0	?	0	1	1	0	0	0	0	1	0	0
Neotatea neblinae	0	0	1	0	2	0	0	?	0	1	1	0	0	0	0	1	0	1
Marila racemosa	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0
Marila laxiflora	0	0	1	0	0	0	1	0	1	0	1	0	2	0	0	0	1	0
Marila plumbaginea	0	0	1	0	0	0	0	0	1	0	1	0	2	0	0	0	0	0
Mahurea exstipulata	0	0	1	0	0	0	0	0	1	0	1	0	2	0	0	0	0	0
Kielmeyera speciosa	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Kielmeyera coriacea	1	1	0	1	0	0	1	0	1	1	1	0	1	0	0	0	0	0
Caraipa densifolia	1	1	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0
Caraipa sp	1	1	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	1
Caraipa savanarum	1	1	0	1	0	1	0	0	1	0	1	0	2	0	0	0	0	0
Haploclathra paniculata	1	1	0	1	1	1	2	0	1	1	1	0	B	0	0	0	0	1
Haploclathra leiantha	1	1	0	1	1	1	2	0	0	0	1	0	1	0	0	0	0	1
Clusiella isthmensis	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Clusiella axillaris	0	0	1	0	0	0	0	1	0	0	0	1	2	0	0	0	0	0
Kayea kunstleri	1	1	0	?	1	1	2	1	1	0	1	0	1	0	0	0	0	0
Kayea elmeri	1	1	0	?	1	1	2	1	1	0	0	0	1	0	0	0	0	0
Kayea borneensis	1	1	0	?	0	1	2	1	1	0	1	0	2	0	0	0	0	0
Mesua ferrea	1	1	0	?	0	0	2	0	1	0	1	0	1	0	0	0	0	0
Poeciloneuron indicum	1	1	0	?	1	1	2	0	1	0	1	0	1	0	0	0	0	0
Calophyllum inophyllum	1	1	0	0	0	1	0	?	1	0	1	0	0	0	0	0	0	0
Calophyllum brasiliense	1	1	0	0	1	1	0	?	1	0	1	0	0	0	0	0	0	0
Calophyllum fibrosum	1	1	0	0	1	1	0	?	1	0	1	0	0	1	0	0	0	0

Table 2-2. Continued

	73	74
Clusia lanceolata	0	0
Hypericum tetrapetalum	?	?
Endodesmia calophylloides	0	0
Mammea subsessifolia	1	0
Mammea siamensis	1	0
Mammea americana	1	0
Neotatea duidae	0	?
Neotatea longifolia	0	0
Neotatea neblinae	0	0
Marila racemosa	1	1
Marila laxiflora	1	1
Marila plumbaginea	1	1
Mahurea exstipulata	0	1
Kielmeyera speciosa	0	0
Kielmeyera coriacea	0	0
Caraipa densifolia	1	0
Caraipa sp	0	0
Caraipa savanarum	0	0
Haploclathra paniculata	0	2
Haploclathra leiantha	0	2
Clusiella isthmensis	0	0
Clusiella axillaris	1	0
Kayea kunstleri	1	0
Kayea elmeri	1	0
Kayea borneensis	1	0
Mesua ferrea	1	0
Poeciloneuron indicum	0	0
Calophyllum inophyllum	1	0
Calophyllum brasiliense	1	0
Calophyllum fibrosum	1	0

State "0" is used to represent the condition found in outgroup taxa, *Clusia lanceolata* and *Hypericum tetrapetalum*. When outgroups differed, the "0" state was arbitrarily assigned to one of the outgroup genera. A = 0&1; B = 1&2; C = 3&4.

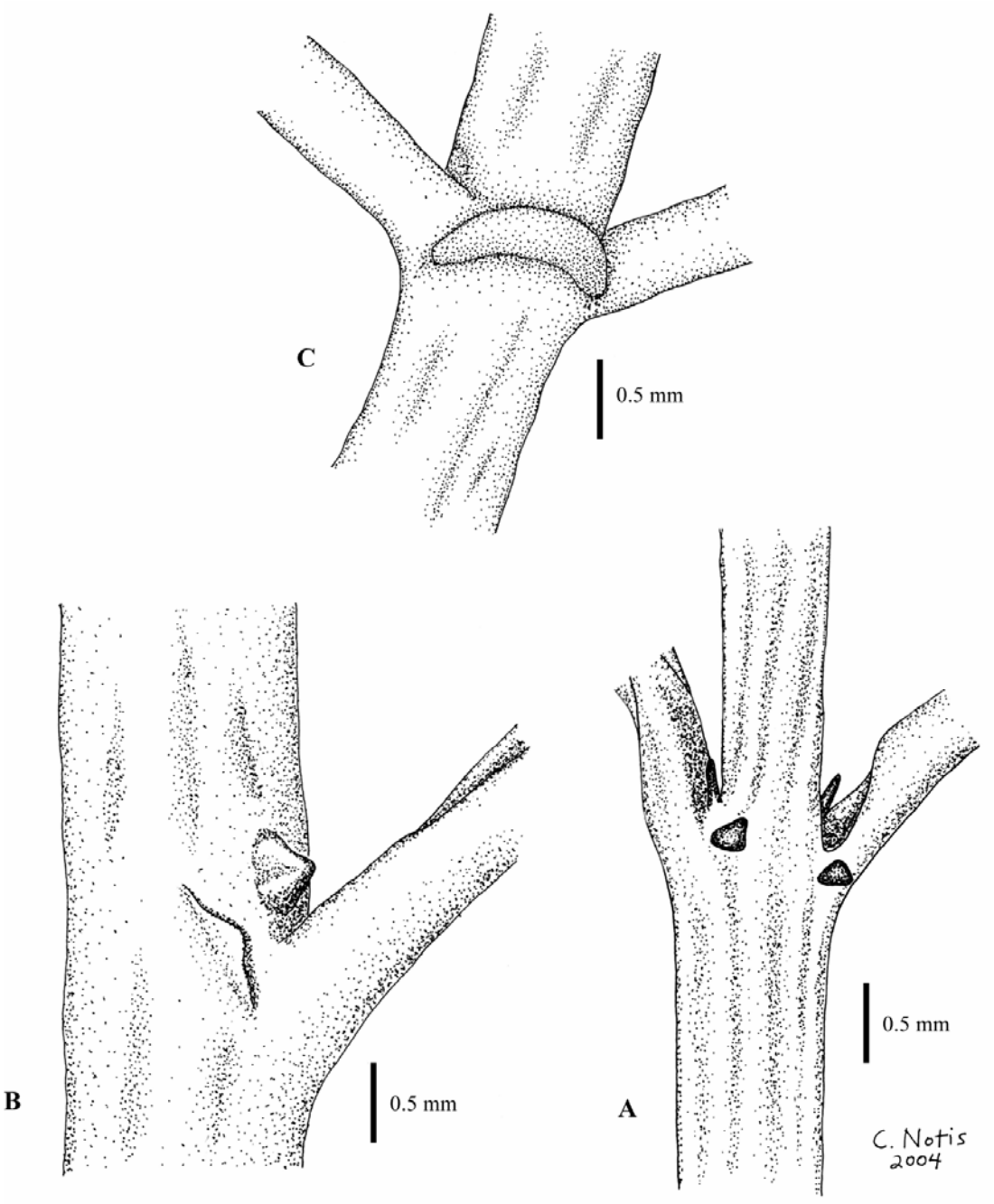


Figure 2-1. Stipuliform structures. A) *Endodesmia calophylloides*. Paired stipuliform structures attached on the stem adjacent to the region where the basal part of the petiole attaches to the stem. B) *Mahurea exstipulata*. Stipuliform structure attached on stem just above point of petiole attachment, adjacent to axillary bud. C) *Clusiella isthmensis*. Stipuliform structure interpetiolar.

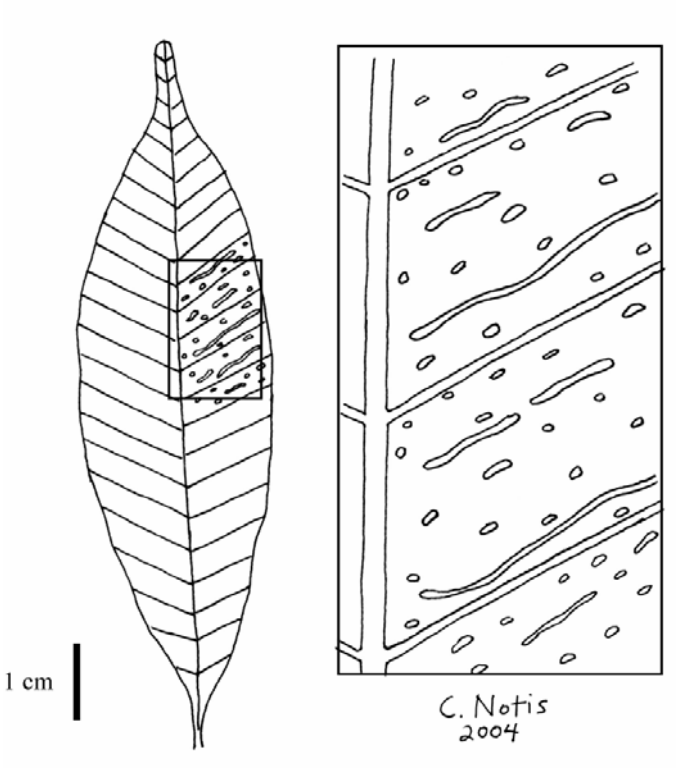


Figure 2-2. Overview of leaf of *Endodesmia calophylloides* showing mesophyll with latex/resin cavities and canals. A similar pattern is found in *Clusiella* spp.

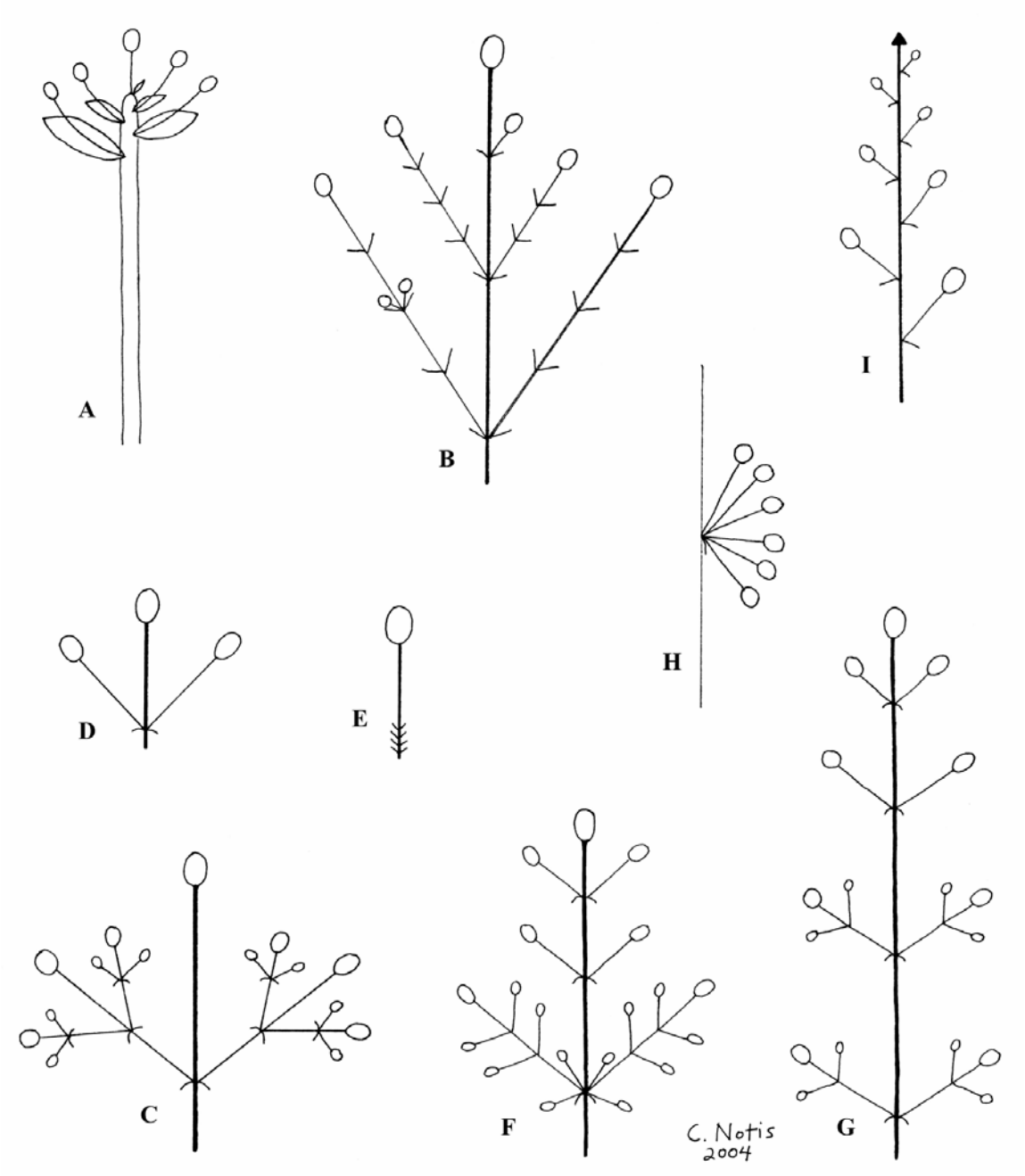


Figure 2-3. Inflorescence architectures of Kielmeyeroideae. A) *Neotatea*-type cyme. B) *Clusiella*-type cyme. C) Cyme, as in *Endodesmia calophylloides* and *Kayea elmeri*. D) Reducded cyme, as in *Kayea kunstleri*. E) Single-flowered cyme, as in *Mesua ferrea*. F-G) Panicle-like cymes, as in *Calophyllum* spp., *Poeciloneuron indicum*, *Kayea borneensis*, *Kielmeyera* spp., *Haploclathra* spp., and *Caraipa* spp. H) Fascicle, as in *Mammea* spp. I) Raceme, as in *Marila* spp.

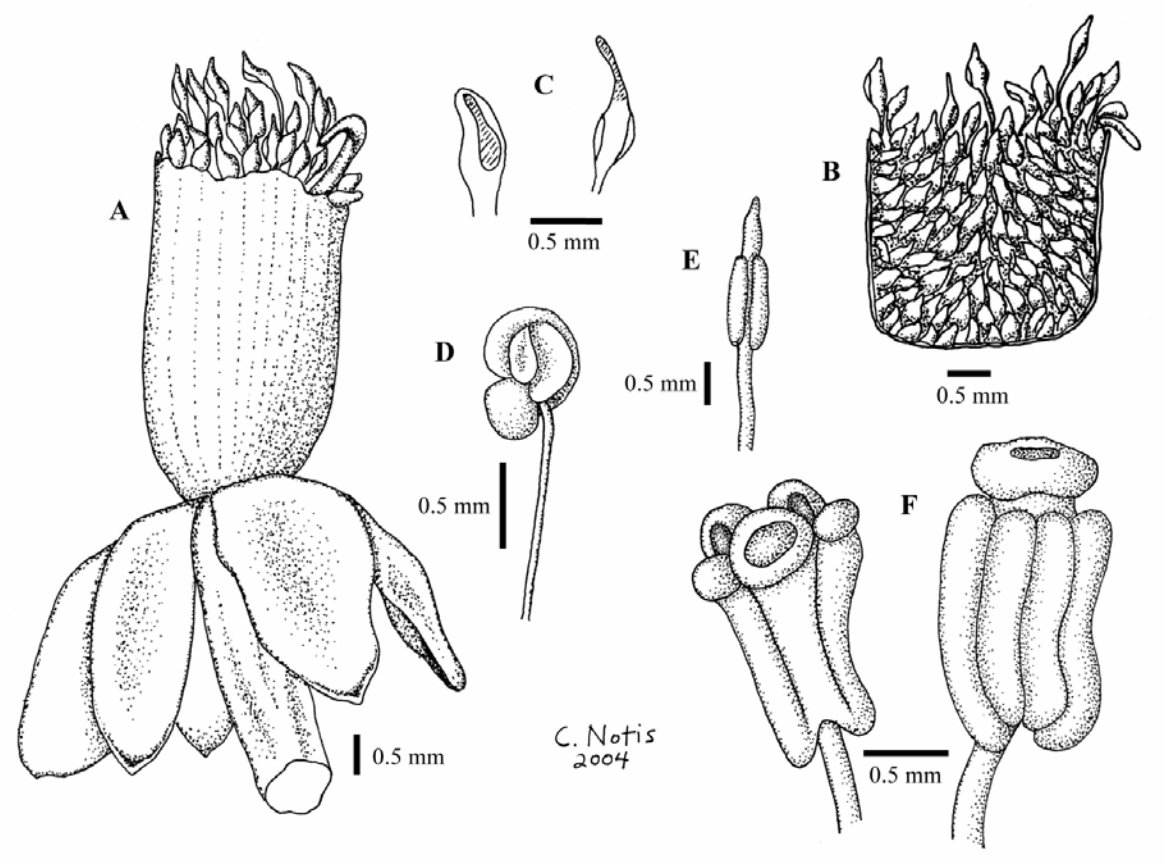


Figure 2-4. Androecial features of Kielmeyeroideae. A-C) *Endodesmia calophylloides*. A) Flower with petals removed. B) Longitudinal section of stamen tube, showing inside surface covered by anthers. C) Two types of anthers, with lines representing resin like substance; nonfertile anther on left and fertile anther on right. D) Stamen of *Kayea borneensis*, showing spherical anther gland at apex of C-shaped theca. E) Stamen of *Marila laxiflora*, showing elongate anther gland at apex. F) Stamen of *Mahurea exstipulata*, illustrating bowl-shaped (crateriform) anther gland.

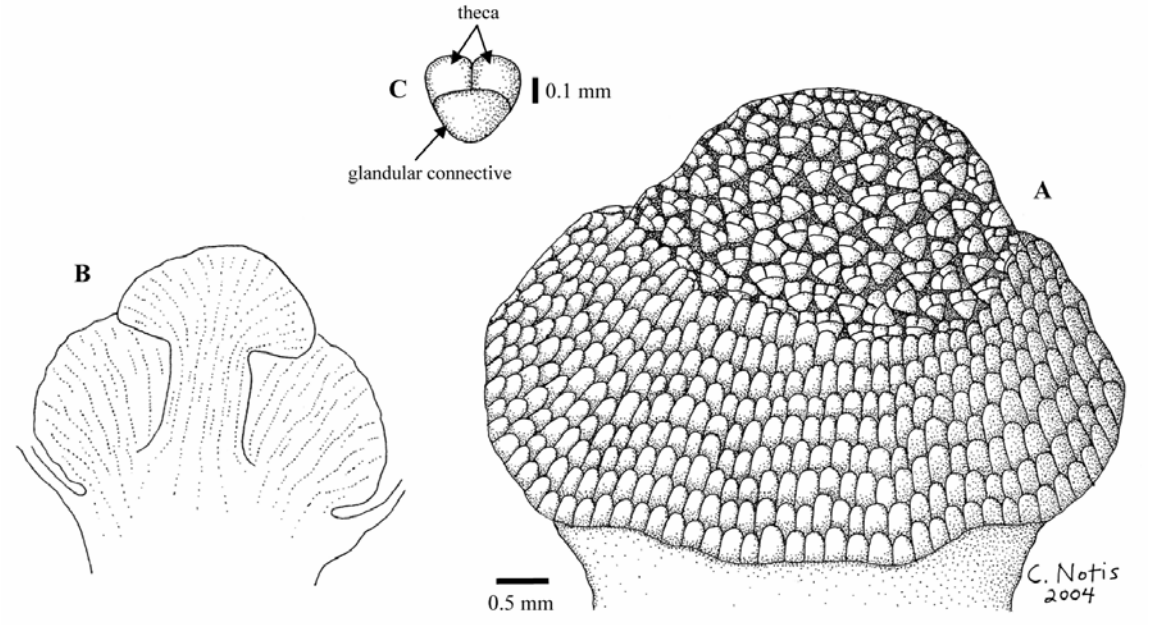


Figure 2-5. Androecium of *Clusiella axillaris*. A) Staminate flower bud with sepals and petals removed. Resin-secreting staminodes surround a monadelphous tube of functional stamens. B) Diagram of longitudinal section through A. C) Close up of functional anther showing glandular connective and theca.

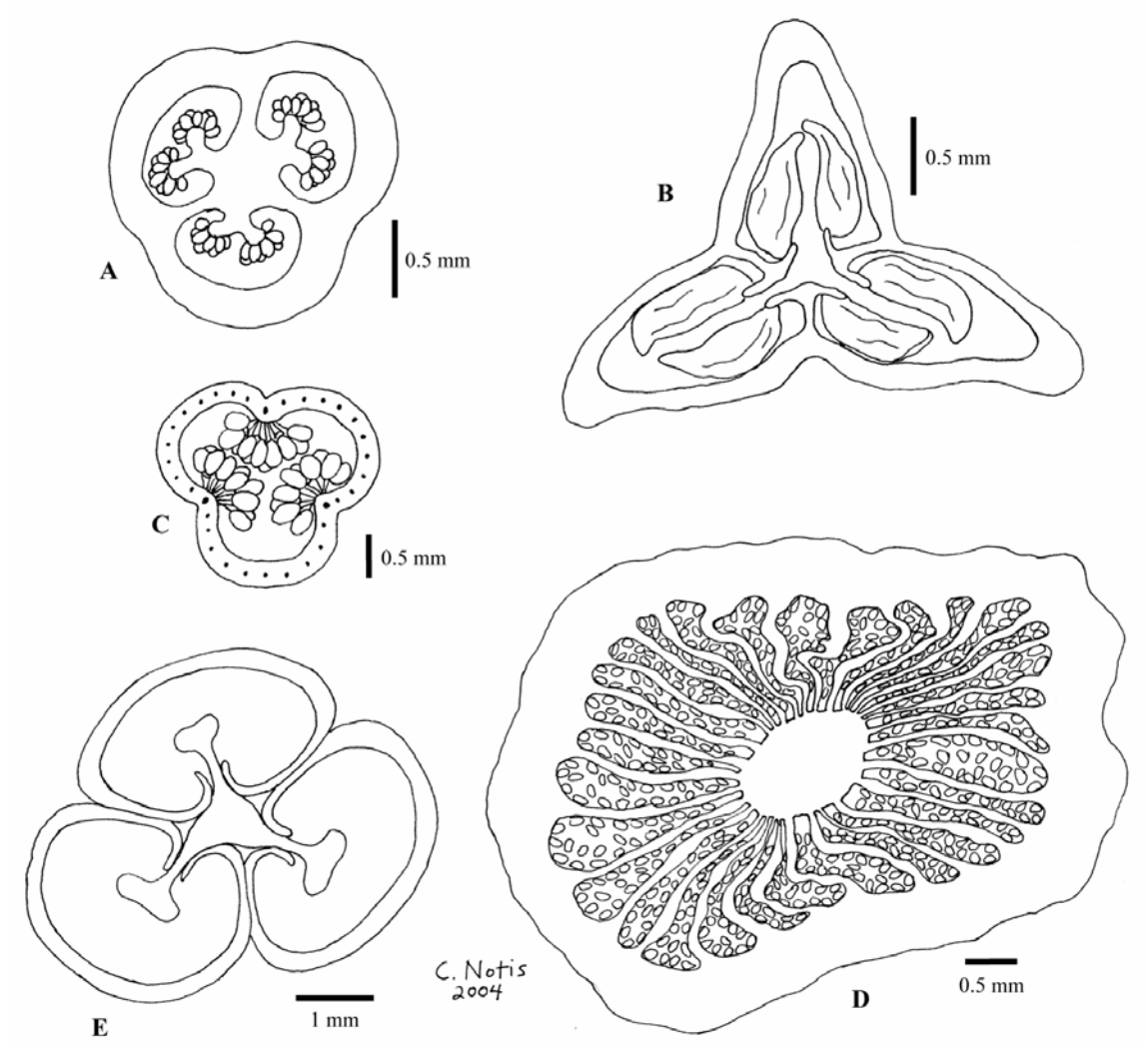


Figure 2-6. Gynoecial features of Kielmeyeroideae. A-D) Placentation types. A) Ovary cross section of *Mahurea exstipulata*, showing axile placentation. B) Ovary cross section of *Kielmeyera speciosa* (hairs on ovary not shown), showing intruded parietal placentation (and falsely septate ovary). C) Ovary cross section of *Hypericum tetrapetalum*, showing parietal placentation and nonseptate ovary. D) Ovary cross section of *Clusiella axillaris*, showing laminar placentation. E) Diagram of young fruit cross section of *Mahurea exstipulata* and *Neotatea* spp. The intruded placentae are bordered by the carpel walls (septae) that have curled in.

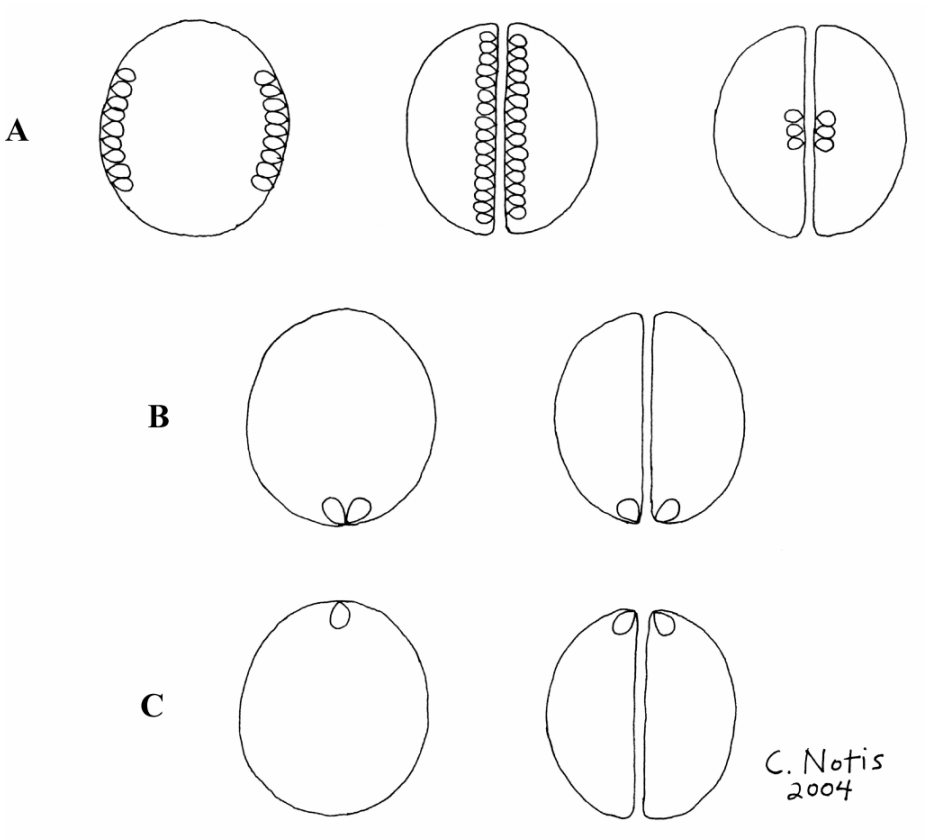


Figure 2-7. Diagrammatic longitudinal sections of ovaries showing ovule position at anthesis. A) Median ovule position. B) Basal ovule position. C) Apical ovule position.

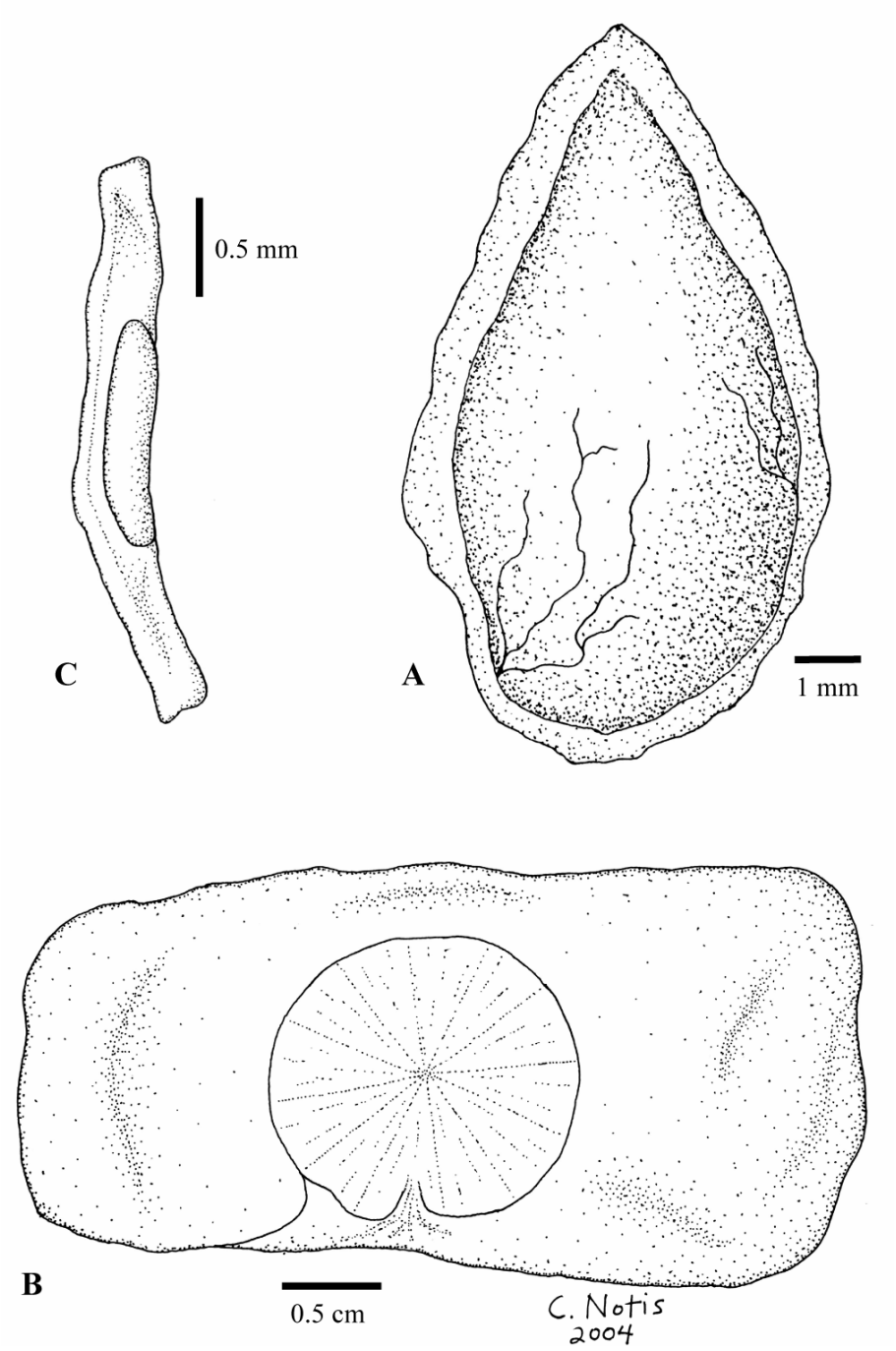


Figure 2-8. Three types of winged seeds found in Kielmeyeroideae. A) *Caraipa densifolia*. The wing is more than two cell layers thick, completely surrounds the seed, and contains no vascular tissue. B) *Kielmeyera coriacea*. The wing is two cell layers thick, completely surrounds the seed, and contains no vascular tissue. C) *Mahurea exstipulata*. The wing is more than two cell layers thick, does not completely surround the seed, and contains vascular tissue.

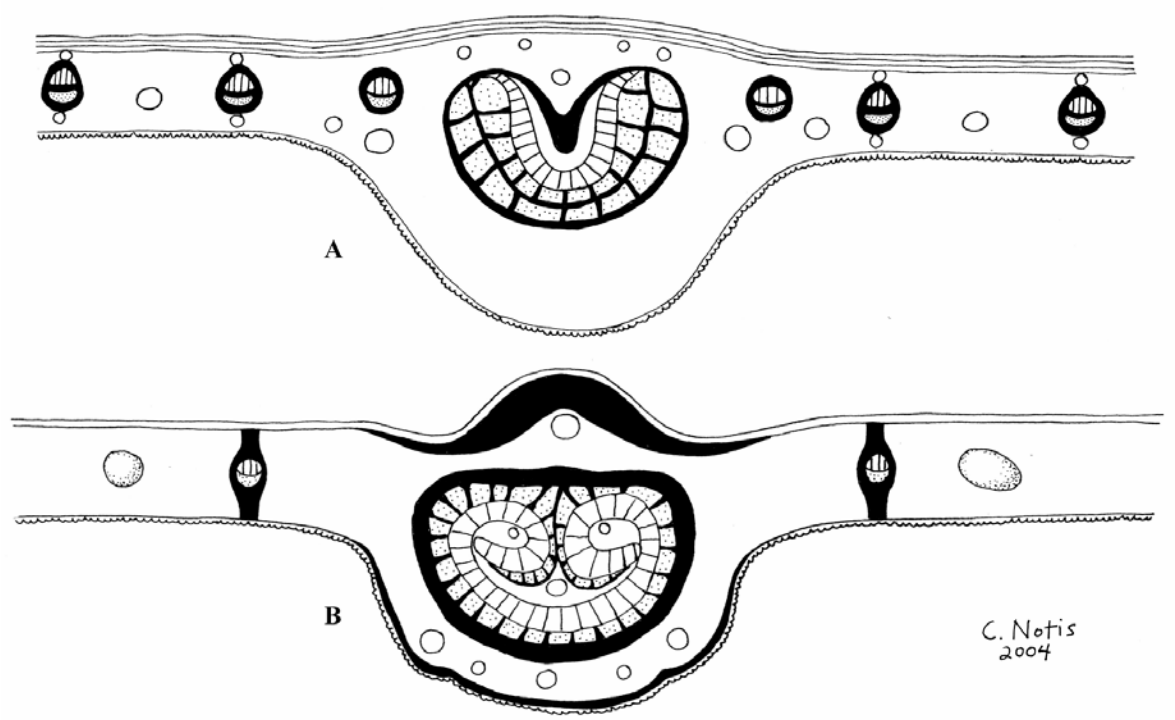


Figure 2-9. Leaf blade cross sections illustrating anatomical characters used in phylogenetic analyses. Xylem is represented by white with closely spaced, narrow, black lines. Phloem is represented by even stipling. Fibers are shown in black. Latex/resin canals are shown as circles, while latex/resin cavities are shown as circles with uneven stipling. A) *Neotatea neblinae*. Canals are present immediately adaxially and abaxially positioned in relation to secondary veins (chr. 64:1). Lateral vascular bundles are included (chr. 63:0). One layer of midrib xylem is present (chr. 67:0). Phloem is doubly partitioned by fibers (chr. 59:2). Hypodermis is present (chr. 70:1). Abaxial epidermis is papillose (chr. 72:1). B) *Haploclathra paniculata*. Cavities are present in mesophyll (chr. 12:1). Lateral vascular bundles are transcurrent (chr. 63:1). Group of fibers adaxial to midrib bundle is present (chr. 60:1). Two layers of midrib xylem are present (chr. 67:1). Phloem is partitioned by fibers (chr. 59:1). Fibers are adjacent to abaxial epidermis, in a continuous band (chr. 61:2). Abaxial epidermis is papillose (chr. 72:1).

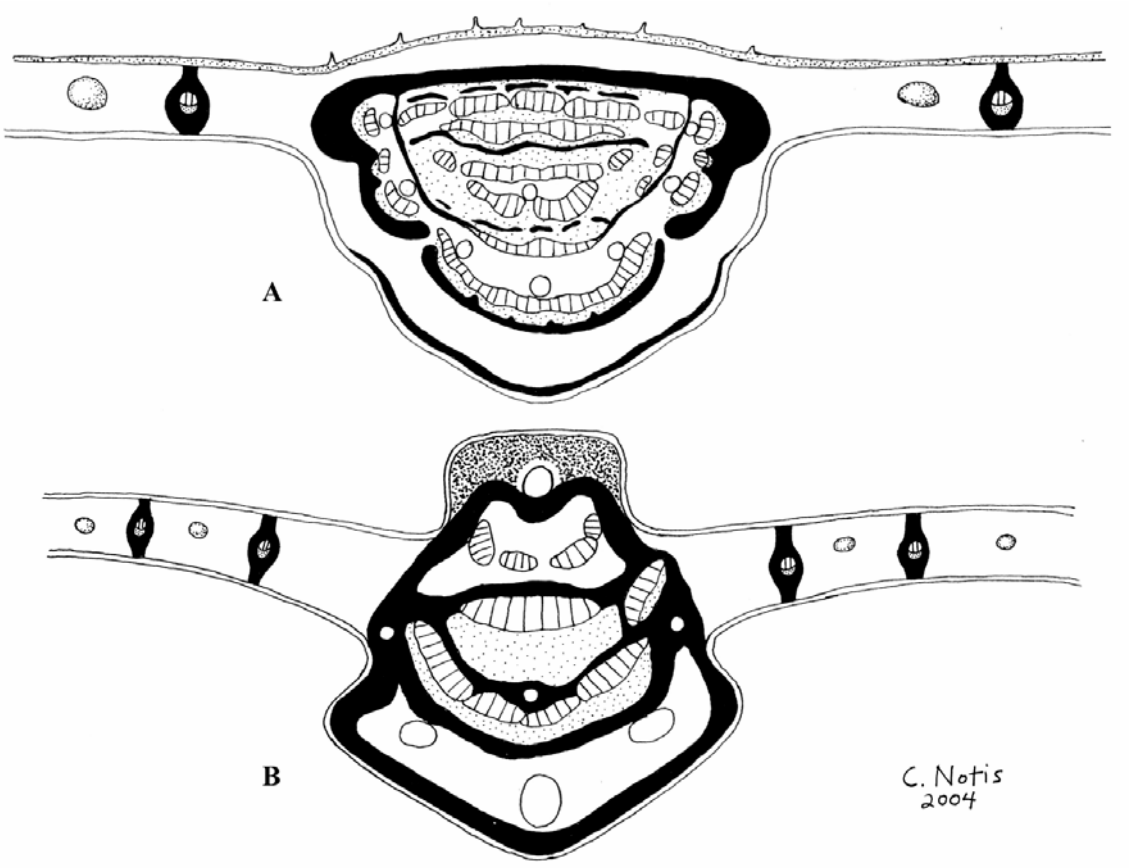


Figure 2-10. Leaf blade cross sections illustrating anatomical characters used in phylogenetic analyses. Xylem is represented by white with closely spaced, narrow, black lines. Phloem is represented by even stipling. Fibers are shown in black. Dark stipling represents less strongly lignified fibers. Latex/resin canals are shown as circles, while latex/resin cavities are shown as circles with uneven stipling. A) *Marila laxiflora*. Cavities are present in mesophyll (chr. 12:1). Lateral vascular bundles are transcurrent (chr. 63:1). Six layers of midrib xylem are present (chr. 67:2). Phloem is not partitioned by fibers (chr. 59:0). Fibers are adjacent to abaxial epidermis, in a continuous band (chr. 61:2). Adaxial epidermis is lignified (chr. 71:1). B) *Kayea borneensis*. Cavities are present in mesophyll (chr. 12:1). Lateral vascular bundles are transcurrent (chr. 63:1). Group of fibers adaxial to midrib bundle is present (chr. 60:1). Three layers of midrib xylem are present (chr. 67:2). Fiber strand is associated with adaxial side of abaxial-most xylem (chr.62:1). Fibers are adjacent to abaxial epidermis, in a continuous band (chr. 61:2).

CHAPTER 3
RESULTS

Analysis 1: *rbcL* of Clusiaceae

Of the 1408 base pairs sequenced in *rbcL*, 410 are variable, and 240 varied in a parsimony-informative manner. The analysis resulted in 1530 most parsimonious trees of length 845 (CI = 0.612, RI = 0.755, RC = 0.462). The *rbcL* data support the monophyly of three major clades: Clusioideae, Hypericoideae + Podostemaceae, and Kielmeyeroideae (Figure 3-1). Kielmeyeroideae, including *Endodesmia calophylloides*, are supported with a bootstrap value of 75%. *Endodesmia calophylloides*, of Endodesmieae, is placed as the sister group to the rest of the subfamily, the Calophylleae, which are strongly supported as a clade (bootstrap support = 95%). In the strict consensus, *Mammea* appears as the sister group to the rest of Calophylleae, but this relationship does not receive bootstrap support >50%. Appearing in the strict consensus tree, but without bootstrap support, is a clade containing *Calophyllum* + *Mesua*, which is a sister to the *Kayea* + *Poeciloneuron* clade.

The Bayesian analysis of *rbcL* (Figure 3-2) also shows support for three major clades within Clusiaceae: Hypericoideae + Podostemaceae, Clusioideae, and Kielmeyeroideae, with posterior probabilities of 100, 100, and 98, respectively. Within Kielmeyeroideae, *Endodesmia* appears sister to Calophylleae with a posterior probability of 100 and *Mammea* appears sister to the remaining Calophylleae with a posterior probability of 71.

The maximum likelihood analysis of *rbcL* also shows the same three major clades within Clusiaceae: Hypericoideae + Podostemaceae, Clusioideae, and Kielmeyeroideae. Two trees were obtained, with likelihood scores of 6792.82386 and 6792.82649. The topologies are identical to that of the Bayesian analysis except in their placement of *Haploclathra*. In one of the maximum likelihood trees, *Haploclathra* appears sister to a clade of *Mahurea* + *Clusiella* + *Marila*, and in the other tree (Figure 3-3), *Haploclathra* appears in a clade with *Kielmeyera* and *Caraipa*.

Analysis 2: *rbcL* + *matK* of Kielmeyeroideae

Combining *rbcL* and *matK* resulted in a total of 1885 characters; 120 of the 279 variable characters were parsimony-informative. The analysis recovered 510 most parsimonious trees of length 361 (CI = 0.831, RI = 0.762, RC = 0.633). *Endodesmia* appears as sister to Calophylleae with 100% bootstrap support, and *Mammea* is placed as sister to the rest of Calophylleae with 78% bootstrap support (Figure 3-4). As in the analysis of *rbcL* alone, a clade containing *Calophyllum* + *Mesua* is sister to *Kayea* + *Poeciloneuron* in all shortest trees, but these relationships again do not receive bootstrap support >50%. *Mesua* appears sister to *Calophyllum* with 86% bootstrap support. *Caraipa*, *Kielmeyera*, and *Haploclathra* form a monophyletic group with 71% bootstrap support.

Bayesian inference resulted in a topology identical to that of parsimony, and therefore the tree is not shown here. Kielmeyeroideae and Calophylleae appear monophyletic, each with posterior probabilities of 100. *Mammea* is placed sister to the rest of Calophylleae with a posterior probability of 92. *Caraipa*, *Kielmeyera*, and *Haploclathra* form a clade with a posterior probability of 100.

The maximum likelihood analysis resulted in a tree with a score of 4912.90425 and a similar topology to that of the parsimony and Bayesian analyses; however, in the maximum likelihood topology, *Marila* appears sister to *Kayea* + *Mahurea*, although without bootstrap support (Figure 3-5).

Analysis 3: ITS of Kielmeyeroideae

The ITS region provided 809 characters, 383 of which are variable; 232 were parsimony-informative. Ten most parsimonious trees were recovered of length 987 (CI = 0.584, RI = 0.658, RC = 0.384). Unlike analyses 1 and 2 (*rbcL* and *rbcL* + *matK*, respectively), ITS shows variation at lower taxonomic levels; however, the ITS analysis shows no bootstrap support for branches along the spine of the tree. All genera appear monophyletic, and most are well supported (Figure 3-6). *Caraipa* + *Haploclathra* + *Kielmeyera* form a clade with 86% bootstrap support. *Poeciloneuron* and *Kayea* are supported only weakly (bootstrap support = 60%).

The topology resulting from Bayesian analysis of ITS sequences (results not shown) is similar to that of parsimony, except that in the Bayesian topology, *Mammea* appears in a weakly supported clade (posterior probability of 64) with *Kayea* and *Poeciloneuron*. All genera are supported by posterior probabilities of 100. *Caraipa*, *Haploclathra*, and *Kielmeyera* are supported as a clade with a posterior probability of 100, within which *Caraipa* and *Haploclathra* are sisters with a posterior probability of 93.

Maximum likelihood analysis of the ITS data set resulted in a tree with a score of 5734.93593. The topology is similar to that of the Bayesian analysis: *Mammea* appears sister to a clade of *Kayea* + *Poeciloneuron*, although without bootstrap support (Figure 3-7).

Analyses 4 and 5: *rbcL* + *matK* + ITS of Kielmeyeroideae

Combining all three gene regions resulted in 2696 characters; 311 of 645 variable characters were parsimony-informative in the unpruned analysis, while 251 of 534 variable characters were parsimony-informative in the pruned analysis. The combined analysis that included all taxa (Analysis 4, *rbcL* + *matK* + ITS, unpruned; Figure 3-5) produced two most parsimonious trees of length 1327 (CI = 0.643, RI = 0.665, RC = 0.427). All genera are monophyletic and are supported by higher bootstrap values than in the previous analyses (Table 3-1). Calophylleae are monophyletic with 100% bootstrap support. *Caraipa*, *Haploclathra*, and *Kielmeyera* form a clade with 95% bootstrap support, *Mesua* is sister to *Calophyllum* with 84% support, and *Kayea* and *Poeciloneuron* are sisters with 72% bootstrap support. While the strict consensus shows resolution along the spine of the tree, these relationships do not receive bootstrap support >50%. The strict consensus places *Mammea* sister to the rest of Calophylleae, within which a clade containing *Mahurea*, *Clusiella*, and *Marila* is sister to a clade of *Caraipa*, *Haploclathra*, and *Kielmeyera* + *Kayea*, *Poeciloneuron*, *Mesua*, and *Calophyllum* (Figure 3-8).

The topology and levels of support in the pruned combined analysis (Analysis 5) are nearly identical to those of the unpruned combined analysis (Analysis 4), and results are therefore not shown here. The only topological difference between analyses 4 and 5 is that in the unpruned combined analysis (Analysis 4; Figure 3-8), the relationships among *Caraipa*, *Haploclathra*, and *Kielmeyera* are unresolved, whereas in the pruned combined analysis (Analysis 5), *Caraipa* and *Kielmeyera* are sister groups with 57% bootstrap support.

Bayesian analyses of the unpruned and pruned combined DNA data sets resulted in similar topologies and levels of support compared with the parsimony analyses of these data sets (Figures 3-9 and 3-10).

The maximum likelihood analysis of the unpruned and pruned combined DNA data sets resulted in nearly identical topologies to that of parsimony and therefore results are not shown here. The only difference in the results is in the relationships among *Kielmeyera*, *Caraipa*, and *Haploclathra*. In strict consensus of the parsimony analysis (Figure 3-8), the relationships among these three genera are unresolved; in the maximum likelihood tree, *Kielmeyera* is sister to a clade of *Caraipa* + *Haploclathra*. The likelihood scores of the unpruned and pruned combined DNA analyses were 10,988.21023 and 9321.34755, respectively. Bootstrap support for clades was equivalent to that of the bootstrap of the parsimony analysis.

Analysis 6: Morphology of Kielmeyeroideae

The morphological analysis was based on 74 variable characters, 64 of which were parsimony-informative. Twenty most parsimonious trees of length 224 were recovered (CI = 0.482, RI = 0.740, RC = 0.357). Kielmeyeroideae are supported as a clade with a bootstrap value of 52% (Figure 3-11). In the strict consensus, all genera except *Caraipa* appear monophyletic with bootstrap support >50%. *Caraipa* and *Haploclathra* form a clade with 58% bootstrap support. *Mesua*, *Calophyllum*, *Kayea*, and *Mammea* form a clade with 72% bootstrap support.

Analyses 7 and 8: DNA + Morphology of Kielmeyeroideae

Combining all three gene regions with the morphological data matrix results in a total of 2770 characters. In the unpruned analysis (Analysis 7), 436 of the 962 variable characters were parsimony-informative; in the pruned analysis (Analysis 8), 306 of the

712 variable characters were parsimony-informative. The unpruned combined DNA + morphology analysis (Analysis 7; Figure 3-12) resulted in four most parsimonious trees of length 1991 (CI = 0.671, RI = 0.648, RC = 0.435). The topology is almost identical to that of the combined DNA analyses without morphology (analyses 4 and 5); in some clades (i.e., *Kielmeyera*, and *Kielmeyera* + *Caraipa* + *Haploclathra*), bootstrap support is slightly lower in the unpruned DNA + morphology combined analysis (Analysis 7), whereas in other clades (i.e., *Marila*, *Marila* + *Mahurea* + *Clusiella* and *Kayea* + *Poeciloneuron*), the bootstrap support is slightly higher when morphology is added (Table 3-1). The only significant difference in support is evident in the *Mesua* + *Calophyllum* clade: this clade received 84% bootstrap support in the unpruned and pruned combined DNA analyses, and received 97% bootstrap support in the unpruned DNA + morphology analysis.

The pruned combined DNA + morphology analysis (Analysis 8; Figure 3-13) resulted in four most parsimonious trees of length 1297 (CI = 0.705, RI = 0.548, RC = 0.386). The topology of the strict consensus is nearly identical to that of the unpruned analysis (Analysis 7), with only two differences: relationships among *Caraipa*, *Haploclathra*, and *Kielmeyera* are unresolved in the pruned analysis (Analysis 8) whereas *Caraipa* and *Haploclathra* are sisters with 56% bootstrap support in the unpruned analysis (Analysis 7). Furthermore, in the strict consensus of the pruned analysis, *Kayea* and *Poeciloneuron* do not form a clade; however, in the bootstrap analysis, *Kayea* and *Poeciloneuron* are supported as sisters with weak support (bootstrap support = 61%).

Analyses 9 and 10: DNA + Morphology of Kielmeryoideae, Including *Neotatea*

Combining all three gene regions with the morphological data matrix, including *Neotatea*, results in a total of 2770 characters. In the unpruned analysis (Analysis 9), 439

of the 962 variable characters were parsimony-informative; in the pruned analysis (Analysis 10), 310 of the 714 variable characters were parsimony-informative. Including *Neotatea* in the unpruned combined DNA + morphology analysis (Analysis 9) resulted in 28 most parsimonious trees of length 2009 (CI = 0.666, RI = 0.654, RC = 0.436). The relationships of *Neotatea* to other genera within Kielmeyeroideae are unresolved in the unpruned analysis (Analysis 9; Figure 3-14).

Adding *Neotatea* to the pruned combined DNA + morphology analysis (Analysis 10; Figure 3-15) resulted in twelve most parsimonious trees of length 1315 (CI = 0.698, RI = 0.569, RC = 0.398). *Neotatea* appears in a clade with *Clusiella*, *Marila*, and *Mahurea* with 69% bootstrap support. *Neotatea* is sister to *Mahurea*, but with only weak support (bootstrap support = 52%).

Character Evolution

Morphological character-state transformations were mapped onto one of the most parsimonious trees (tree #2) from Analysis 7 (DNA + morphology, unpruned; Figure 3-16). Kielmeyeroideae are distinguished from *Clusia lanceolata* by 15 characters. An informal survey of Clusioideae and Hypericoideae provides evidence that none of these characters is likely synapomorphic for Kielmeyeroideae. However, having a nonfasciculate androecium might be a synapomorphy for Kielmeyeroideae; although most members of Clusieae do not have fasciculate androecia, most of the remaining members of Clusioideae (Garcinieae and Symphonieae), as well as most Hypericoideae, do have fasciculate androecia.

Following is a nonexhaustive list of putative morphological synapomorphies for clades within Kielmeyeroideae. Equivocal, very homoplasious character state transformations (i.e., 0/1 → 0/1) are not mentioned except where this condition is only an

artifact of missing morphological data from taxa that were included in the molecular data set, but not the morphological data set. Character states changing from equivocal to unequivocal (i.e., 0/1 → 1) are mentioned. Unequivocal, but homoplasious, character-state transformations (i.e., 0 → 1) are designated by an asterisk. Unequivocal, unique, character-state transformations (i.e., 0 → 1) are designated by two asterisks. Refer to Table 2-1 for a description of character states.

Endodesmia has the following potential morphological synapomorphies: a nonfunctional terminal bud (chr. 1:1*), presence of stipuliform structures (chr. 8:1*), eucamptodromous secondary venation (chr. 10:0), quincuncial calyx aestivation (chr. 22:1*), sepals not enclosing petals (chr. 24:1), five petals (chr. 27:0*), asymmetrical petals (chr. 28:1**), *Endodesmia*-type stamen dimorphism (chr. 31:2**), *Endodesmia*-type monadelphous stamens (chr. 32:3**), one carpel (chr. 37:1), apical placentation (chrs. 38:4** and 42:2**), a narrow stigma (chr. 45:1*), a swollen pedicel (chr. 48:1**), and an indehiscent fruit (chr. 50:2).

Potential synapomorphies for Calophylleae include having a terminal bud without scales (chr. 2:1), absence of latex/resin canals in mesophyll (chr. 13:1*), fused styles (chr. 44:1), presence of transcurrent lateral bundles in leaf blade (chr. 63:1*), and marginal lignification of leaf blade (chr. 65:1*).

Mammea may be diagnosed by several putative morphological synapomorphies including an axillary inflorescence position (chr. 15:1*), fasciculate inflorescence (chr. 17:5**), absence of terminal flower (chr. 18:1*), being androdioecious (21:0), having two sepals (chr. 23:3*), sepals that enclose the petals before anthesis (chr. 24:0), fused sepals (chr. 25:1*), decussate corolla aestivation (chr. 26:2*), basal placentation (chrs.

38:3 and 42:1), the absence of a persistent calyx (chr. 46:1*), indehiscent fruit (chr. 50:2), and the presence of druse crystals in the petiole (chr. 73:1).

Putative synapomorphies for the remaining members of Calophylleae (all genera except *Mammea*) are: having brochidodromous secondary venation (chr. 10:1), a panicle-like cyme inflorescence (chr. 17:1), being monoecious (chr. 21:1), and two layers of leaf midrib xylem (chr. 67:1*).

The clade containing *Kayea*, *Poeciloneuron*, *Mesua*, and *Calophyllum* has the following potential synapomorphies: basal placentation (chrs. 38:3 and 42:1) and the presence of a group of fibers adaxial to the leaf midrib bundle (60:1). A likely synapomorphy for *Kayea* and *Poeciloneuron* is narrow stigmas (chr. 45:1*). Potential synapomorphies for *Kayea* include sepals that enclose the petals before anthesis (chr. 24:0), the outer whorl of sepals fused and the inner whorl free (chr. 25:2**), imbricate corolla aestivation (chr. 26:0*), four carpels (chr. 37:4**), a style length to ovary length ratio greater than or equal to six (chr. 43:1*), an accrescent calyx (chr. 47:1*), a fiber strand associated with adaxial side of abaxial-most xylem of leaf midrib (chr. 62:1*), and the presence of druse crystals in the petiole (chr. 73:1). *Poeciloneuron* may be diagnosed by the following putative morphological synapomorphies: an indumentum of unbranched, unicellular hairs (chr. 4:1*), the presence of stipuliform structures (chr. 8:1*), quincuncial calyx aestivation (chr. 22:1), five sepals (chr. 23:1), sepals not enclosing the petals before anthesis (chr. 24:1), five petals (chr. 27:0), 15 stamens (chr. 29:1**), the absence of anther glands (chr. 34:0), anthers with terminal pores (chr. 36:1**), free styles (chr. 44:0*), septicidal capsule (chr. 50:0), abaxial phloem in leaf midrib partitioned by fibers (chr. 59:1), and the absence of druse crystals in the petiole (chr. 73:0). *Calophyllum* and

Mesua have several potential morphological synapomorphies, including the absence of colleters (chr. 7:1*), axillary inflorescence position (chr. 15:1*), the absence of bracteoles (chr. 19:1*), opposite or decussate corolla aestivation (chr. 26:2*), filaments fused at base (chr. 32:1), and the absence of anther glands (chr. 34:0). *Mesua* is recognized by the following putative synapomorphies: a nonfunctional terminal bud (chr. 1:1*), immersed axillary bud (chr. 3:2**), cyme inflorescence (chr. 17:0*), the presence of a U-shaped partition in the ovary (chr. 39:1**), septifragal capsule (chr. 50:1), the absence of a group of fibers adaxial to the midrib bundle (chr. 60:0), and the presence of a continuous band of fibers adjacent to the abaxial epidermins (chr. 61:2). *Calophyllum* can be recognized by many potential synapomorphies, including a terminal bud without scales (chr. 2:0), an indumentum of multicellular hairs (chr. 5:1*), eucamptodromous secondary venation (chr. 10:0*), tertiary venation that is not evident (chr. 11:0*), absence of latex/resin cavities in mesophyll (chr. 12:0*), presence of latex/resin canals in mesophyll (chr. 13:0*), intersecondary veins that are replaced by canals (chr. 14:1*), one ovule per gynoecium (chr. 41:2**), absence of persistent calyx (chr. 46:1*), bony endocarp (chr. 49:1**), indehiscent fruit (chr. 50:2), absence of fibers adjacent to abaxial epidermis (chr. 61:0), and a single layer of leaf midrib xylem (chr. 67:0*).

The clade containing *Caraipa*, *Haploclathra*, *Kielmeyera*, *Clusiella*, *Mahurea*, and *Marila* has a few potential morphological synapomorphies, including quincuncial calyx aestivation (chr. 22:1*), five petals (chr. 27:0*), three carpels (chr. 37:3*), axile or intruded-parietal placentation (chr. 38:0), and median ovule position (chr. 42:0). Potential synapomorphies for the clade containing *Kielmeyera*, *Caraipa*, and *Haploclathra* include an indumentum of multicellular hairs (chr. 5:1*), alternate leaves

(chr. 6:1), sepals that enclose the petals before anthesis (chr. 24:1), cordate cotyledons (58:1**), and the absence of druse crystals (chr. 73:0). Putative synapomorphies for *Kielmeyera* include more than 15 ovules per carpel (chr. 41:1), absence of a persistent calyx (chr. 46:1), and a seed with a wing two cell layers thick, going completely around the seed, with no vascular tissue (chr. 51:2**). *Caraipa* and *Haploclathra* are united by a terminal bud without scales (chr. 2:0*), a septifragal capsule (chr. 50:1*), a seed with a wing several cell layers thick, going completely around the seed, with no vascular tissue (chr. 51:1**), and the presence of a group of fibers adaxial to the leaf midrib bundle (60:1*). One possible synapomorphy for *Caraipa* is bowl-shaped anther glands (chr. 35:1). *Haploclathra* may be diagnosed by the following putative synapomorphies: opposite leaves (chr. 6:0), slightly connate filaments (chr. 32:1), absence of anther glands (chr. 34:0*), basal ovule position (chr. 42:1*), abaxial phloem in leaf midrib partitioned by fibers (chr. 59:1), the presence of a continuous band of fibers adjacent to the abaxial epidermis (chr. 61:2*), a papillose epidermis (chr. 72:1*), and petiole bundle an arch with dorsal groupings of xylem and phloem (chr. 74:2**).

The clade containing *Clusiella*, *Mahurea*, and *Marila* has the following potential synapomorphies: more than 15 ovules per carpel (chr. 41:1), a testa vasculature of unbranched or braided raphal bundle (chr. 54:0), presence of an exotegmen (chr. 55:0*), presence of endosperm in the mature seeds (chr. 56:0*), and an embryo less than four mm long (chr. 57:0*). *Clusiella* has many potential synapomorphies, including the presence of an interpetiolar stipuliform structure (chr. 8:2**), presence of latex/resin canals in the mesophyll (chr. 13:0*), sympodial shoot growth (chr. 16:1**), *Clusiella*-type cyme (chr. 17:2**), bracteoles with an abaxial gland (chr. 20:1**), dioecy (chr.

21:0*), imbricate calyx aestivation (chr. 22:2*), *Clusiella*-type stamen dimorphism (chr. 31:1**), *Clusiella*-type monadelphous stamens (chr. 32:2**), ten to 30 carpels (chr. 37:5**), laminar placentation with ovules scattered on the partitions (chr. 38:2**), indehiscent fruit (chr. 50:2), absence of transcurrent lateral bundles (chr. 63:0*), absence of marginal lignification (chr. 65:0*), and absence of a marginal canal in leaf blade (chr. 66:1**). *Mahurea* and *Marila* have the following potential synapomorphies: percurrent tertiary venation (chr. 11:2**), septicidal capsule (chr. 50:0), and a petiole bundle with three layers of xylem and phloem (chr. 74:1*). Potential synapomorphies of *Mahurea* include an indumentum of unbranched, unicellular hairs (chr. 4:1), alternate leaves (chr. 6:1*), presence of stipuliform structures (chr. 8:1*), eucamptodromous secondary venation (chr. 10:0*), free filaments (chr. 32:0), bowl-shaped anther glands (chr. 35:1*), intruded placentae bordered by in-curled carpel walls (chr. 40:1**), a seed with an elongate wing several cell layers thick, not going completely around the seed, with a peripheral vascular bundle (chr. 51:3**), three to six layers of xylem in the leaf midrib (chr. 67:2) and the absence of druse crystals in the petiole (chr. 73:0). *Marila* can be diagnosed by the following likely synapomorphies: an axillary inflorescence (chr. 15:1*), a raceme (chr. 17:4**), absence of a terminal flower (chr. 18:1*), fused sepals (chr. 25:1), imbricate corolla aestivation (chr. 26:0), plumose seeds (chr. 52:1**), and the presence of druse crystals in the petiole (chr 73:1).

Morphological character-state transformations mapped onto a representative most parsimonious tree from Analysis 9 (DNA + morphology with *Neotatea*, pruned) are shown for the clade containing *Neotatea* (Figure 3-17). The clade containing *Clusiella*, *Marila*, *Mahurea*, and *Neotatea* has the same synapomorphies as does the clade of

Clusiella, *Mahurea*, and *Marila* in Analysis 7 (Figure 3-16). Putative synapomorphies for *Marila*, *Mahurea*, and *Neotatea* are the presence of unicellular hairs (chr. 4:1*), percurrent tertiary venation (chr. 11:2*), sepals that enclose the petals before anthesis (24:0*), septicidal capsule (chr. 50:0*), and a petiole bundle with three layers of xylem and phloem (chr. 74:1*). *Mahurea* and *Neotatea* are united by the following potential synapomorphies: alternate leaves (chr. 6:1*), free filaments (chr. 32:0*), intruded placentae bordered by in-curled carpel walls (chr. 40:1**), and a seed with an elongate wing several cell layers thick, not completely surrounding the seed, with a peripheral vascular bundle (chr. 51:3**). Potential synapomorphies of *Neotatea* are the absence of colleters (chr. 7:1), tertiary venation that is not evident (chr. 11:0*), absence of latex/resin cavities in the mesophyll (chr. 12:0*), presence of latex/resin canals (chr. 13:0*), intersecondary veins that are replaced by canals (chr. 14:1*), *Neotatea*-type inflorescence (chr. 17:3**), absence of bracteoles (chr. 19:1*), imbricate calyx aestivation (chr. 22:2*), anthers longer than six mm (chr. 33:2**), phloem of leaf midrib doubly partitioned by fibers (chr. 59:2**), lateral bundles of leaf midrib not transcurrent (chr. 63:0), presence of canals immediately adaxially and abaxially positioned in relation to secondary veins (chr. 64:1**), a single layer of leaf midrib xylem (chr. 67:0*), a lamina with a hypodermis (chr. 70:0*), and a petiole bundle with vascular tissue arched to circular (chr. 74:0).

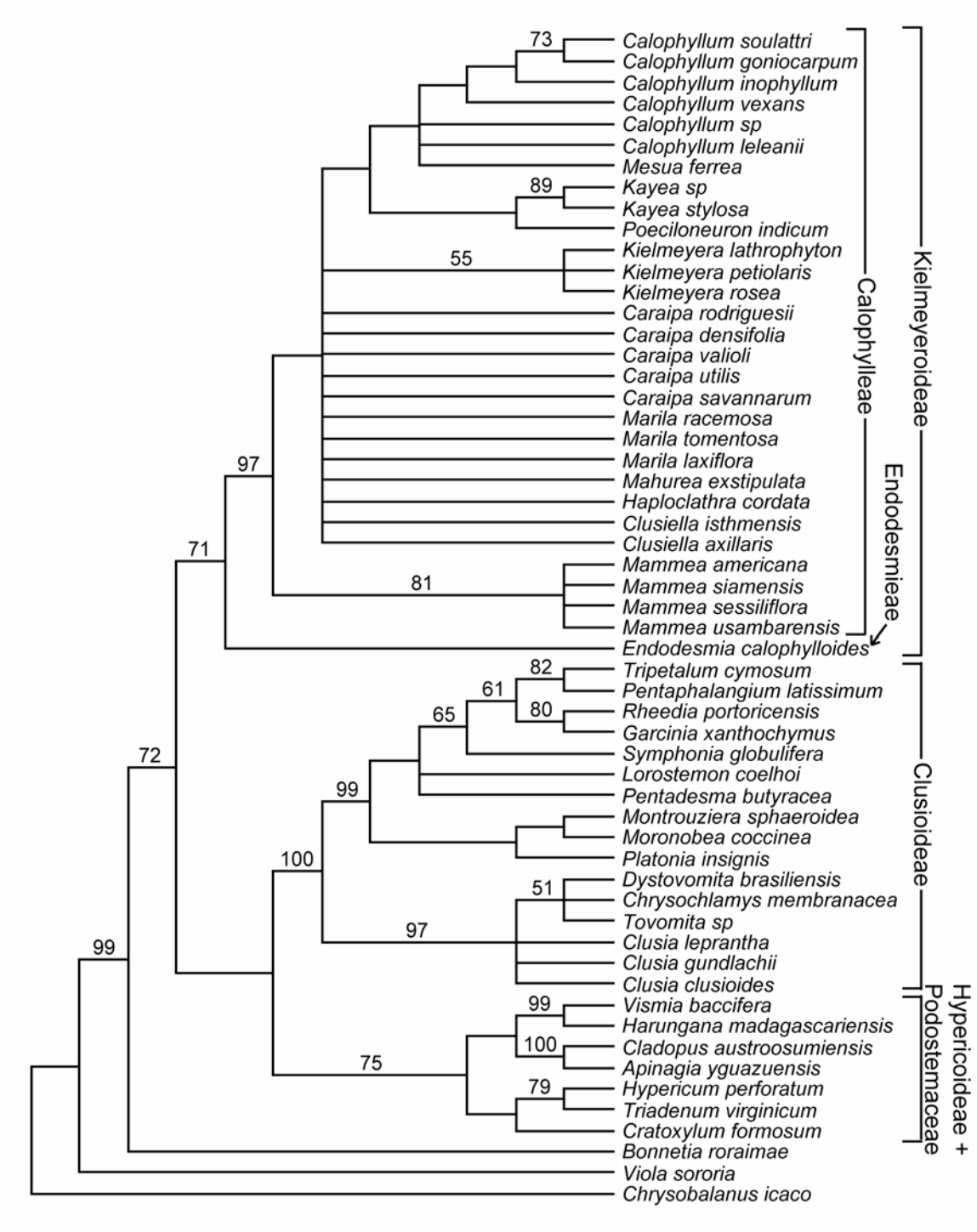


Figure 3-1. Strict consensus of 1530 most parsimonious trees of length 845 from Analysis 1 (*rbcL* alone). CI = 0.612, RI = 0.755, RC = 0.462, HI = 0.388; of 1408 aligned positions, 240 are parsimony-informative. Numbers above branches represent bootstrap values. *Kayea* sp. was originally labeled as "*Mesua* sp." in the Gustafsson et al. (2002) analysis, but is probably a species of *Kayea* (see Discussion).

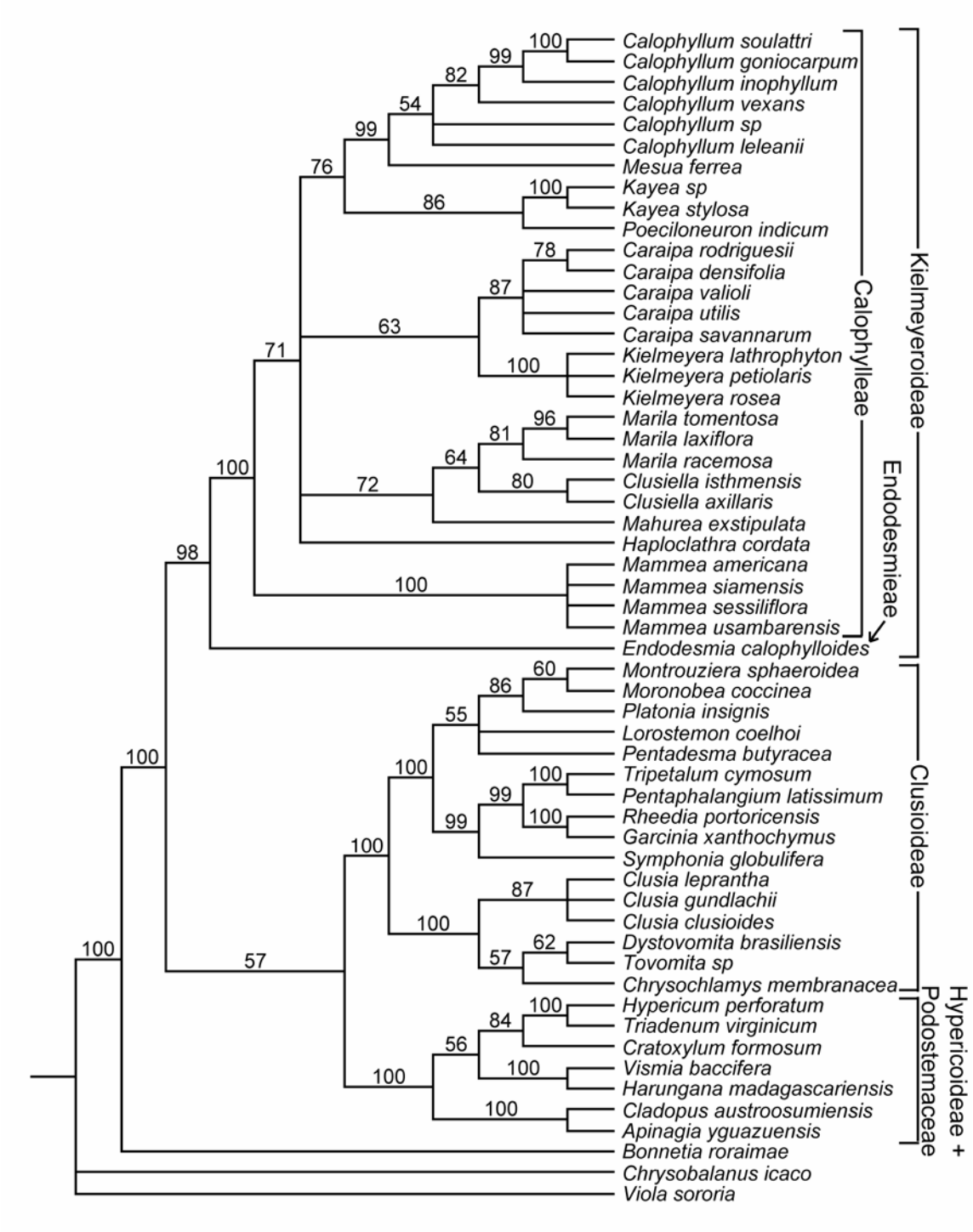


Figure 3-2. Majority-rule consensus tree based on *rbcL* data for Clusiaceae (Analysis 1). Bayesian analysis was run using the GTR+I+Γ substitution model. The analysis employed four chains of two million generations. Trees produced during the burn-in phase were not used to produce the consensus tree. Numbers above branches are the percent of the time that the clade occurs among the sampled trees (i.e., the posterior probability).

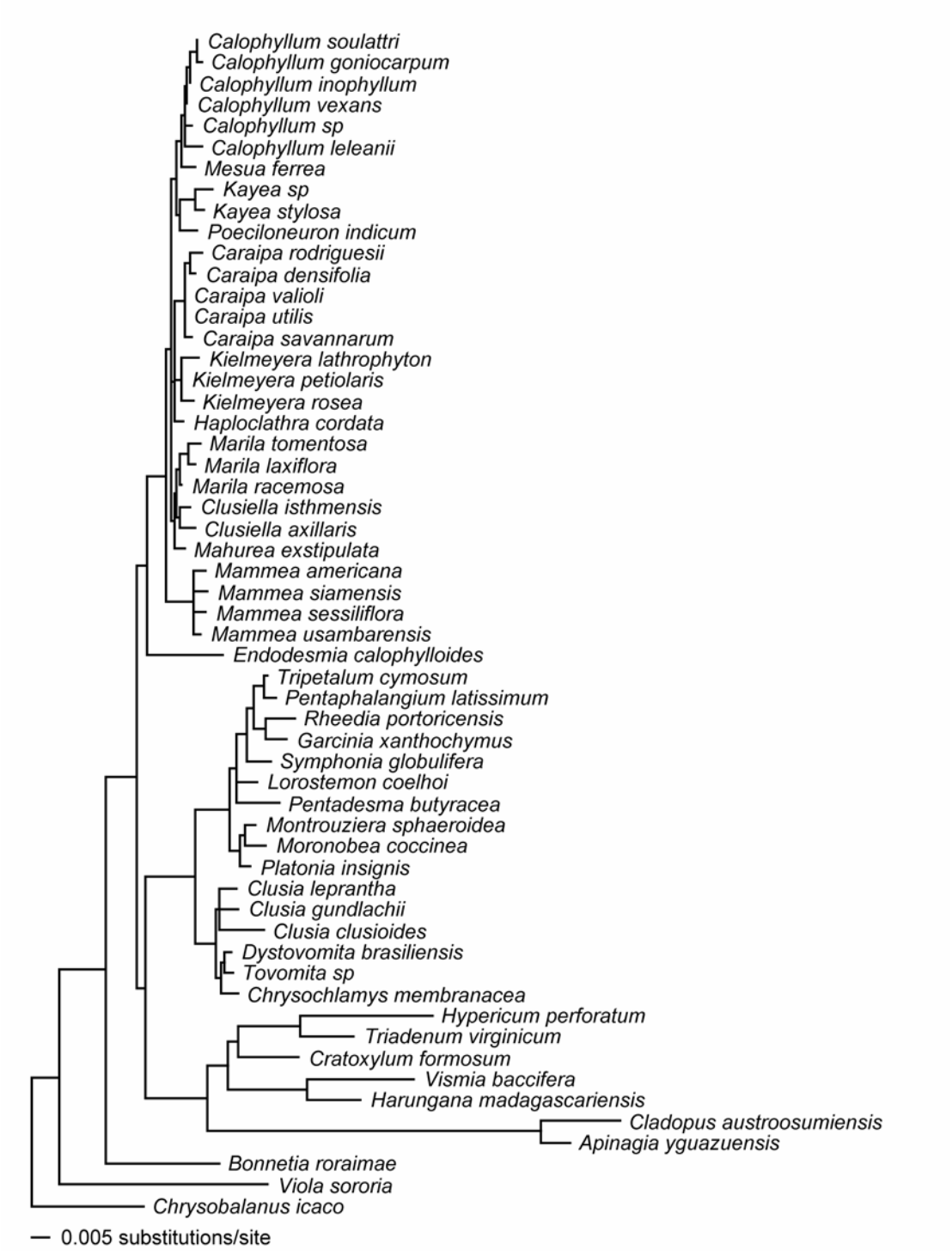


Figure 3-3. Maximum likelihood tree of *rbcL* sequences across Clusiaceae (Analysis 1). Likelihood score = 6792.82649. Maximum likelihood analysis employed the GTR+I+Γ substitution model.

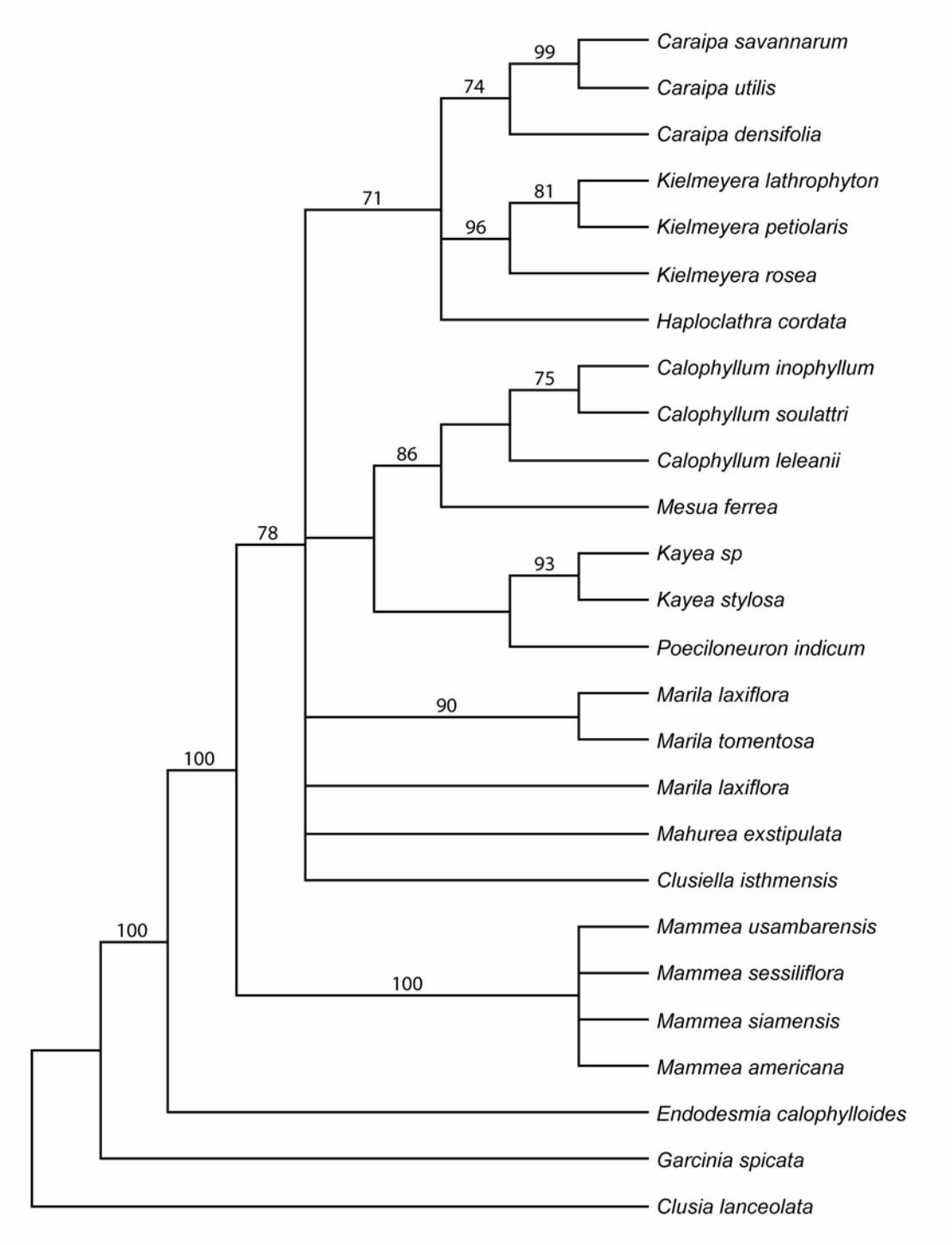


Figure 3-4. Strict consensus of 510 most parsimonious trees of length 361 from Analysis 2 (*rbcL* + *matK*). CI = 0.831, RI = 0.762, RC = 0.633, HI = 0.169; of 1885 aligned positions, 120 are parsimony-informative. Numbers above branches represent bootstrap values. *Kayea* sp. was originally labeled as "*Mesua* sp." in the Gustafsson et al. (2002) analysis, but is probably a species of *Kayea* (see Discussion).

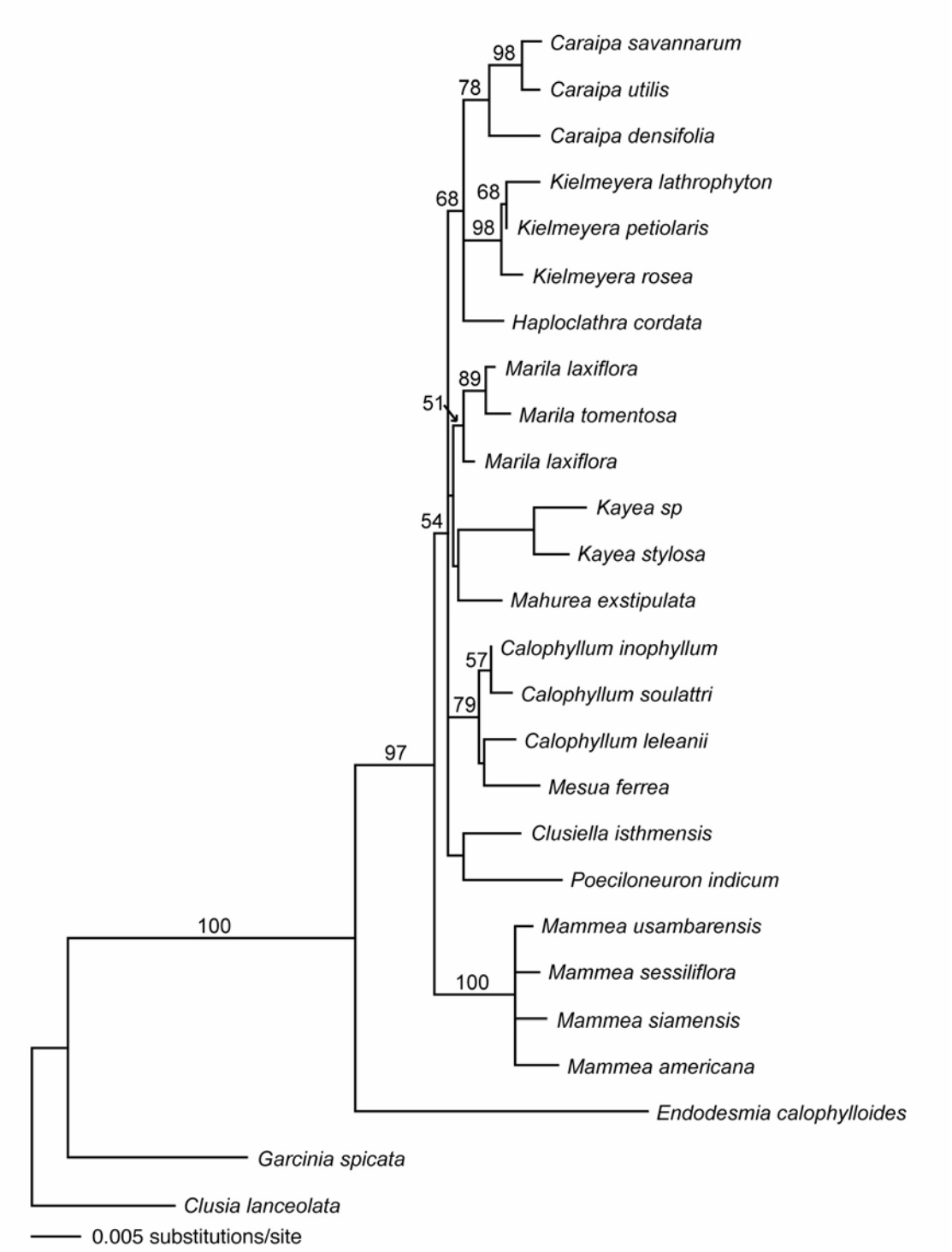


Figure 3-5. Maximum likelihood tree of *rbcL* + *matK* data set (Analysis 2). Likelihood score = 4912.90425. Maximum likelihood analysis employed the GTR+I+Γ substitution model. Numbers above branches represent bootstrap values.

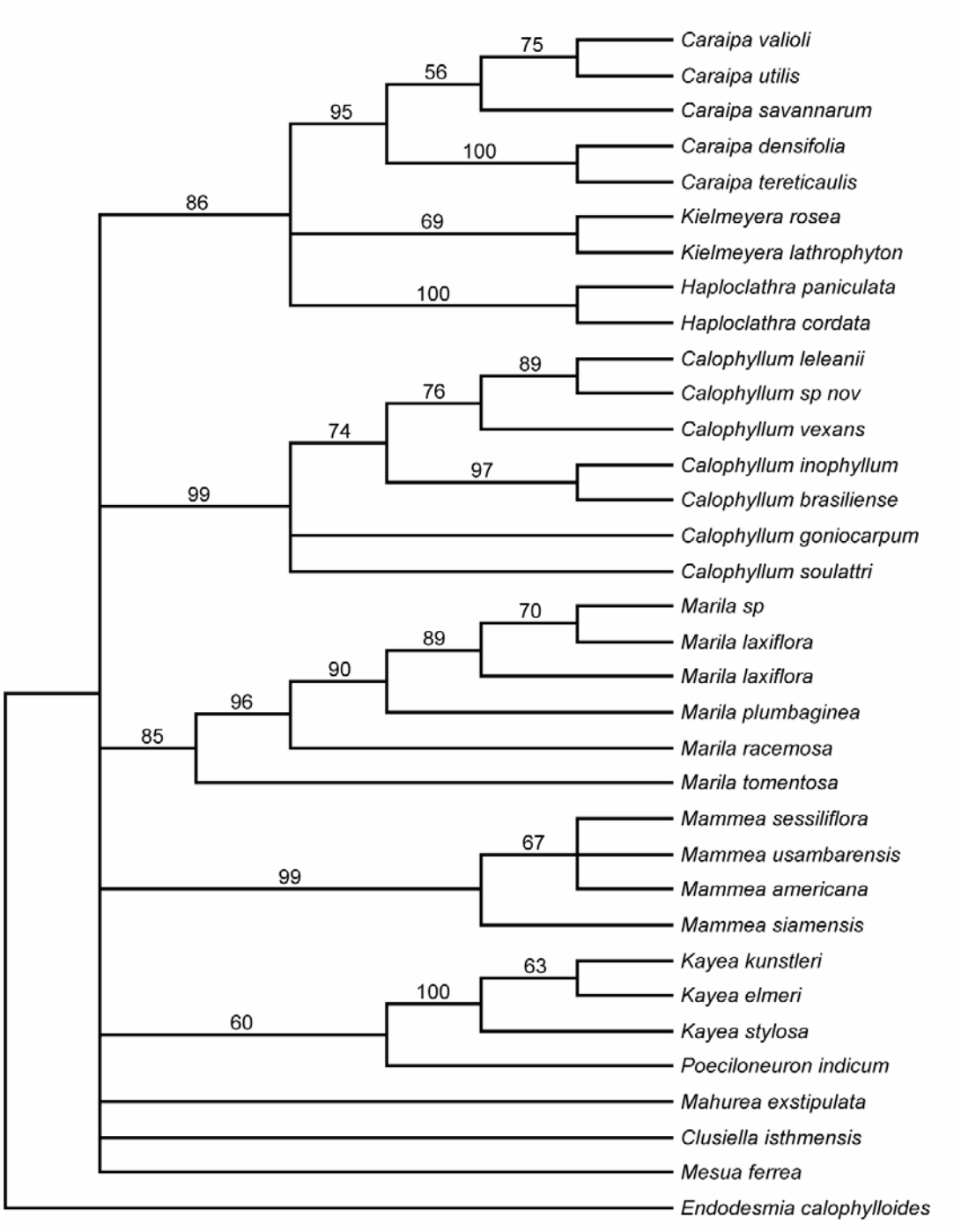


Figure 3-6. Strict consensus of 10 most parsimonious trees of length 987 from Analysis 3 (ITS alone). CI = 0.584, RI = 0.658, RC = 0.384, HI = 0.416; of 809 aligned positions, 232 are parsimony-informative. Numbers above branches represent bootstrap values.

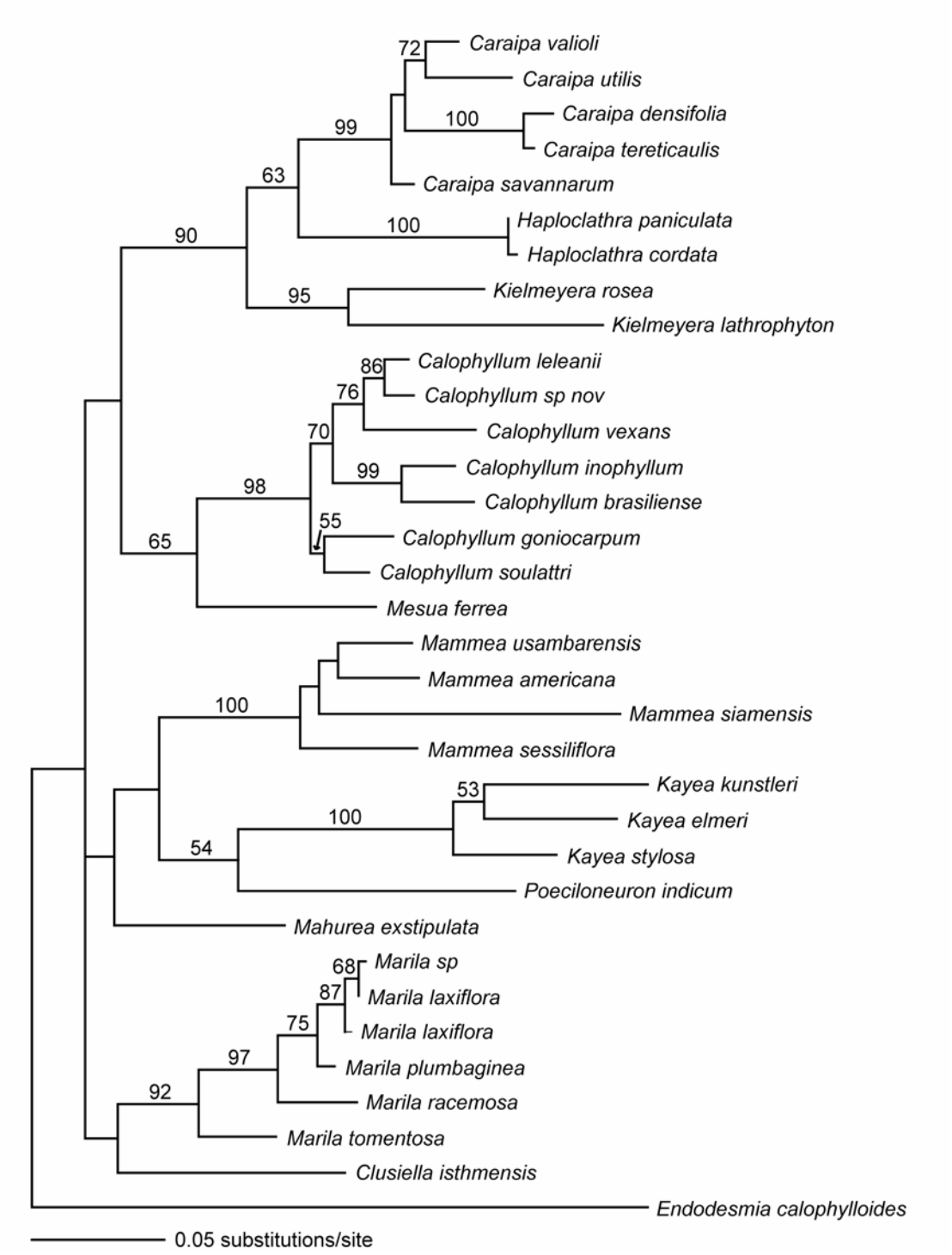


Figure 3-7. Maximum likelihood tree of ITS sequences (Analysis 3). Likelihood score = 5734.93593. Maximum likelihood analysis employed the GTR+I+Γ substitution model. Numbers above branches represent bootstrap values.

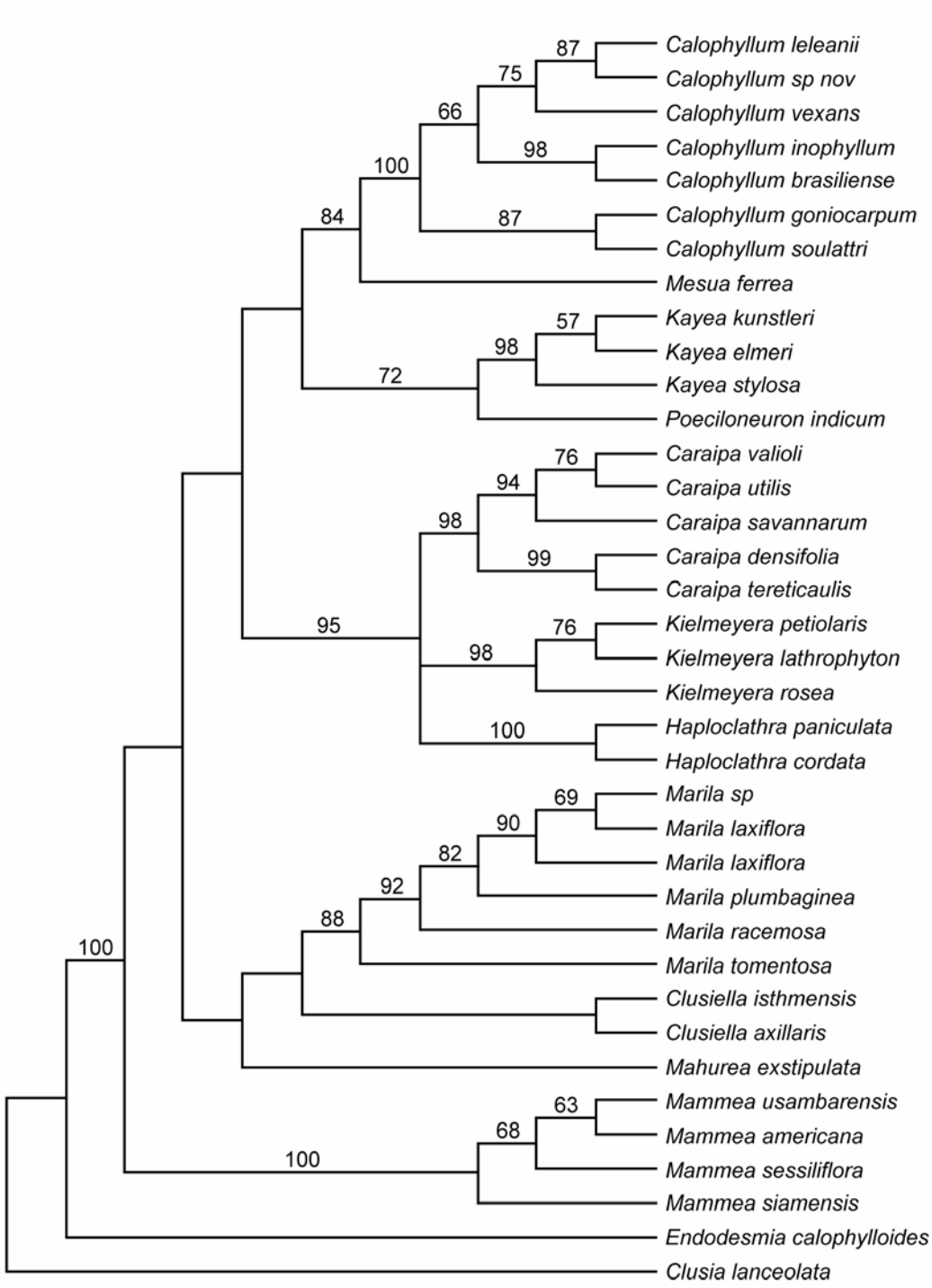


Figure 3-8. Strict consensus of 2 most parsimonious trees of length 1326 from Analysis 4 (*rbcL* + *matK* + ITS, unpruned). CI = 0.643, RI = 0.665, RC = 0.427, HI = 0.357; of 2696 aligned positions, 311 are parsimony-informative. Numbers above branches represent bootstrap values.

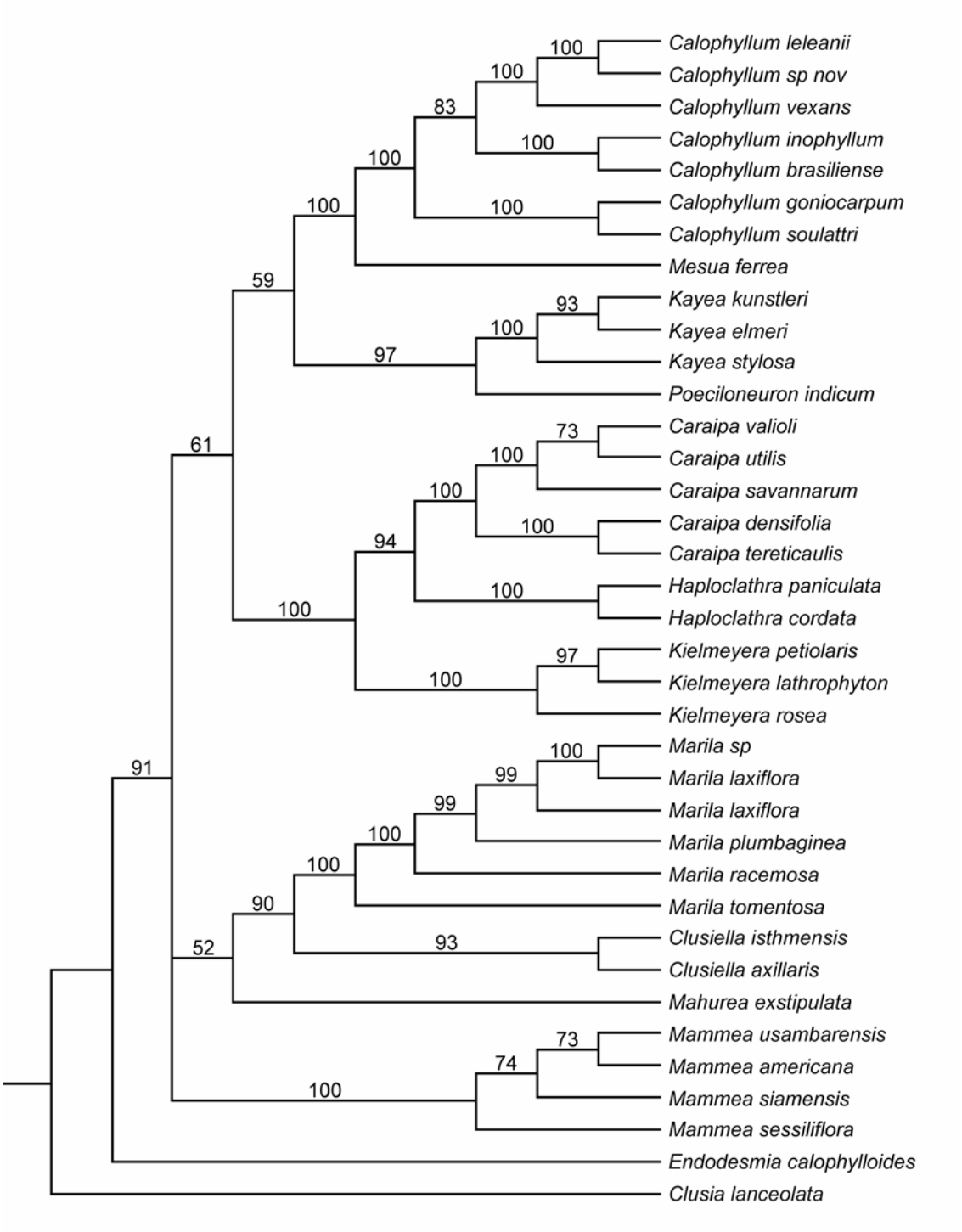


Figure 3-9. Majority-rule consensus tree based on the unpruned *rbcL* + matK + ITS data set (Analysis 4). Bayesian analysis was run using the GTR+I+Γ substitution model. The analysis employed four chains of two million generations. Trees produced during the burn-in phase were not used to produce the consensus tree. Numbers above branches are the percent of the time that the clade occurs among the sampled trees (i.e., the posterior probability).

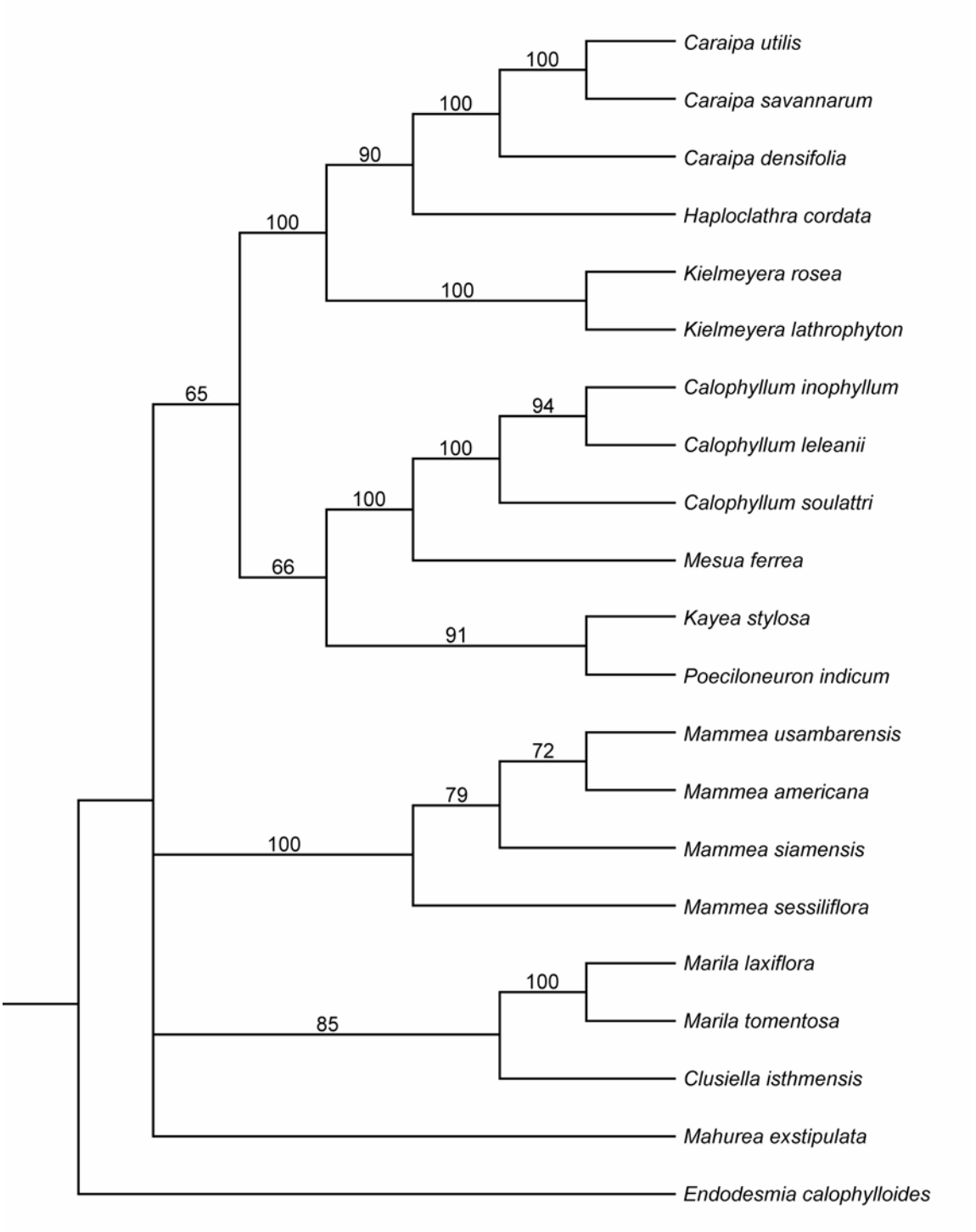


Figure 3-10. Majority-rule consensus tree based on the pruned *rbcL* + matK + ITS data set (Analysis 5). Bayesian analysis was run using the GTR+I+Γ substitution model. The analysis employed four chains of two million generations. Trees produced during the burn-in phase were not used to produce the consensus tree. Numbers above branches are the percent of the time that the clade occurs among the sampled trees (i.e., the posterior probability).

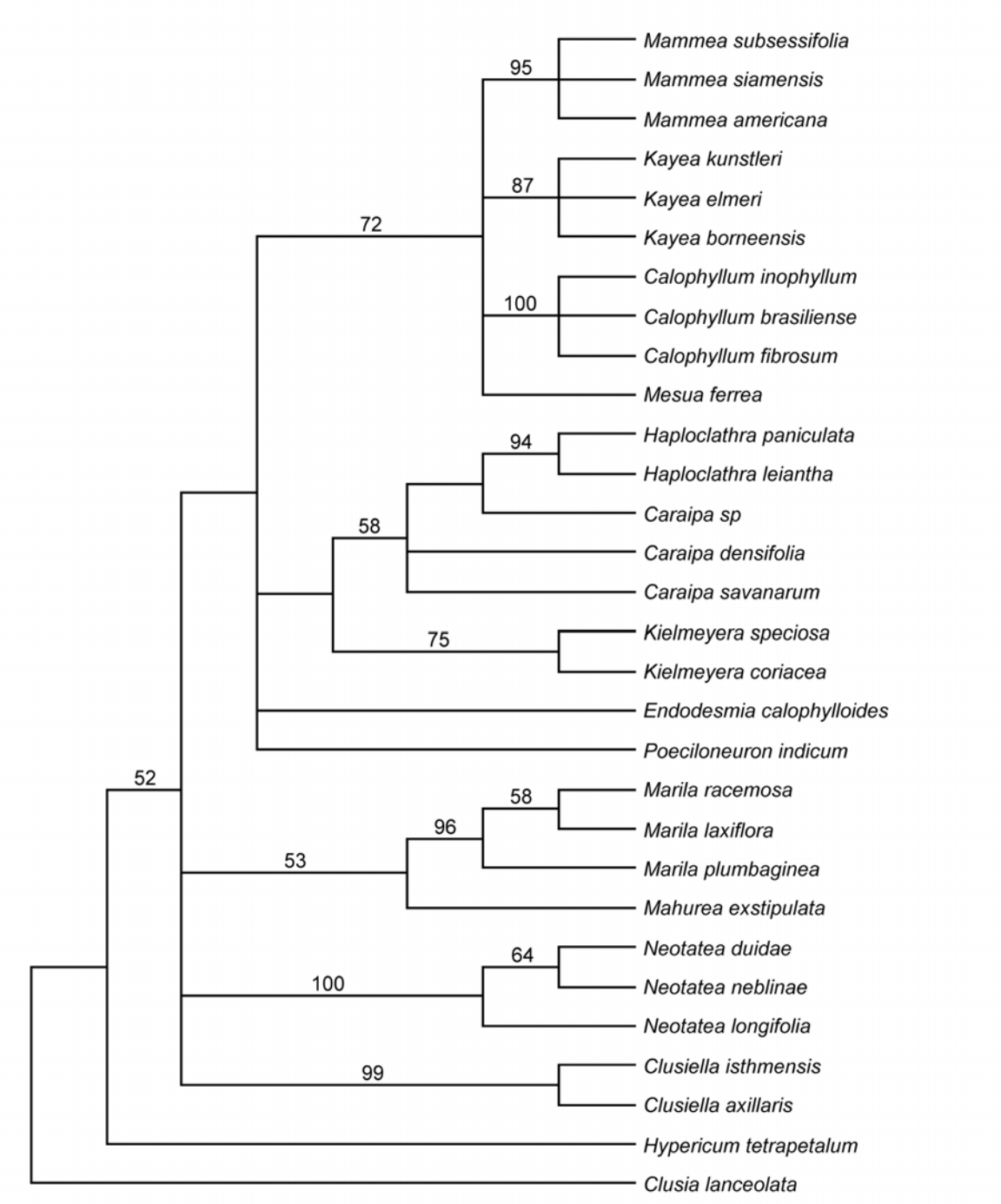


Figure 3-11. Strict consensus of 61 most parsimonious trees of length 227 from Analysis 6 (morphology alone). CI = 0.482, RI = 0.740, RC = 0.357, HI = 0.518; of 74 characters, 64 are parsimony-informative. Numbers above branches represent bootstrap values.

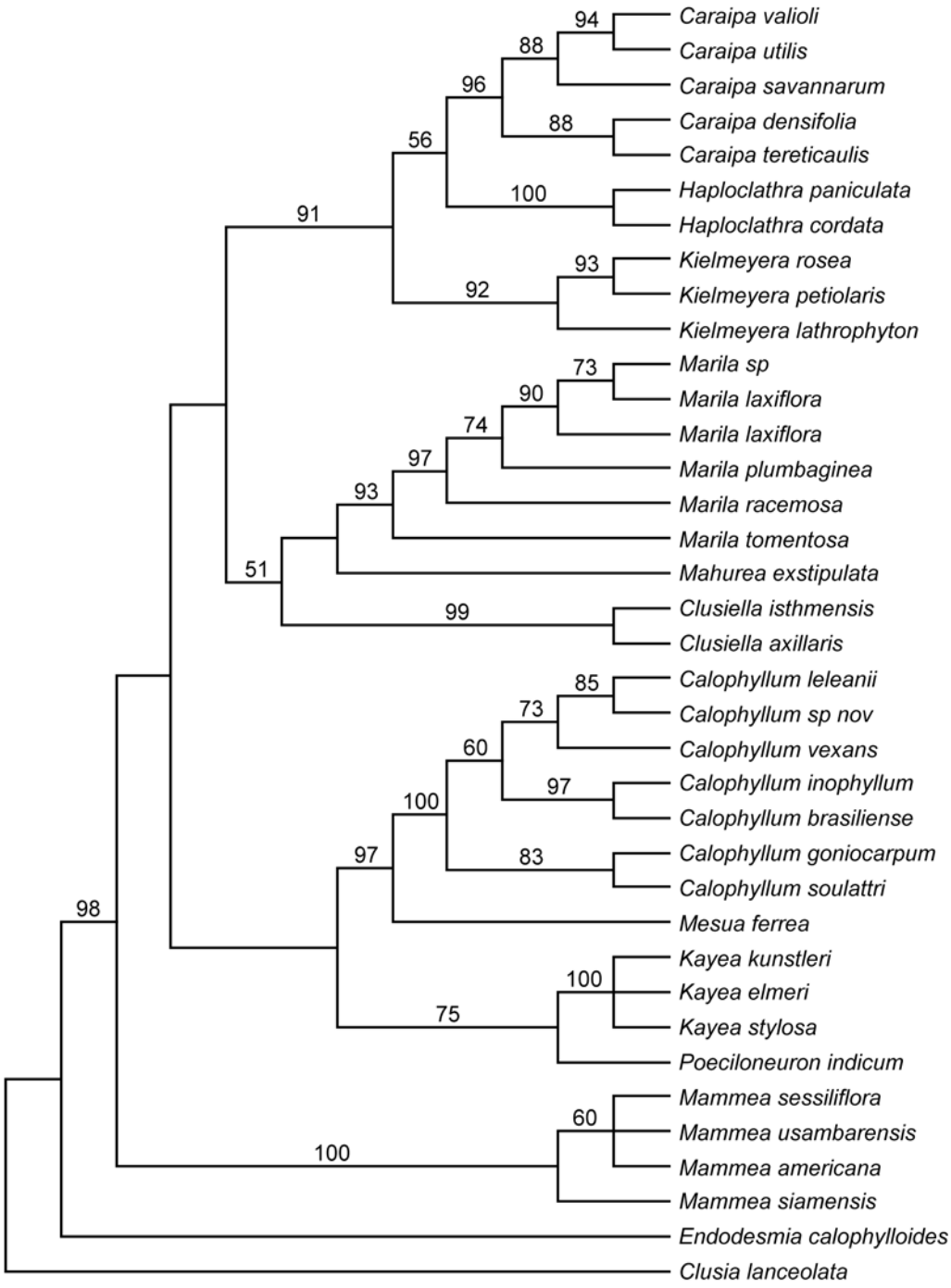


Figure 3-12. Strict consensus of 4 most parsimonious trees of length 1990 from Analysis 7 (DNA + morphology, unpruned). CI = 0.671, RI = 0.648, RC = 0.435, HI = 0.329; of 2770 characters, 436 are parsimony-informative. Numbers above branches represent bootstrap values.

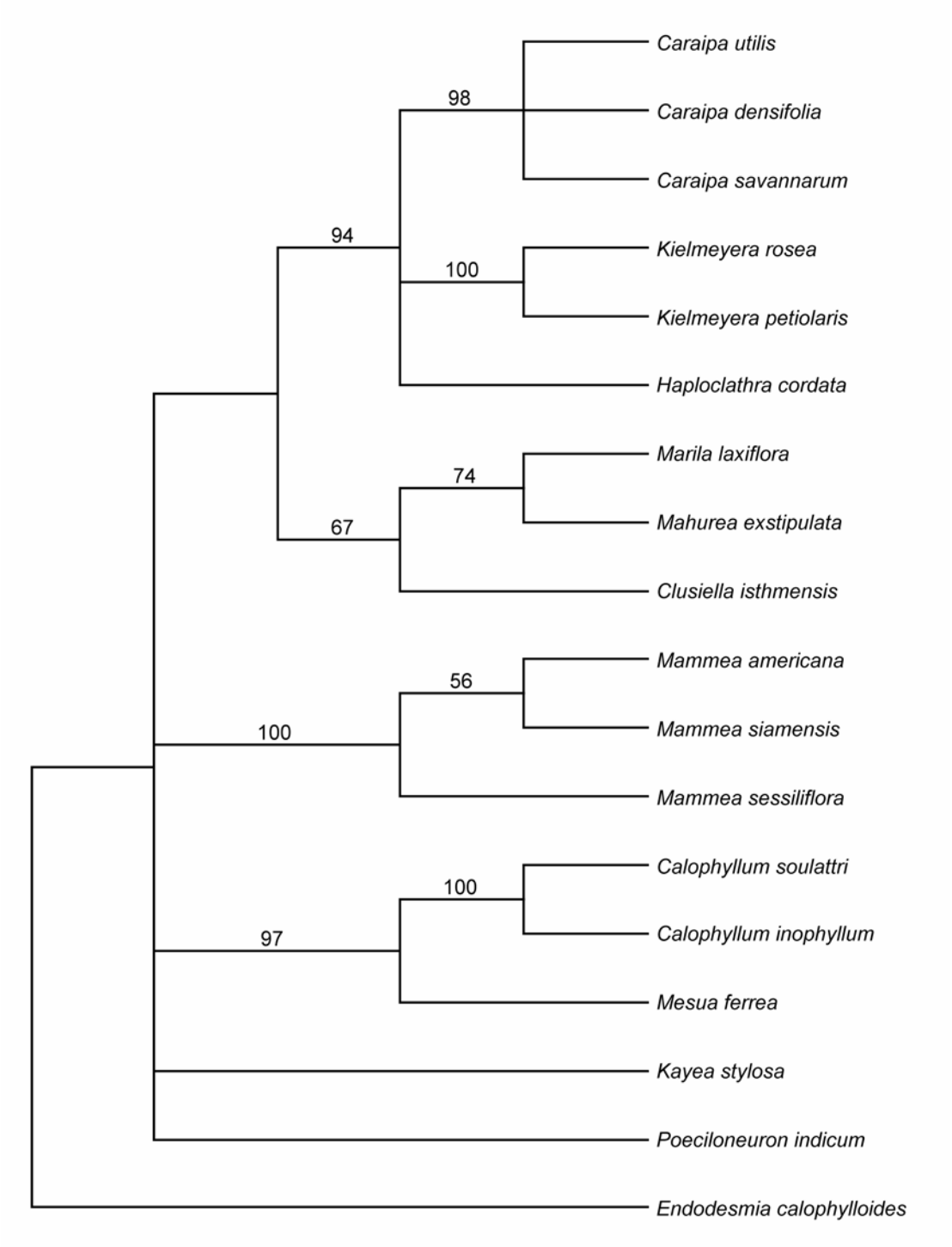


Figure 3-13. Strict consensus of 4 most parsimonious trees of length 1297 from Analysis 8 (DNA + morphology, pruned). CI = 0.705, RI = 0.548, RC = 0.386, HI = 0.295; of 2770 characters, 306 are parsimony-informative. Numbers above branches represent bootstrap values.

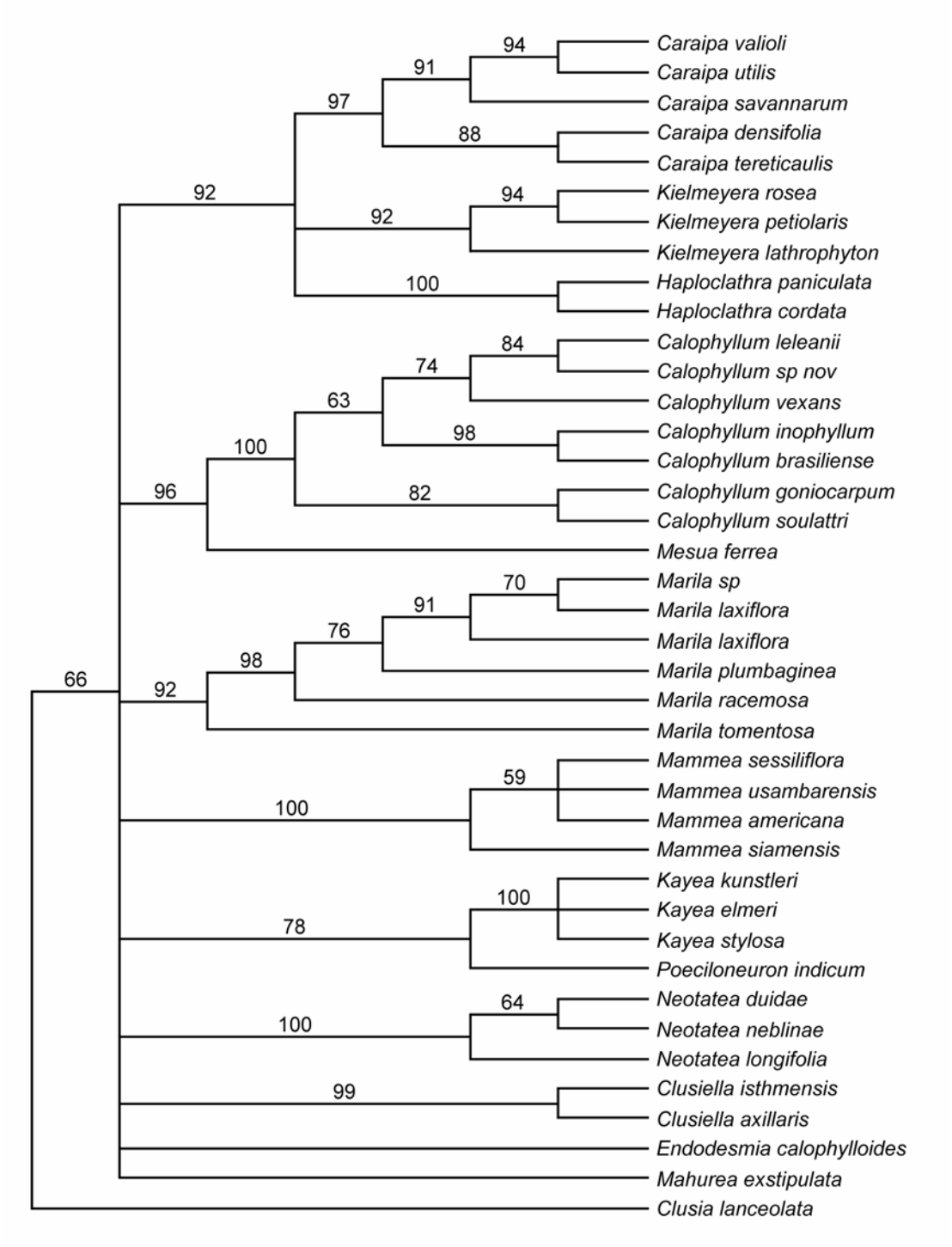


Figure 3-14. Strict consensus of 28 most parsimonious trees of length 2009 from Analysis 9 (DNA + morphology, unpruned, including *Neotatea*). CI = 0.666, RI = 0.654, RC = 0.436, HI = 0.334; of 2770 characters, 439 are parsimony-informative. Numbers above branches represent bootstrap values.

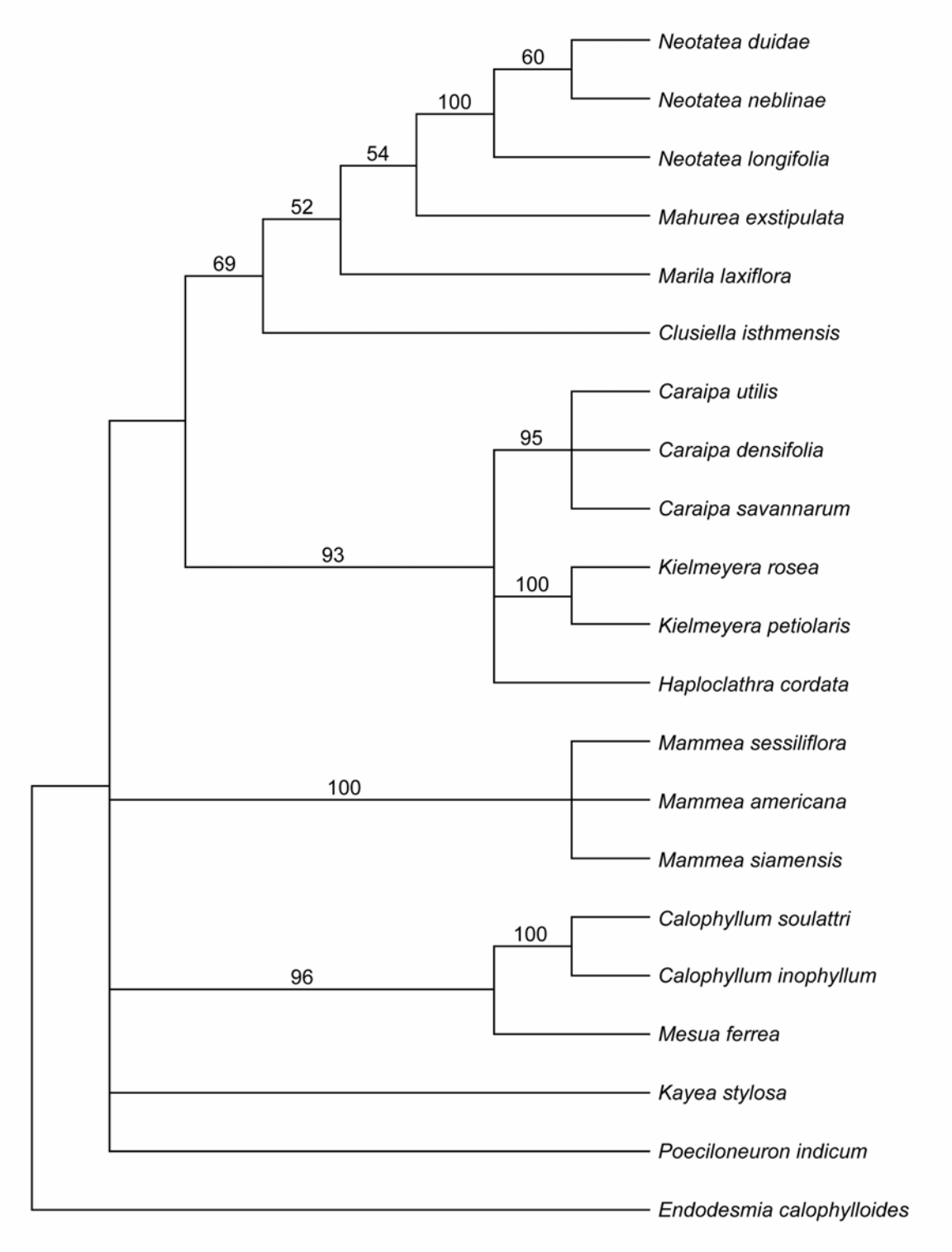
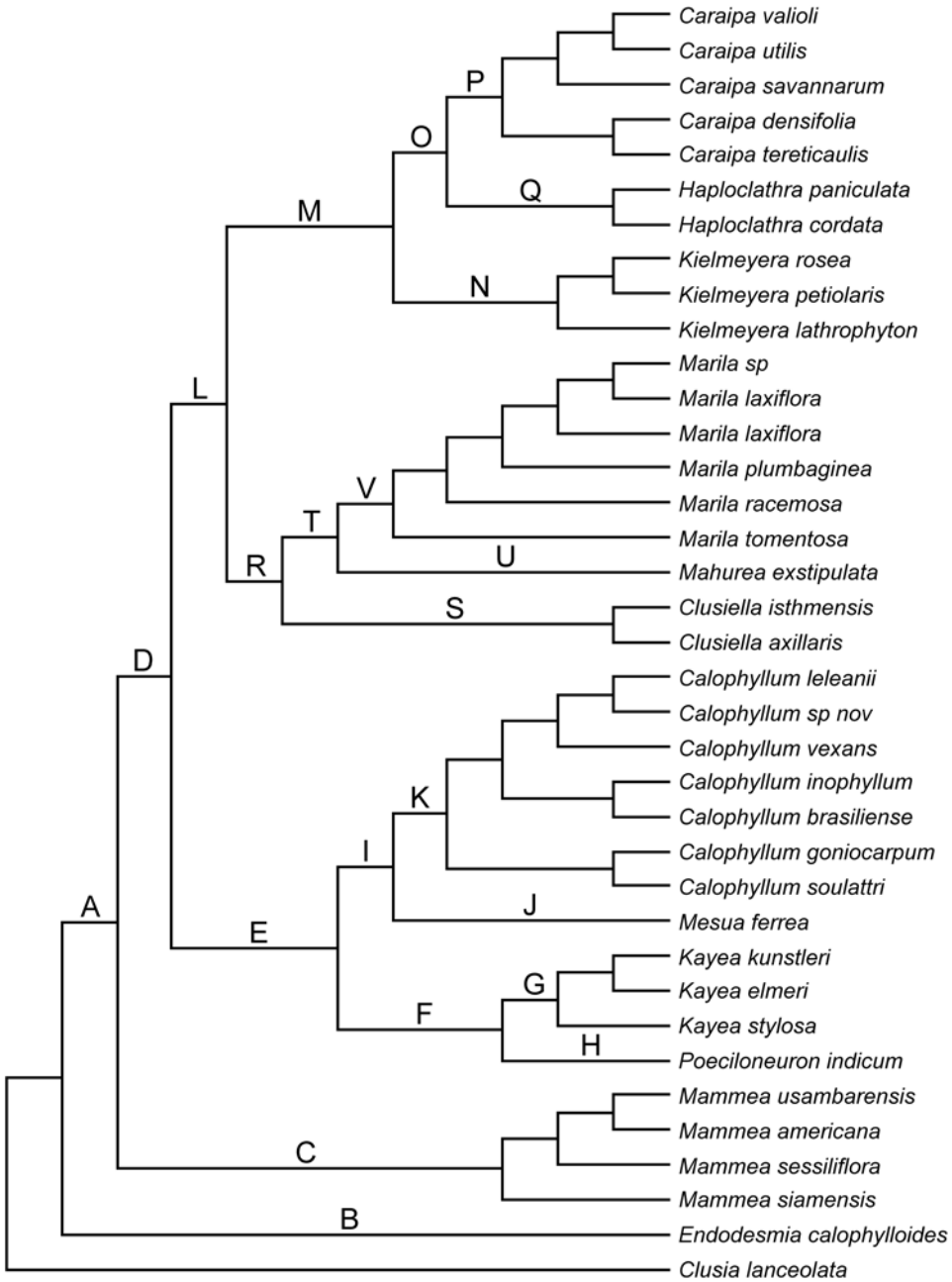


Figure 3-15. Strict consensus of 12 most parsimonious trees of length 1315 from Analysis 10 (DNA + morphology, pruned, including *Neotatea*). CI = 0.698, RI = 0.569, RC = 0.398, HI = 0.302; of 2790 characters, 310 are parsimony-informative. Numbers above branches represent bootstrap values.

Figure 3-16. Morphological character-state transformations mapped onto one of the most parsimonious trees (tree #2) from Analysis 7 (DNA + morphology, unpruned). Equivocal, very homoplasious character-state transformations (i.e., 0/1 → 0/1) are not included in this list. See Table 2-1 for a description of characters and character states. **A**: 2(0/1→1), 13(0→1), 44(0/1→1), 63(0→1), 65(0→1); **B**: 1(0→1), 8(0→1), 10(0/1→1), 22(0→1), 24(0/1→1), 27(1→0); 28(0→1), 31(0→2), 32(0→3), 37(0/1/2→1), 38(0/3/4→4), 42(0/1/2→2), 45(0→1), 48(0→1), 50(0/2→2); **C**: 15(0→1), 17(0/1/5→5), 18(0→1), 21(0/1→0), 23(1→3), 24(0/1→0), 25(0→1), 26(1→2), 38(0/3→3), 42(0/1→1, 46(0→1), 50(0/2→2), 73(0/1→1); **D**: 10(0/1→1), 17(0/1/5→1), 21(0/1→1), 67(0→1); **E**: 38(0/3→3), 42(0/1→1), 60(0→1); **F**: 45(0→1); **G**: 23(1/2→2), 24(0/1→0), 25(0→2), 26(1→0), 37(2→4), 43(0→1), 47(0→1), 62(0→1), 73(0/1→1); **H**: 4(0→1), 8(0→1), 22(2→1), 23(1/2→1), 24(0/1→1), 27(0/1→0), 29(0→1), 32(0/1→0), 34(0/1→0), 36(0→1), 44(1→0), 50(0/2→0), 59(0/1→1), 73(0/1→0); **I**: 7(0→1), 15(0→1), 19(0→1), 26(1→2), 32(0/1→1), 34(0/1→0)73(0/1→1); **J**: 1(0→1), 3(1→2), 17(1→0), 39(0→1), 50(0/1/2→1), 60(1→0), 61(0/2→2); **K**: 2(0/1→0), 5(0→1), 10(1→0), 11(1→0), 12(1→0), 13(1→0), 14(0→1), 41(0→2), 46(0→1), 49(0→1), 50(0/1/2→2), 61(0/2→0), 67(1→0); **L**: 22(0→1), 27(1→0), 37(2→3), 38(0/3→0), 42(0/1→0); **M**: 5(0→1), 6(0→1), 24(0/1→1), 58(0→1), 73(0/1→0); **N**: 41(0/1→1), 46(0/1→1), 51(0/1/2→2); **O**: 2(1→0), 50(0/2→1), 51(0/1/2→1), 54(0/1→1), 60(0→1); **P**: 35(0/1→1); **Q**: 6(1→0), 32(0/1→1), 34(1→0), 42(0→1), 59(0→1), 61(0→2), 72(0→1), 74(0→2); **R**: 41(0/1→1), 54(0/1→0), 55(1→0), 56(1→0), 57(1→0); **S**: 8(0→2), 13(1→0), 16(0→1), 17(1→2), 20(0→1), 21(1→0), 22(1→2), 31(0→1), 32(0/1→2), 37(3→5), 38(0→2), 50(0/2→2), 63(1→0), 65(1→0), 66(0→1); **T**: 11(1→2), 50(0/2→0), 74(0→1); **U**: 4(0/1→1), 6(0→1), 8(0→1), 10(1→0), 32(0/1→0), 35(0→1), 40(0→1), 51(0→3), 67(1/2→2), 73(0/1→0; **V**: 15(0→1), 17(1→4), 18(0→1), 25(0→1), 26(1→0), 52(0→1), 73(0/1→1).


Caraipa valioli
Caraipa utilis
Caraipa savannarum
Caraipa densifolia
Caraipa tereticaulis
Haploclathra paniculata
Haploclathra cordata
Kielmeyera rosea
Kielmeyera petiolaris
Kielmeyera lathrophyton
Marila sp
Marila laxiflora
Marila laxiflora
Marila plumbaginea
Marila racemosa
Marila tomentosa
Mahurea exstipulata
Clusiella isthmensis
Clusiella axillaris
Calophyllum leleanii
Calophyllum sp nov
Calophyllum vexans
Calophyllum inophyllum
Calophyllum brasiliense
Calophyllum goniocarpum
Calophyllum soulattri
Mesua ferrea
Kayea kunstleri
Kayea elmeri
Kayea stylosa
Poeciloneuron indicum
Mammea usambarensis
Mammea americana
Mammea sessiliflora
Mammea siamensis
Endodesmia calophylloides
Clusia lanceolata
A
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V

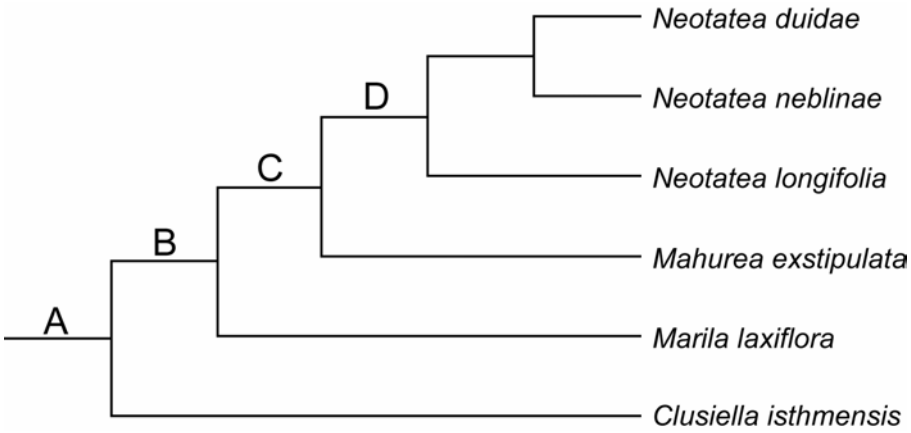

Figure 3-17. Morphological character-state transformations mapped onto one of the most parsimonious trees (tree #1) from Analysis 9 (DNA + morphology with *Neotatea*, pruned) showing the clade containing *Neotatea*. Equivocal, very homoplasious character-state transformations (i.e., 0/1 → 0/1) are not included in this list. See Table 2-1 for a description of characters and character states. **A**: 41(0/1→1), 42(0/1→0), 54(0/1→0), 55(1→0), 56(1→0), 57(1→0); **B**: 4(0→1), 11(1→2), 24(1→0), 50(2→0), 67(1→2); **C**: 6(0→1), 32(1→0), 40(0→1), 51(0→3); **D**: 7(0/1→1), 11(2→0), 12(1→0), 13(1→0), 14(0→1), 17(1→3), 19(0→1), 22(1→2), 33(0→2), 59(0→2), 63(0/1→0), 64(0→1), 67(2→0), 70(0→1), 74(0/1→0).

Table 3-1. Bootstrap support values by parsimony analysis

	Bootstrap Support					
Clade	*rbcL*	*matK*	*matK* + *rbcL*	ITS	ITS + *matK* + *rbcL*, unpruned	ITS + *matK* + *rbcL*, pruned
Kielmeyeroideae	75	100	100	NA	—	NA
Calophylleae	95	98	100	—	100	—
Mammea + rest of Calophylleae	57	52	78	—	—	—
Kayea	91	NA	93	100	99	NA
Kayea + *Poeciloneuron*	—	—	—	60	72	70
Mammea	88	99	100	99	100	100
Marila	—	—	—	85	88	97
Marila + *Mahurea* + *Clusiella*	—	—	—	—	—	—
Neotatea + *Mahurea* + *Marila* + *Clusiella*	NA	NA	NA	NA	NA	NA
Calophyllum	—	—	—	99	100	99
Mesua + *Calophyllum*	—	64	86	—	84	84
Haploclathra	NA	NA	NA	100	100	NA
Kielmeyera	53	97	96	69	98	98
Caraipa	—	52	74	95	98	95
Caraipa + *Haploclathra*	—	—	—	—	—	—
Haploclathra + *Kielmeyera* + *Caraipa*	—	57	71	86	100	92

Table 3-1. Continued.

	Bootstrap Support				
Clade	morpho.	DNA + morpho w/o *Neotatea,* unpruned	DNA + morpho w/o *Neotatea,* pruned	DNA + morpho w/ *Neotatea,* unpruned	DNA + morpho w/ *Neotatea,* pruned
Kielmeyeroideae	52	—	NA	—	NA
Calophylleae	—	98	—	66	—
Mammea + rest of Calophylleae	—	—	—	—	—
Kayea	87	100	NA	100	NA
Kayea + *Poeciloneuron*	—	75	61	78	—
Mammea	95	100	100	100	100
Marila	96	93	NA	92	NA
Marila + *Mahurea* + *Clusiella*	—	51	67	—	—
Neotatea + *Mahurea* + *Marila* + *Clusiella*	—	NA	NA	—	69
Calophyllum	100	100	100	100	100
Mesua + *Calophyllum*	—	97	97	96	96
Haploclathra	94	100	NA	100	NA
Kielmeyera	75	92	100	92	100
Caraipa	—	96	98	97	95
Caraipa + *Haploclathra*	58	56	—	55	—
Haploclathra + *Kielmeyera* + *Caraipa*	—	91	94	92	93

Bootstrap support generally increases with combination of data sets. A dash (—) signifies that the clade is not supported by a bootstrap value >50%.

CHAPTER 4
DISCUSSION

Analytical Issues

The topologies from each data set analyzed separately did not generally conflict with each other. The only conflict involves the position of *Mammea* as supported by morphology compared to *rbcL* and *matK* nucleotide sequences. Morphology alone (Figure 3-8) places *Mammea* in a clade with *Calophyllum*, *Mesua*, and *Kayea*, while the analyses using *rbcL* alone (Figures 3-1 through 3-3) and *matK* alone (results not shown) place *Mammea* as sister to the rest of Calophylleae (i.e., all Kielmeyeroideae except *Endodesmia*). In each case, the position of *Mammea* in the separate analyses is not well-supported.

Combining the three gene regions into one data set increased internal support (Table 3-1) and decreased the resulting number of most parsimonious trees. For example, *Kayea* and *Poeciloneuron* are sisters with 72% bootstrap support in the parsimony unpruned combined DNA analysis (Figure 3-5), whereas this clade does not receive more than 60% bootstrap support in any of the separate analyses (Figures 3-1 and 3-4). *Marila* does not receive bootstrap support >50% in the *rbcL* and *matK* separate analyses and receives 85% support in the ITS analysis; in the unpruned combined DNA analysis, *Marila* is supported with 89% bootstrap support. Furthermore, the clade of *Kielmeyera* + *Caraipa* + *Haploclathra* is supported by 95% bootstrap support in the unpruned combined DNA analysis, whereas this clade does not receive support greater than 86% in the separate analyses. While the sister-group relationship of *Mammea* to the

rest of Calophylleae is not supported in the combined DNA analyses, it is supported by 78% bootstrap support in the *rbcL* + *matK* analysis (Figure 3-3). The total evidence approach (i.e., combining all data sets into a single matrix for analysis) was also shown to have positive results in the large data set analyses of Soltis et al. (1998) and Chase et al. (1998).

The Value of Morphology

The value of morphology in phylogenetic studies has recently been questioned by Scotland et al. (2003). These authors argue that one of the major constraints in using morphology involves the limited number of unambiguous characters provided by this time-consuming approach. It is interesting to note that in this study, morphology alone provides 64 parsimony-informative characters, while the *matK* region used (488 bp) provides only 55 parsimony-informative characters and *rbcL* (1408 bp, of Kielmeyeroideae) provides 66 parsimony-informative characters. The number of informative characters provided by including 809 bp of ITS is much greater (232) than for the other data sets, but the homoplasy index is also higher for ITS (0.416, compared to 0.205 for *rbcL* and 0.116 for *matK*). Of the four separate data sets (*rbcL*, *matK*, ITS, and morphology), the morphological characters had the highest homoplasy index (0.518). Despite the greater amount of homoplasy in the morphological characters, combining morphology with the three molecular data sets (Figures 3-9 through 3-12) results in values of support equivalent to those in the combined DNA analyses (Table 3-1). In some cases, bootstrap support is higher when morphology is added. For example, a clade containing *Clusiella*, *Mahurea*, and *Marila*, present in the strict consensus of the combined molecular data set (but without bootstrap support >50%), receives bootstrap support of 51% (in the unpruned analysis) and 67% (in the pruned analysis) with the

addition of morphology to the combined molecular data set (Figures 3-9 and 3-10). The sister-group relationship of *Calophyllum* and *Mesua* also receives higher support (97% vs. 84%) with the addition of morphology to the combined DNA analyses (Figures 3-9 and 3-10). In the analysis that included only morphological characters, every genus except for *Caraipa* (which was placed in a clade with *Haploclathra*) appeared monophyletic.

Scotland et al. (2003) stated that "much of the useful morphological diversity has already been scrutinized." This statement, which discourages morphological phylogenetics, is misleading. While it may be true that many of the morphological attributes of an organism have been observed at some earlier point in the taxonomic history of the organism, these characters may not have been applied in a phylogenetic context. Characters that have been stressed in historical classifications do not always turn out to be the appropriate (i.e., accurate indicators of phylogenetic relationships). The historical classification of *Clusiella* illustrates this point. Characters that had "already been scrutinized" and stressed in Engler's (1925) classification of *Clusiella* (resiniferous staminodes, cupuliform stigmas, epiphytic habit) were characters that incorrectly placed the genus near *Clusia*. Only when numerous morphological characters were examined in a phylogenetic context did the taxonomic placement of *Clusiella* within Kielmeyeroideae become clear. For example, the presence of stipuliform structures, resin cavities, anther glands, seeds without an aril, and an embryo with large cotyledons support this placement.

Scotland et al. (2003) did support the systematic use of anatomical characters, after the necessary rigorous and critical studies. The present investigation does show that

anatomical characters can be phylogenetically useful (i.e., presence/absence of transcurrent lateral bundles, position of resin canals relative to vascular bundles, presence/absence of fibers adaxial to the midrib bundle, and presence/absence of fibers partitioning the phloem). Through observations of just leaf and petiole anatomy, 16 characters were discovered, and 13 of these are phylogenetically informative. But why only study the anatomy of "few morphological characters," as Scotland et al. suggest? If the anatomy of other parts of the plant (i.e., stems, roots, flowers, fruits, etc.) were observed, it seems likely that many additional, phylogenetically useful, anatomical characters could have been found.

In a response to the Scotland et al. (2003), Jenner (2004) stated that, in the case of well-studied seed plants, the assertion that much of the morphological diversity has already been scrutinized may be true. The present study provides evidence that, not even in the case of seed plants, is this statement valid. Several morphological characters, which had never before been used phylogenetically in a published work, were shown to be phylogenetically informative.

The use of morphology in the phylogenetic analysis of Kielmeyeroideae allowed the inclusion of *Neotatea*, a genus endemic to the tepuis of northern South America. DNA could not be obtained from *Neotatea* because the herbarium specimens were originally collected in alcohol. Because only morphological characters could be used to infer its phylogenetic relationships, including *Neotatea* in the analysis is similar to including a fossil taxon (Gandolfo, 2002; Hermsen et al., 2003). Although its position does not receive strong support (Figure 3-12), the placement of *Neotatea* as the sister group to *Mahurea*, in a clade with *Clusiella* and *Marila*, is logical based on seed and fruit

characters (Figure 3-14) and is a good hypothesis of relationships until molecular data for *Neotatea* can be acquired.

The inclusion of morphological data also allowed clear comparison of phylogenetic results with traditional classifications of Kielmeyeroideae and determination of putative morphological synapomorphies for the genera of Kielmeyeroideae (see below and Results).

Taxonomic History

The taxonomic placement of several genera of Kielmeyeroideae has been a source of confusion throughout the taxonomic history of this group. The genera that are primarily South American in distribution have been especially problematic. While Engler's (1925) treatment of Clusiaceae included *Kielmeyera*, *Mahurea*, *Marila*, *Caraipa*, and *Haploclathra* (in Kielmeyeroideae), other workers had different placements for these genera. Hutchinson (1959, 1969) placed *Kielmeyera*, *Marila,* and *Caraipa* in Bonnetiaceae, while Maguire (1972) also included *Haploclathra*, *Mahurea,* and *Neotatea*, but not *Marila*, in Bonnetiaceae. Kubitzki (1978) included *Caraipa* and *Mahurea* in Bonnetiaceae and Field (1978) placed *Haploclathra* in Theaceae, which he included as part of Bonnetiaceae. Cronquist (1981) tentatively placed *Kielmeyera* and *Neotatea* within Bonnetioideae (Theaceae), stating that although these genera have alternate leaves like Bonnetioideae and other Theaceae, they have the resin ducts and anatomy of Clusiaceae. In the morphological cladistic analysis of Clusiaceae-Bonnetiaceae-Elatinaceae of Stevens (unpubl.), *Marila*, *Caraipa*, *Kielmeyera*, *Haploclathra*, *Neotatea*, and *Mahurea* appear as basal branches within Kielmeyeroideae (Clusiaceae). The *rbcL*-based phylogeny of Clusiaceae by Gustafsson et al. (2002) confirms the placement of *Marila*, *Mahurea*, *Kielmeyera*, and *Caraipa* within

Kielmeyeroideae, but does not support a basal position of these genera. The results presented here, based on *rbcL* sequences across Clusiaceae, including a larger sampling of Kielmeyeroideae (Figures 3-1 through 3-3), confirm the placement of *Marila*, *Mahurea*, *Neotatea*, *Kielmeyera*, *Caraipa*, and *Haploclathra* within Kielmeyeroideae. These New World genera, in addition to *Clusiella*, likely form one of three main clades within Calophylleae (Figures 3-2, 3-3, 3-8, 3-9, 3-12, 3-13, and 3-15).

The taxonomic placement of *Clusiella* has also been a cause of debate. When first described, Planchon and Triana (1860) placed *Clusiella* in their tribe Clusieae based on its sessile, cupuliform stigmas and non-ascendent ovules. Engler (1925) also believed that *Clusiella* belonged in Clusioideae, and placed the genus next to *Clusia*. Besides stigma shape and ovule orientation, *Clusiella* and *Clusia* are similar in having an epiphytic habit, resiniferous androecium, and dioecious habit. Hammel (1999) noted that *Clusiella* has many characteristics (i.e., stipuliform structures, bud scales, resin cavities in the leaf, psilate pollen exine, baccate fruits, small foveolate seeds without an aril, and an embryo with large cotyledons) that are not common in clusioid genera, and thus he questioned its placement in Clusioideae. Stevens (in press) placed *Clusiella* in its own tribe, Clusielleae, sister to the rest of Clusioideae. In the *rbcL*-based phylogeny of Gustafsson et al. (2002), *Clusiella* appears within a well-supported Kielmeyeroideae, but its relationship to other genera of the subfamily is not supported. In the separate molecular parsimony analyses of the present study (Figures 3-1 and 3-6), *Clusiella*'s position within Kielmeyeroideae is unresolved. In the combined molecular DNA analyses, *Clusiella* appears in a clade with Mahurea and Marila, but without bootstrap support >50% (Figure 3-8). The Bayesian analysis of *rbcL* data across Clusiaceae

(Figure 3-2) weakly supports a clade of *Mahurea*, *Clusiella*, and *Marila* with a posterior probability of 72. In the maximum likelihood analysis of *rbcL* (Figure 3-3) and *rbcL* + *matK* + ITS (results not shown), *Clusiella* appears in a clade with *Marila* and *Mahurea*. When the morphological characters are added to the combined molecular data set (analyses 7-10), *Clusiella* appears with weak to moderate bootstrap support in a clade with *Marila* and *Mahurea* (Figures 3-12 and 3-13), or with *Marila*, *Mahurea*, and *Neotatea,* in the pruned analysis that includes *Neotatea* (Figure 3-15). This clade is diagnosed by several seed and fruit characters, such as an unbranched or braided raphal bundle in the testa, the absence of an exotegmen, presence of endosperm in ripe seeds, a small embryo, and gynoecia having more than 15 ovules per carpel.

Based on its unisexual flowers, drupaceous fruit, and large embryo, Robson (1978) included *Mammea* in Clusioideae, tribe Garcinieae. The large embryos of Garcinieae and *Mammea* are only superficially similar; the embryo of *Garcinia* is composed almost entirely of a swollen hypocotyl, while that of *Mammea* is mainly two immense cotyledons (Stevens, 1980). *Mammea* was properly placed in Kielmeyeroideae by Takhtajan (1997) and Stevens (in press). In the morphological cladistic analysis of Stevens (unpubl.), *Mammea* appears in a clade with other primarily Old World genera (e.g., *Calophyllum*, *Mesua*, *Kayea*, and *Poeciloneuron*). *Mammea*, *Calophyllum*, *Mesua*, *Kayea*, and *Poeciloneuron* all have basal placentation and usually two or four carpels. In my analysis based solely on morphological characters (Figure 3-8), *Mammea* appears in a clade with *Calophyllum*, *Mesua*, and *Kayea*. *Mammea* appears distinct from these genera (and sister to all other Calophylleae) in the *rbcL* phylogeny of Gustafsson et al. (2002), although without bootstrap support. With an increased taxon sampling of *Mammea* and

other Kielmeyeroideae, this relationship still does not receive support greater than 50% in the parsimony analysis of *rbcL* sequences (Figure 3-1). The Bayesian analysis of *rbcL* data (Figure 3-2) places *Mammea* sister to the remaining Calophylleae with a posterior probability of 71. By adding an additional gene region, the sister-group relationship of *Mammea* + remaining Calophylleae receives stronger bootstrap support (Analysis 2, *rbcL* + *matK*; Figures 3-4 and 3-5). The Bayesian analysis of the *rbcL* + *matK* data set supports the sister group relationship of *Mammea* + remaining Calophylleae with a posterior probability of 92 (results not shown). In the combined DNA + morphology analyses (Figures 3-9 through 3-12), the position of *Mammea* is again unresolved.

The position of Endodesmieae (which includes two monotypic genera, *Endodesmia* and *Lebrunia*) within Clusiaceae has been questioned due to the morphological similarities of the genera of Endodesmieae to both Clusioideae and Kielmeyeroideae. Vegetatively, Endodesmieae are similar to Clusioideae in that they are glabrous, possess resin canals, and have lateral vascular bundles in the leaf blade that are not transcurrent; however, the presence of large seeds with large cotyledons allies Endodesmieae with Kielmeyeroideae. Stevens (in press) tentatively placed Endodesmieae sister to Calophylleae within Kielmeyeroideae. For the first time, *Endodesmia* is included here in a molecular phylogenetic analysis, and its placement is confirmed. In both Analysis 1 (*rbcL*; Figures 3-1 through 3-3) and Analysis 2 (*rbcL* + *matK*; Figures 3-4 and 3-5), *Endodesmia* appears sister to the remaining Kielmeyeroideae, the Calophylleae, with high support.

In the *rbcL* phylogeny of Gustafsson et al. (2002), *Kayea stylosa* and *Mesua* sp. appear as sister taxa; this is likely an artifact of a misidentification of their "*Mesua* sp.,"

which is probably a species of *Kayea*. *Kayea* has been included in *Mesua* in the past (Kostermans, 1969), but was properly separated from *Mesua* by Bentham (1863) and Anderson (1874) based on stigma shape and ovary type. *Kayea* has four narrow stigma lobes while *Mesua* has two broad stigma lobes. The ovary of *Kayea* is composed of a single locule (but is four-carpellate) and contains four or more ovules. *Mesua* has a two-loculate (two-carpellate) ovary with four ovules. Despite its history of being treated as closely related to *Mesua*, *Kayea* is actually more closely related to the Western Ghats endemic *Poeciloneuron*, while *Mesua* is the sister to *Calophyllum* (Figures 3-1 through 3-10 and 3-12 through 3-15). *Kayea* and *Poeciloneuron* both have narrow stigmas, and *Mesua* and *Calophyllum* have broad stigma lobes.

Character Evolution

One of the probable synapomorphies for the Clusiaceae is the presence of resin or other exudates in secretory canals or cavities (Stevens, 2001; Judd et al., 2002). Determining which of these (i.e., canals or cavities) is the ancestral condition cannot be answered until we have an understanding of subfamilial relationships within Clusiaceae. In the strict consensus of the *rbcL* family-level parsimony analysis (Figure 3-1), Kielmeyeroideae are sister to a Clusioideae + Hypericoideae clade, but this relationship receives no bootstrap support. The Bayesian analysis also shows this topology, but the probability is low (Figure 3-2). Within Kielmeyeroideae, if it is assumed that the presence of canals is the ancestral state, it is most parsimonious to assume that canals were lost in the ancestor of Calophylleae and re-evolved in three different lineages: *Neotatea*, *Clusiella*, and *Calophyllum* (Figure 4-1). It is important to note that while the four species of *Mammea* included in my analysis do not have canals, some species (i.e., *M. vatoensis*, *M. nervosa*, and *M. mirabilis*) are reported to have canals (Stevens,

unpubl.), thus possibly making the loss of canals, as assumed by parsimony, occur one node up in the cladogram. Assuming that the absence of resin cavities is the ancestral state, then resin cavities evolved in the ancestor of Kielmeyeroideae and have been lost two separate times, in *Neotatea* and *Calophyllum* (Figure 4-2). It is interesting that *Endodesmia*, which is sister to the rest of Kielmeyeroideae, possesses both resin cavities and canals.

Having opposite leaves is one character that is often given as a diagnostic feature of Clusiaceae, but within Kielmeyeroideae, alternate leaves evolved two or three times. Alternate leaves evolved once in the *Mahurea-Neotatea* clade and once or twice in the *Kielmeyera-Caraipa-Haploclathra* clade (Figure 4-3). The change to alternate leaves could have occurred in the ancestor of *Kielmeyera-Caraipa-Haploclathra* with a reversal to opposite leaves in *Haploclathra* or alternate leaves could have evolved in parallel in *Kielmeyera* and *Caraipa* (Figure 4-3).

Some of the anatomical characters used in this analysis are highly homoplasious (i.e., presence of druse crystals in the petiole); however, despite their homoplasy, many of the anatomical characters varied in a phylogenetically informative manner. The presence of a lignified leaf margin and transcurrent lateral bundles in the leaf blade are two features that likely evolved in the ancestor of Calophylleae and were lost only sporadically within this clade (Figures 4-4 and 4-5). Petiole bundle architecture is another anatomical character that is phylogenetically useful. A petiole bundle with three layers of xylem and phloem is probably a synapomorphy for *Marila*, and a petiole bundle comprised of an arch with dorsal groupings of xylem and phloem is unique to *Haploclathra* (Figure 4-6).

A noteworthy feature of many members of Kielmeyeroideae is the presence of an apical gland on the anthers. The shape of the anther gland may be spherical to elongate or bowl-shaped (crateriform). The anther glands were described by Stevens (in press) as containing "latex, resin, or other material;" other reports (i.e., Gustafsson et al., 2002) describe the glands as containing "oily fluids." I observed that the anther glands of *Endodesmia* secrete a shiny, resin-like substance; in the anther glands of other taxa, the secretion is not evidently resin-like and may indeed be oils of some kind. Work needs to be done to characterize the substances secreted by these anther glands.

The presence of anther glands may be a synapomorphy for Kielmeyeroideae; however, many members of Hypericoideae also have anther glands (Stevens, in press), but their homology with those of Kielmeyeroideae is uncertain. Although anther glands are present in *Symphonia* (Stevens, unpubl.), most Clusioideae lack anther glands. If we assume the absence of anther glands is ancestral, then anther glands evolved once in the ancestor of Kielmeyeroideae and were lost several times within this subfamily (Figure 4-7). The absence/presence of anther glands may vary intragenerically (i.e., *Mammea*, *Kielmeyera*, and *Kayea*), be consistently absent within a genus (i.e., *Haploclathra*, *Poeciloneuron*, *Mesua*, and *Calophyllum*), or be consistently present within a genus (i.e., *Marila*, *Mahurea*, *Clusiella*, and *Caraipa*).

Little is known about the pollination biology of members of Kielmeyeroideae. Taxa that secrete resin via anther glands (i.e., *Endodesmia* and possibly others) or staminodes (i.e., *Clusiella*) are likely pollinated by resin-collecting bees. The bees use the resin for nest construction (Armbruster, 1984; Oliveira et al., 1996). The resin polymerizes slowly and provides the bees with a waterproof protection and probably

antiviral and antimicrobial activity for their larvae (Oliveira et al., 1996). Anther glands that may contain oils might attract pollinators by fragrance (Ribeiro et al., 1999) or be used in nest construction.

Carpel number varies greatly within Kielmeyeroideae, but the variation is phylogenetically informative (Figure 4-8). Having two carpels is likely the ancestral state in the Calophylleae, and the two-carpellate taxa do not form one clade. *Kayea*, whose closest relatives have two carpels, apparently experienced a doubling of its carpel number to four. The presence of three carpels is a likely synapomorphy for the clade containing *Kielmeyera*, *Caraipa*, *Haploclathra*, *Clusiella*, *Mahurea*, *Neotatea*, and *Marila*. Within this clade, there is a secondary increase in carpel number in *Clusiella*.

Fruit type within Clusiaceae is homoplasious, as it is in many other angiosperm families (Kron et al., 2003; Judd et al., 2002; Wilson et al., 2001). The number of times a fleshy, indehiscent fruit has evolved within Kielmeyeroideae is unclear. Using a DELTRAN optimization and assuming that capsular fruits are ancestral, a fleshy, indehiscent fruit may have evolved five different times in Kielmeyeroideae (Figure 4-9). The fibrous, drupaceous fruits of *Calophyllum* are dispersed by birds, bats, or sometimes water in the case of strand species (Stevens, in press). Most of the fleshy fruits of *Mammea* are dispersed by mammals (Stevens, in press). The seeds of *Clusiella isthmensis* have been found in a fecal sample of a thrush-like manakin (*Schiffornis turdinus*) in Costa Rica (A. Boyle, pers. comm.). Most species of *Kayea* have capsular fruits, but in some species (i.e., *K. elmeri*), the calyx becomes highly accrescent and surrounds the fruit, making it indehiscent. Septifragal capsules evolved twice within Kielmeyeroideae: in *Mesua* and in the *Caraipa-Haploclathra* clade.

Winged seeds have probably evolved three separate times within Kielmeyeroideae (Figure 4-10). By examining their morphology and anatomy, it is clear that all winged seeds in the group are not homologous. The wing on the seeds of *Mahurea* and *Neotatea* does not completely surround the seed, is several cell layers thick, and contains a peripheral vascular bundle. The wing on the seeds of *Kielmeyera* completely surrounds the seed, is two cell layers thick, and does not have any vascular tissue. *Caraipa* and *Haploclathra* have a seed wing that completely surrounds the seed, is several cell layers thick, and does not contain vascular tissue.

The small, dry seeds of *Marila* and the winged seeds of *Mahurea*, *Neotatea*, *Kielmeyera* are wind-dispersed (Stevens, in press). The narrowly winged seeds of *Caraipa* and *Haploclathra* are wind dispersed or possibly water-dispersed. Many species of *Caraipa* and *Haploclathra* live in periodically flooded habitats of the Amazonian region (Kubitzki, 1989; Vasquez, 1993), in which the production of a water-dispersed seed would be advantageous.

Seed and embryo characters are shown to vary in a phylogenetically informative manner. The loss of an exotegmen occurred in the ancestor of Kielmeyeroideae and a reversal to the presence of an exotegmen occurred once within the subfamily, in the *Clusiella-Mahurea-Neotatea-Marila* clade (Figure 4-11). The presence of endosperm in mature seeds shows the same evolutionary pattern: a loss of endosperm occurred in the presumed common ancestor of Kielmeyeroideae and a reversal to presence of endosperm occurred in the *Clusiella-Mahurea-Neotatea-Marila* clade (Figure 4-12). Having an embryo less than four mm long is another synapomorphy for the *Clusiella-Mahurea-Neotatea-Marila* clade; all other members of Kielmeyeroideae have an embryo greater

than four mm in length (Figure 4-13). Cotyledon shape is also a useful character: cordate cotyledons are unique to the clade containing *Kielmeyera*, *Caraipa,* and *Haploclathra* (Figure 4-14).

Biogeography

The ancestral distribution of Kielmeyeroideae is unclear based on my reconstructions (Figure 4-15). *Endodesmia* (along with the other genus of Endodesmieae, *Lebrunia*) is restricted to tropical Africa. *Mammea*, whose center of diversity is in Madagascar (Stevens, unpubl.), also has representatives in Africa and in the Neotropics. According to Stevens (unpubl.), "phenetically primitive" species of *Mammea* exist in both Central America and Africa. Taxon sampling for *Mammea* (a genus of about 70 species) must be increased before its biogeographical pattern becomes apparent. *Kielmeyera*, *Caraipa*, *Haploclathra*, *Clusiella*, *Mahurea*, *Neotatea*, and *Marila* make up an entirely New World clade within Kielmeyeroideae. The other major clade within Kielmeyeroideae, composed of *Kayea*, *Poeciloneuron*, *Mesua*, and *Calophyllum*, is entirely Old World except for some species of *Calophyllum*. *Calophyllum inophyllum* is distributed in both the Neotropics and Paleotropics, while *Calophyllum brasiliense*, along with about ten other species not included in this study, are restricted to the Neotropics. It is clear from Figure 4-15 that *Calophyllum* originated in the Paleotropics, and subsequently spread to the Neotropics.

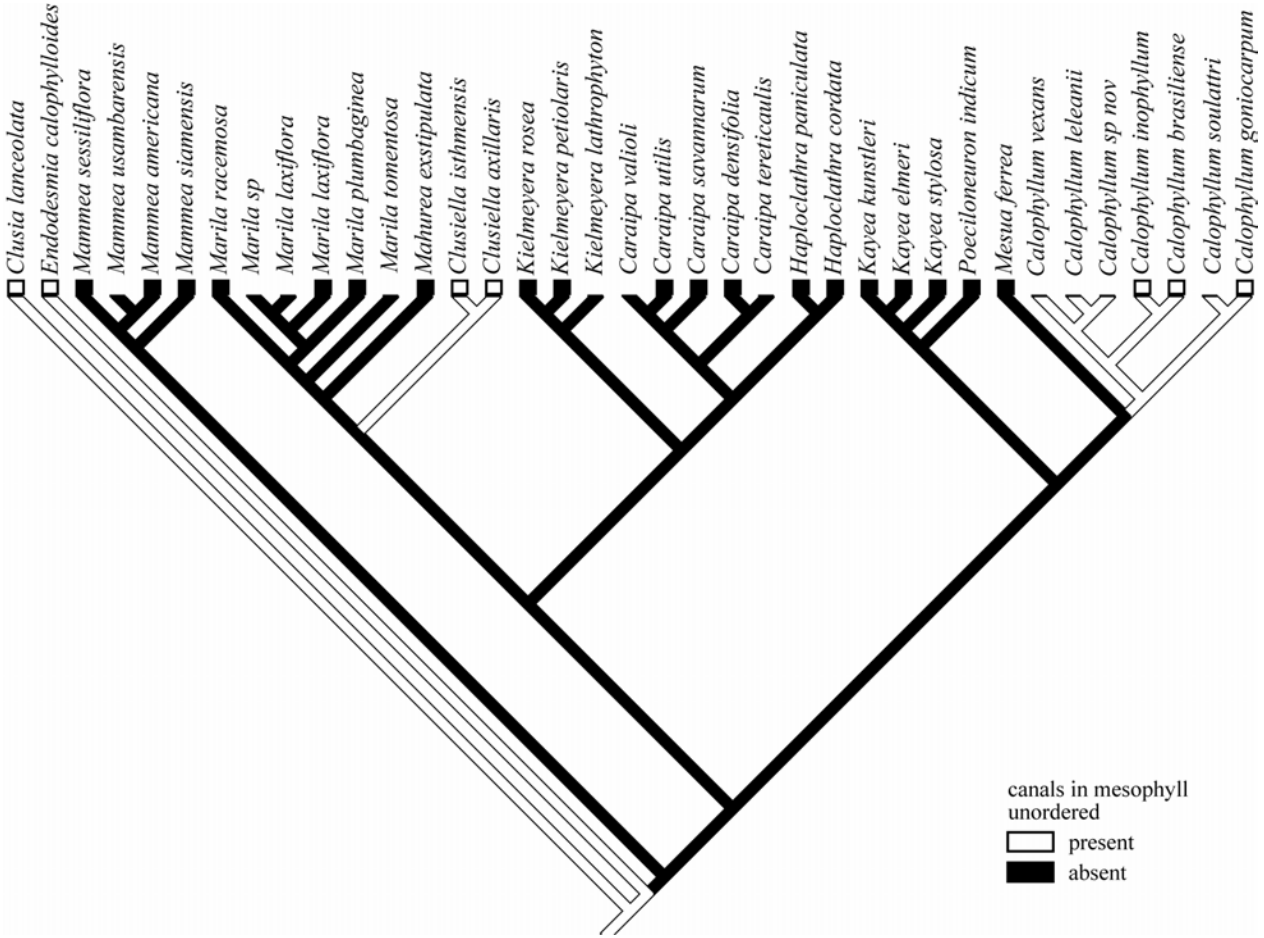

Figure 4-1. Character-state distribution for resin/latex canals in leaf mesophyll within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has canals in the leaf mesophyll. MacClade was set to show all most parsimonious states at each node.

Figure 4-2. Character state distribution for resin/latex cavities in leaf mesophyll within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, does not contain cavities in the mesophyll. MacClade was set to show all most parsimonious states at each node.

Figure 4-3. Character-state distribution for leaf arrangement within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has alternate leaves. MacClade was set to show all most parsimonious states at each node.

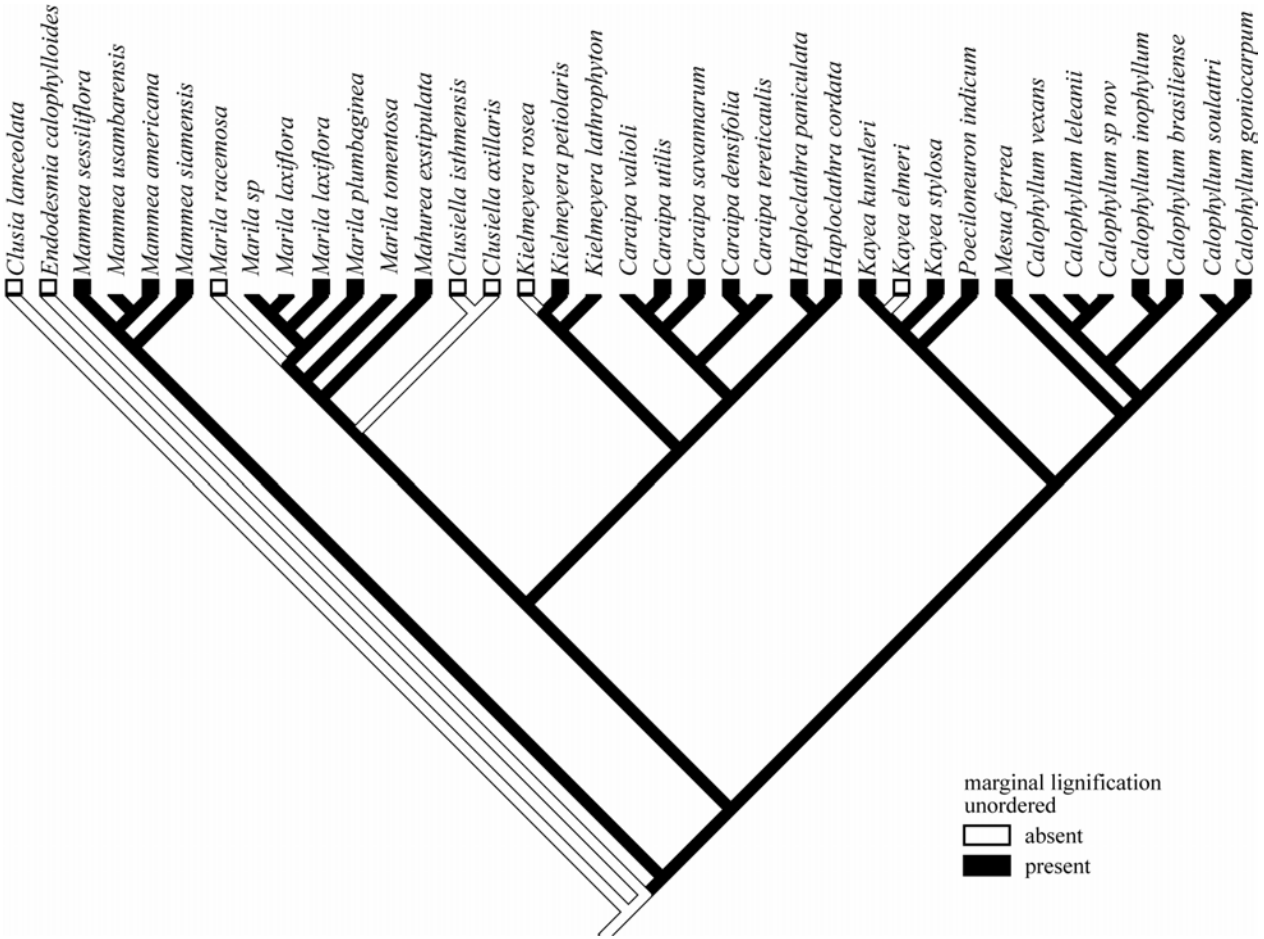

Figure 4-4. Character-state distribution for lignification of the leaf margin within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has lignified margins. MacClade was set to show all most parsimonious states at each node.

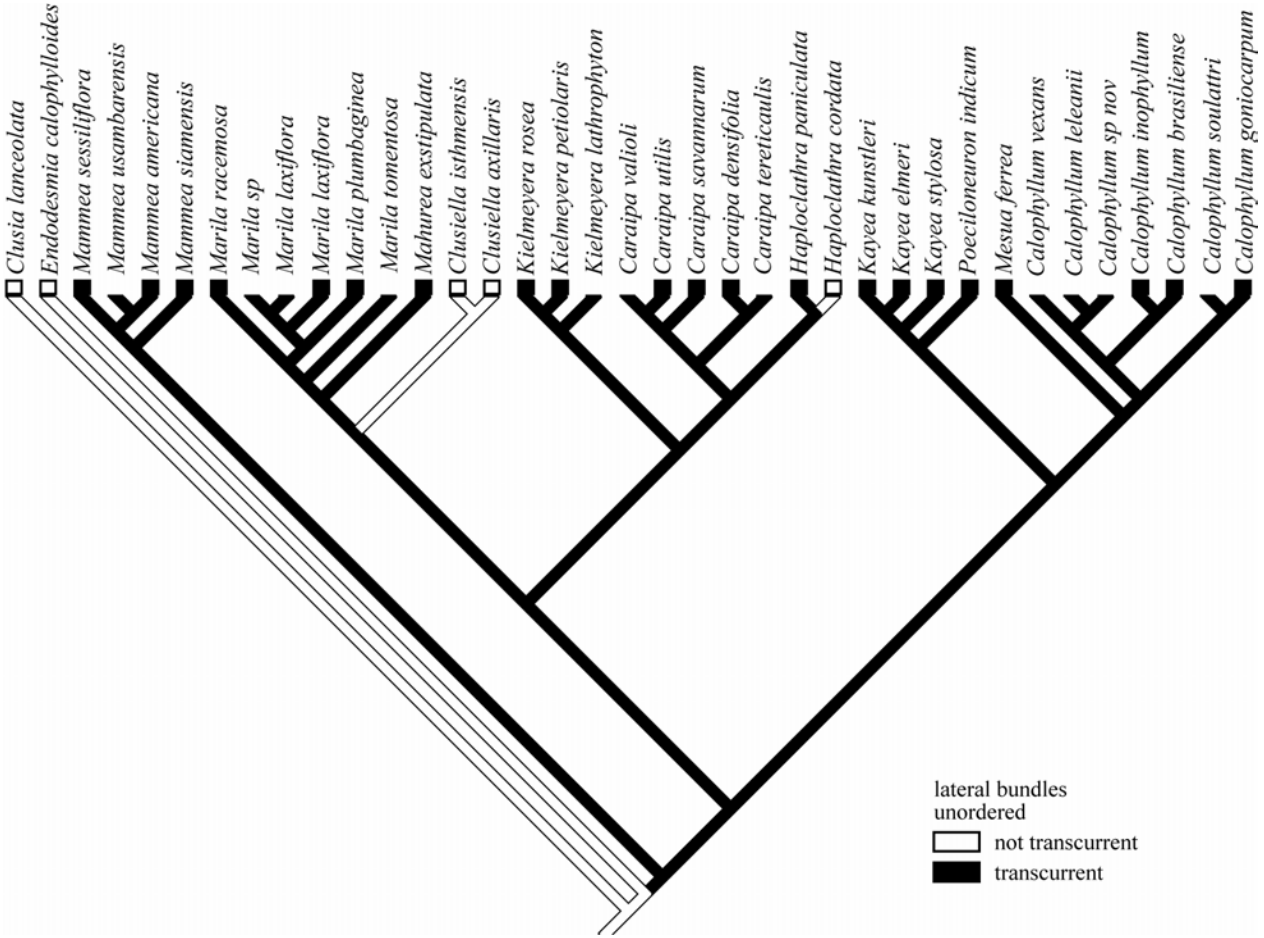

Figure 4-5. Character-state distribution for transcurrent lateral bundles in the leaf blade within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has lateral bundles that are not transcurrent. MacClade was set to show all most parsimonious states at each node.

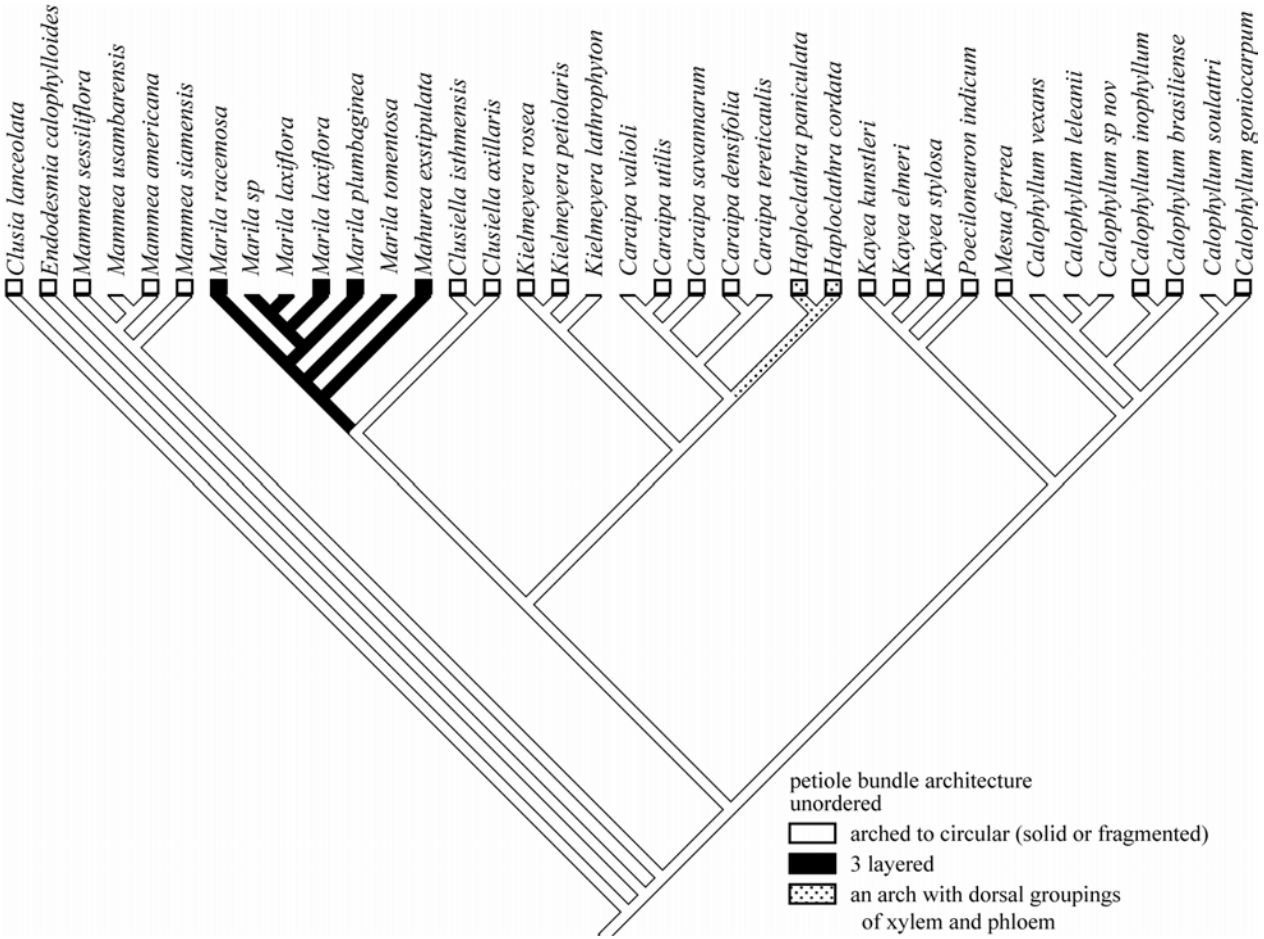

Figure 4-6. Character-state distribution for petiole bundle architecture within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has an arched petiole bundle architecture. MacClade was set to show all most parsimonious states at each node.

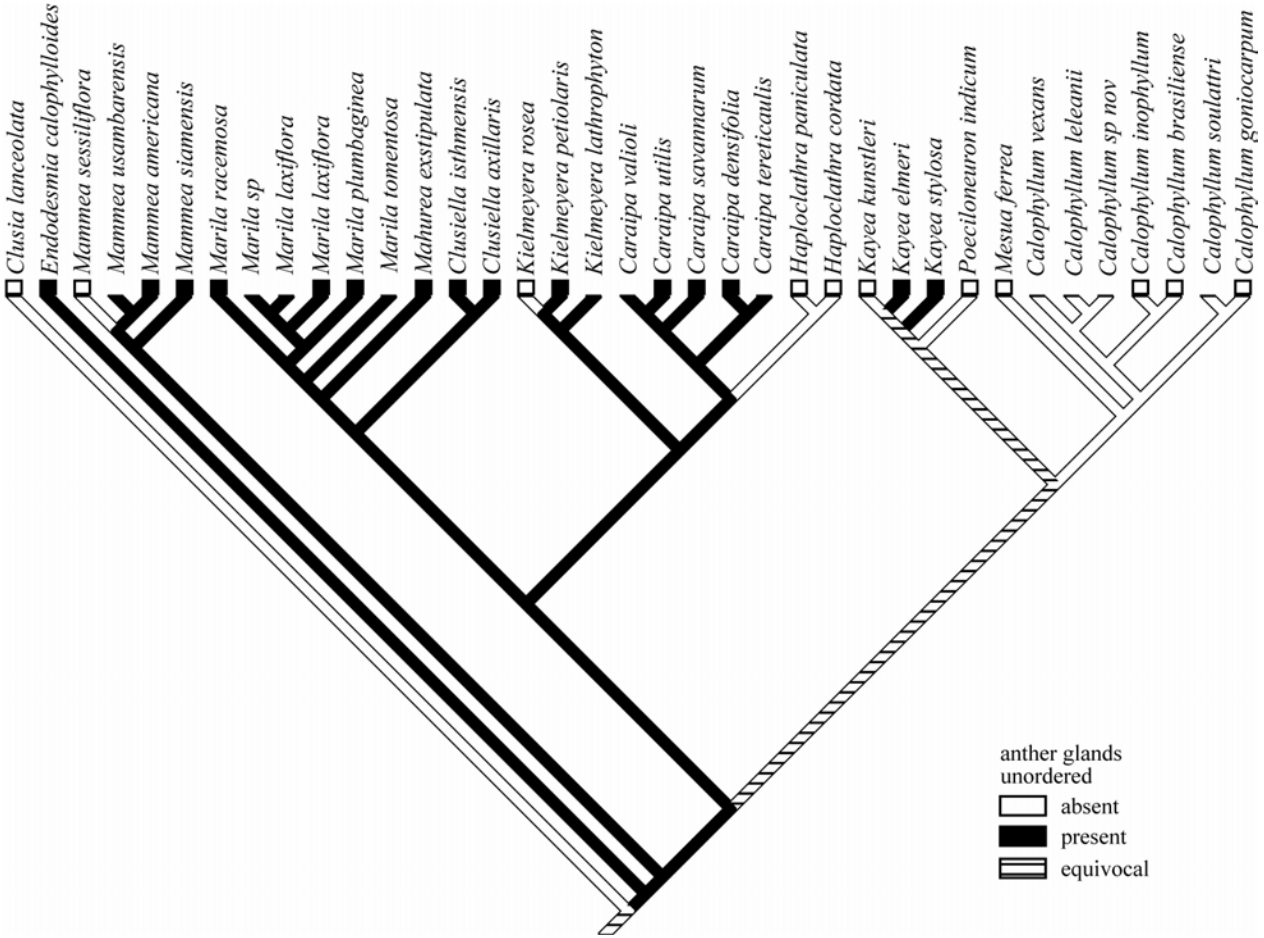

Figure 4-7. Character-state distribution for anther glands within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has anther glands. MacClade was set to show all most parsimonious states at each node.

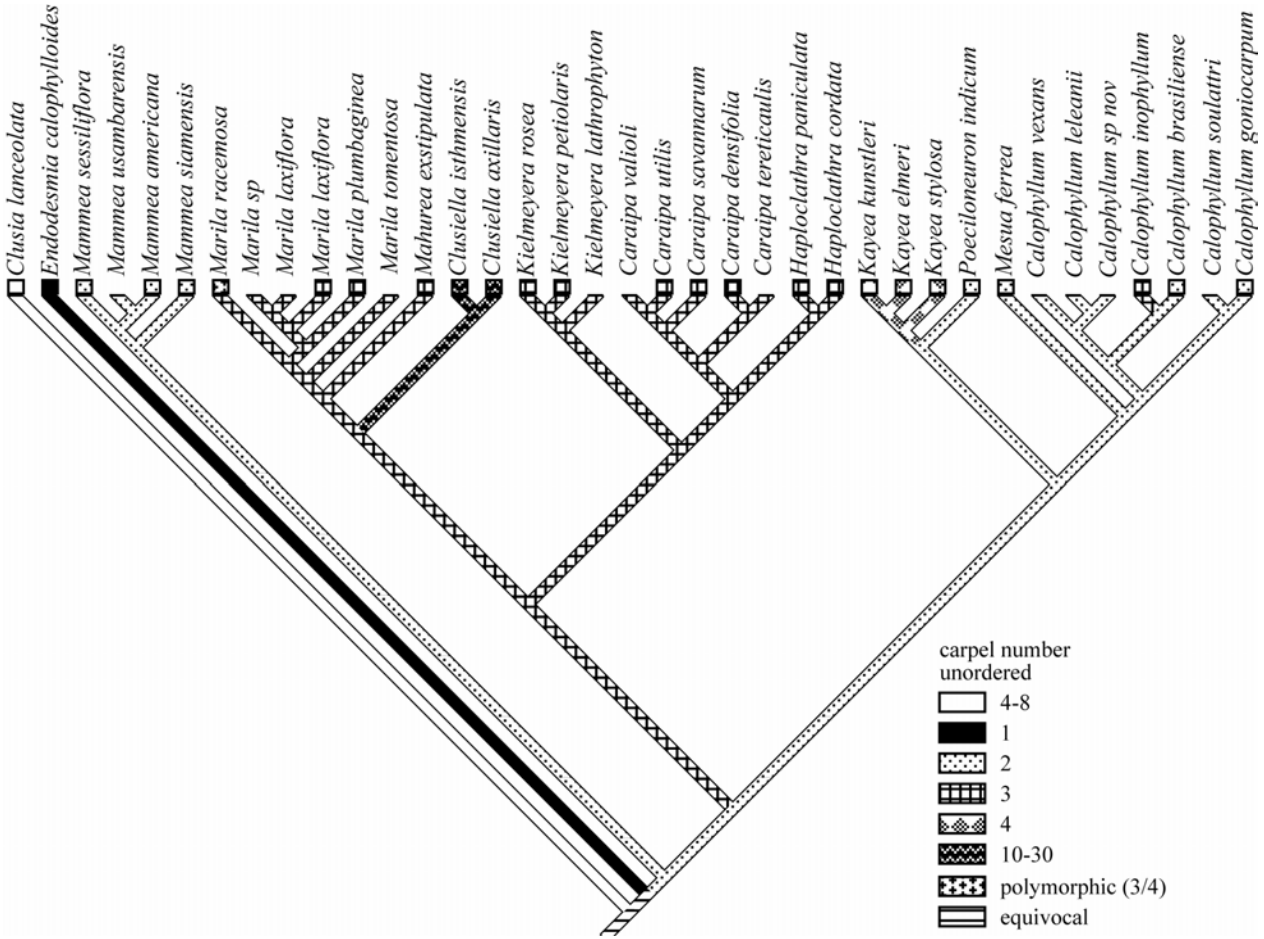

Figure 4-8. Character-state distribution for carpel number within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has three carpels. MacClade was set to show all most parsimonious states at each node.

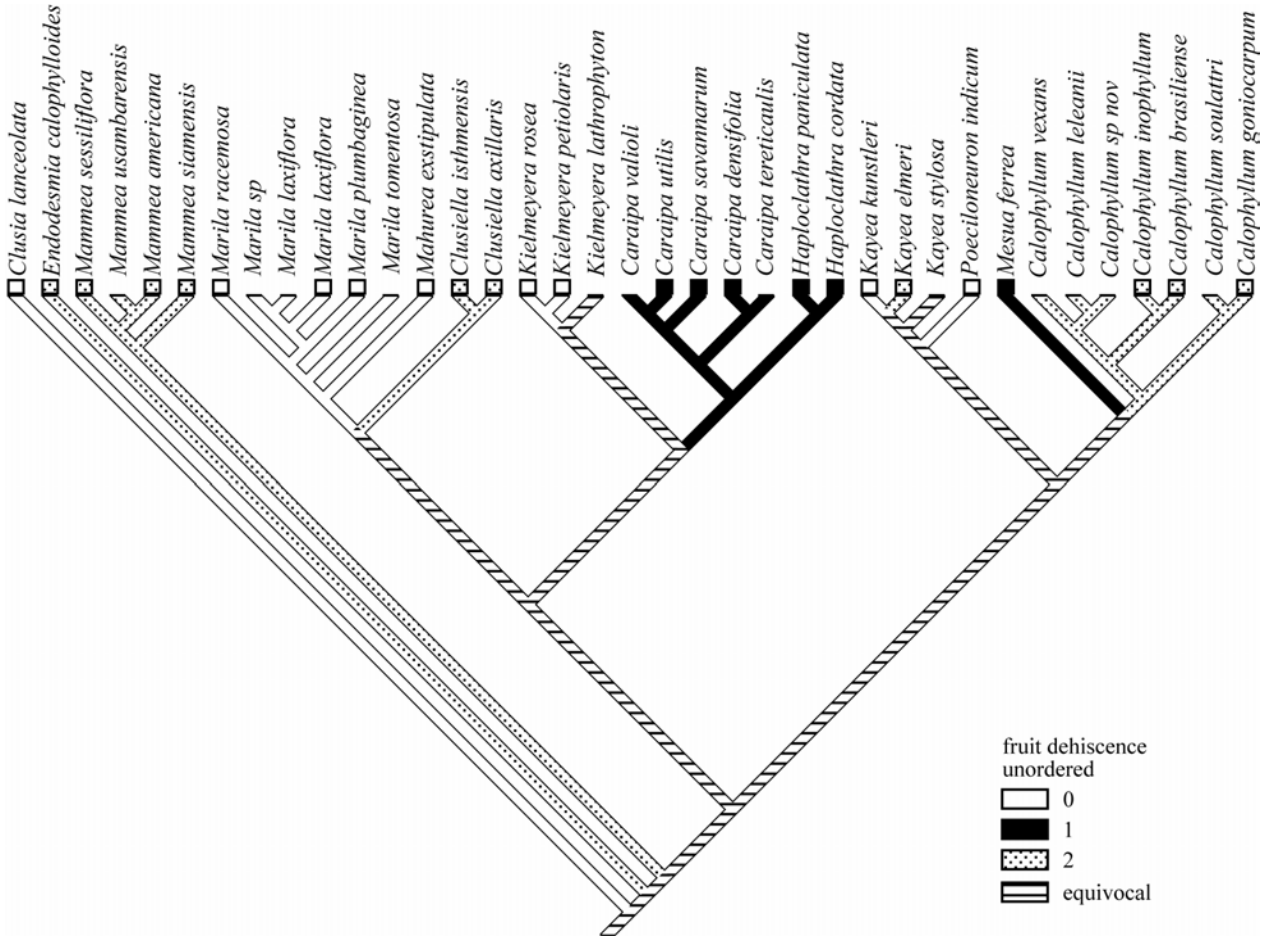

Figure 4-9. Character-state distribution for fruit type within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has a septicidal capsule. MacClade was set to show all most parsimonious states at each node.

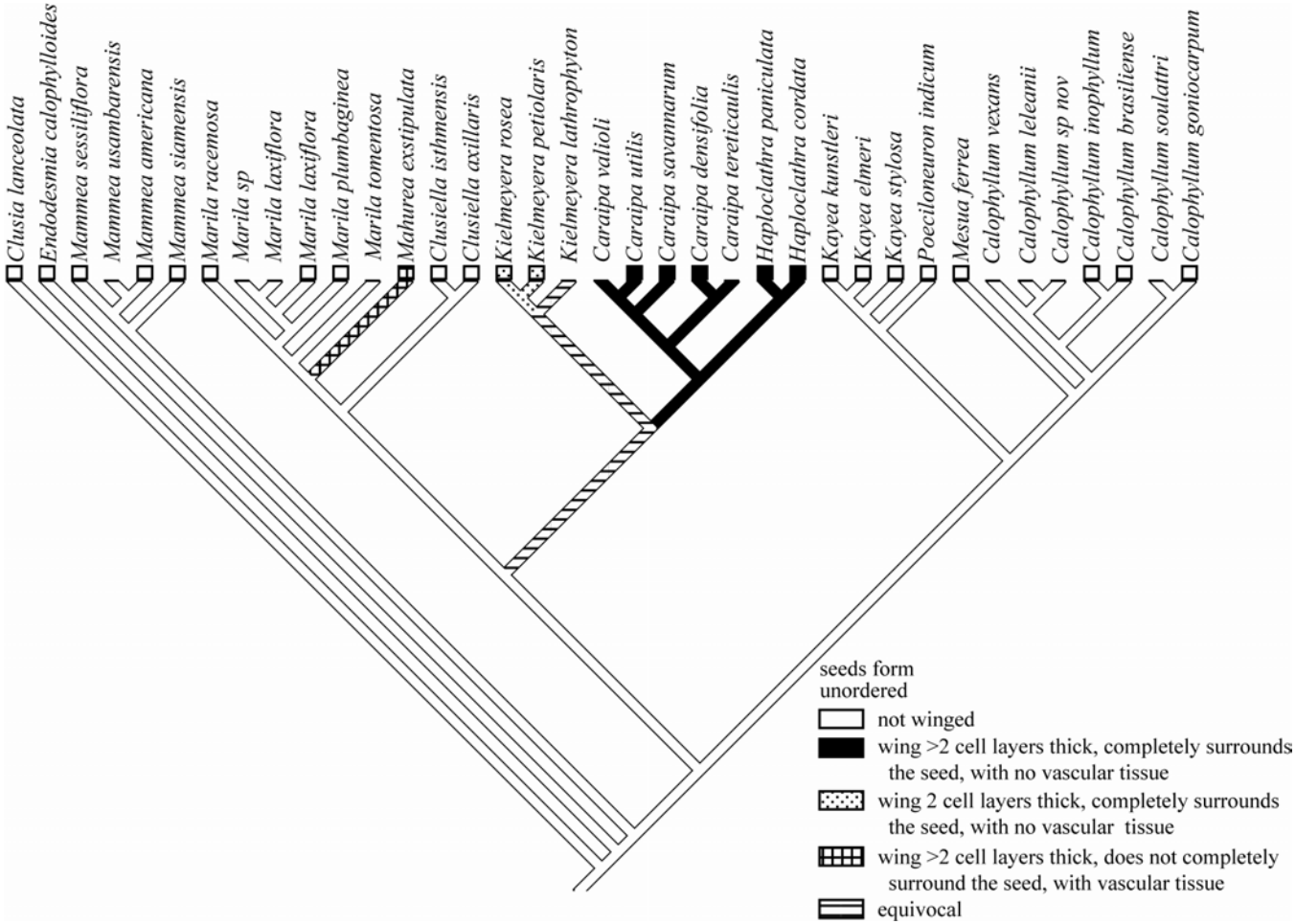

Figure 4-10. Character-state distribution for seed form within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has seeds with a wing like that of *Mahurea*. MacClade was set to show all most parsimonious states at each node.

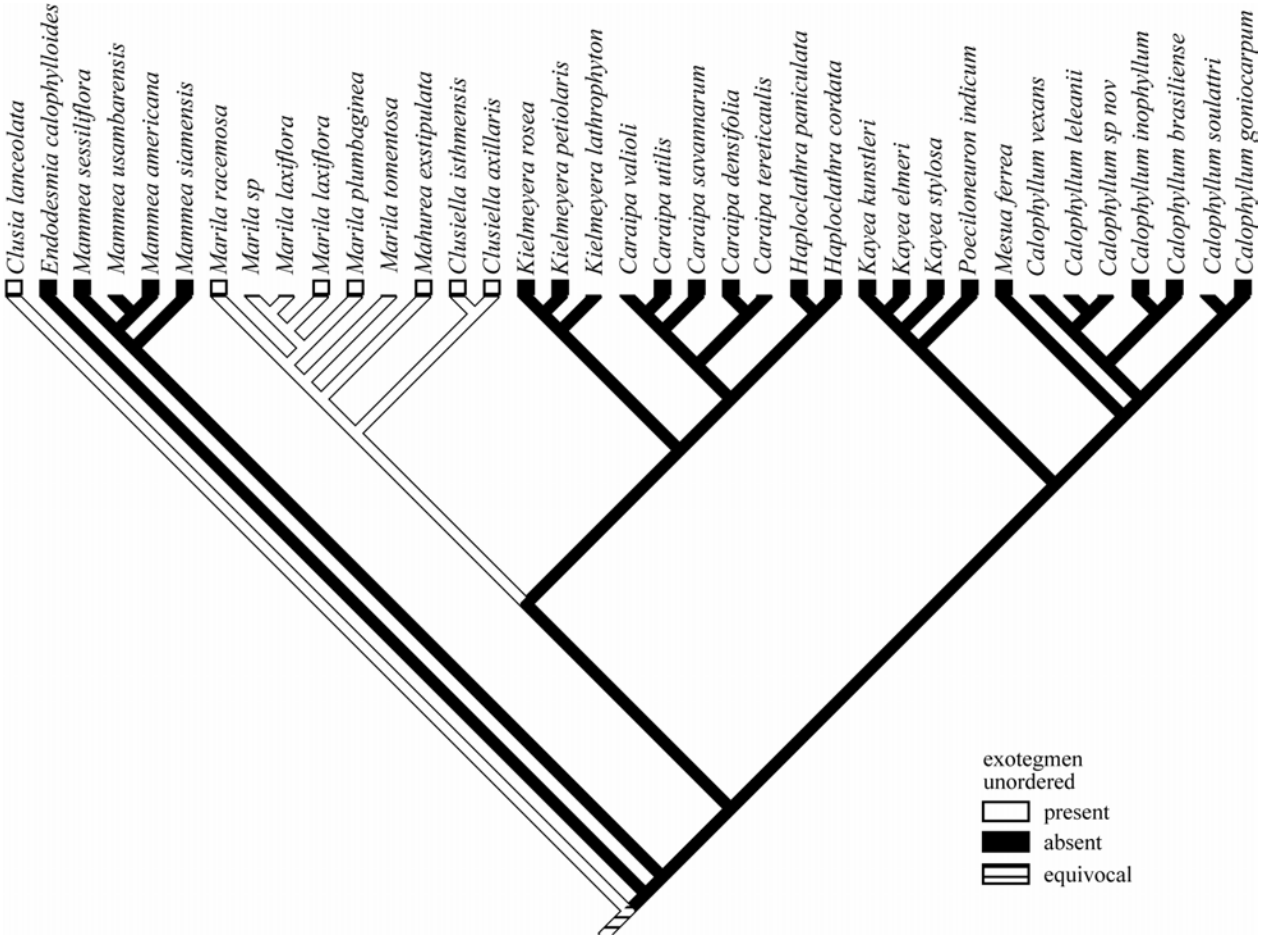

Figure 4-11. Character-state distribution for exotegmen presence within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has an exotegmen. MacClade was set to show all most parsimonious states at each node.

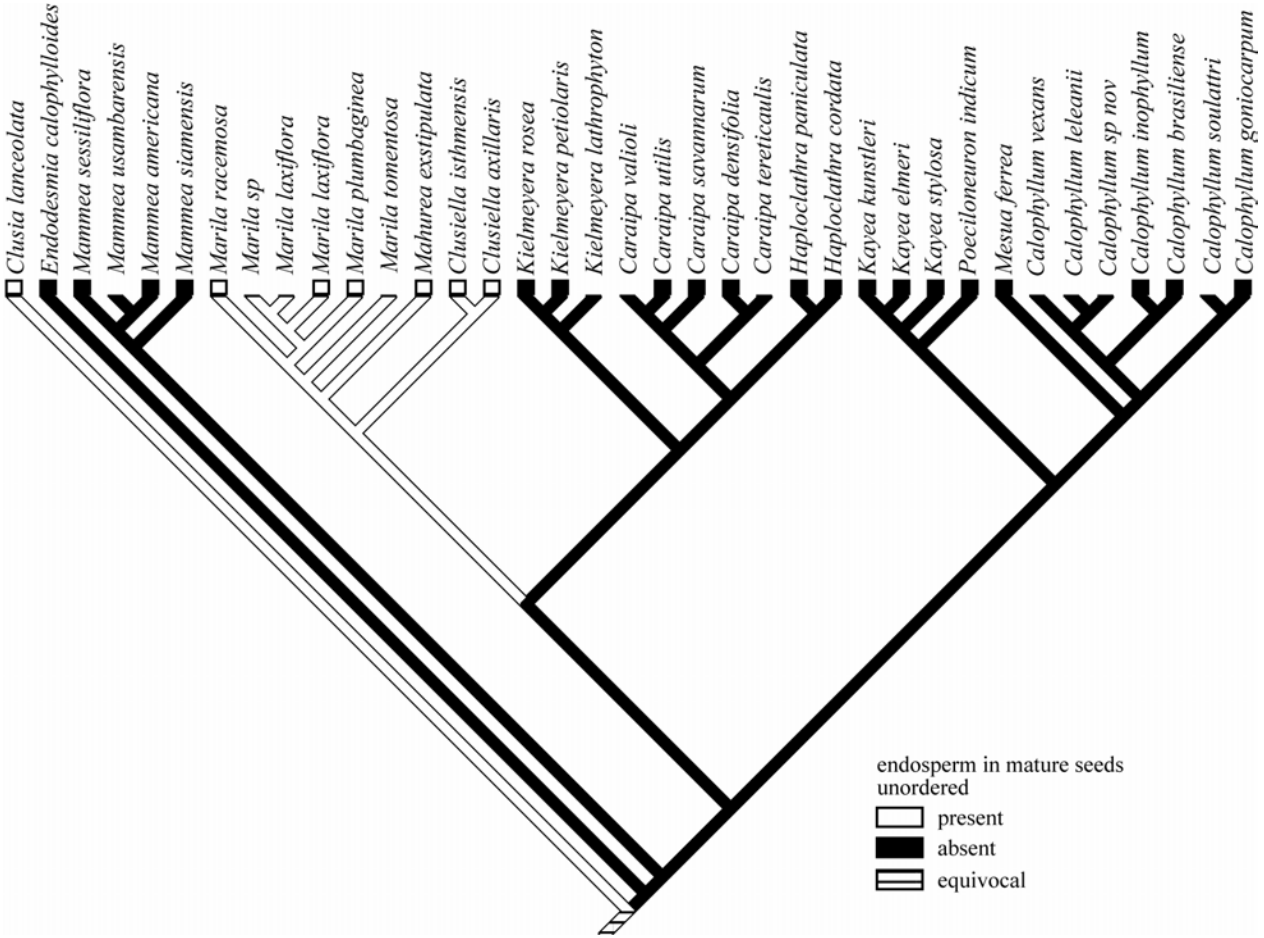

Figure 4-12. Character-state distribution for endosperm in mature seeds within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has endosperm in the ripe seeds. MacClade was set to show all most parsimonious states at each node.

Figure 4-13. Character-state distribution for embryo length within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has an embryo of less than 4 mm long. MacClade was set to show all most parsimonious states at each node.

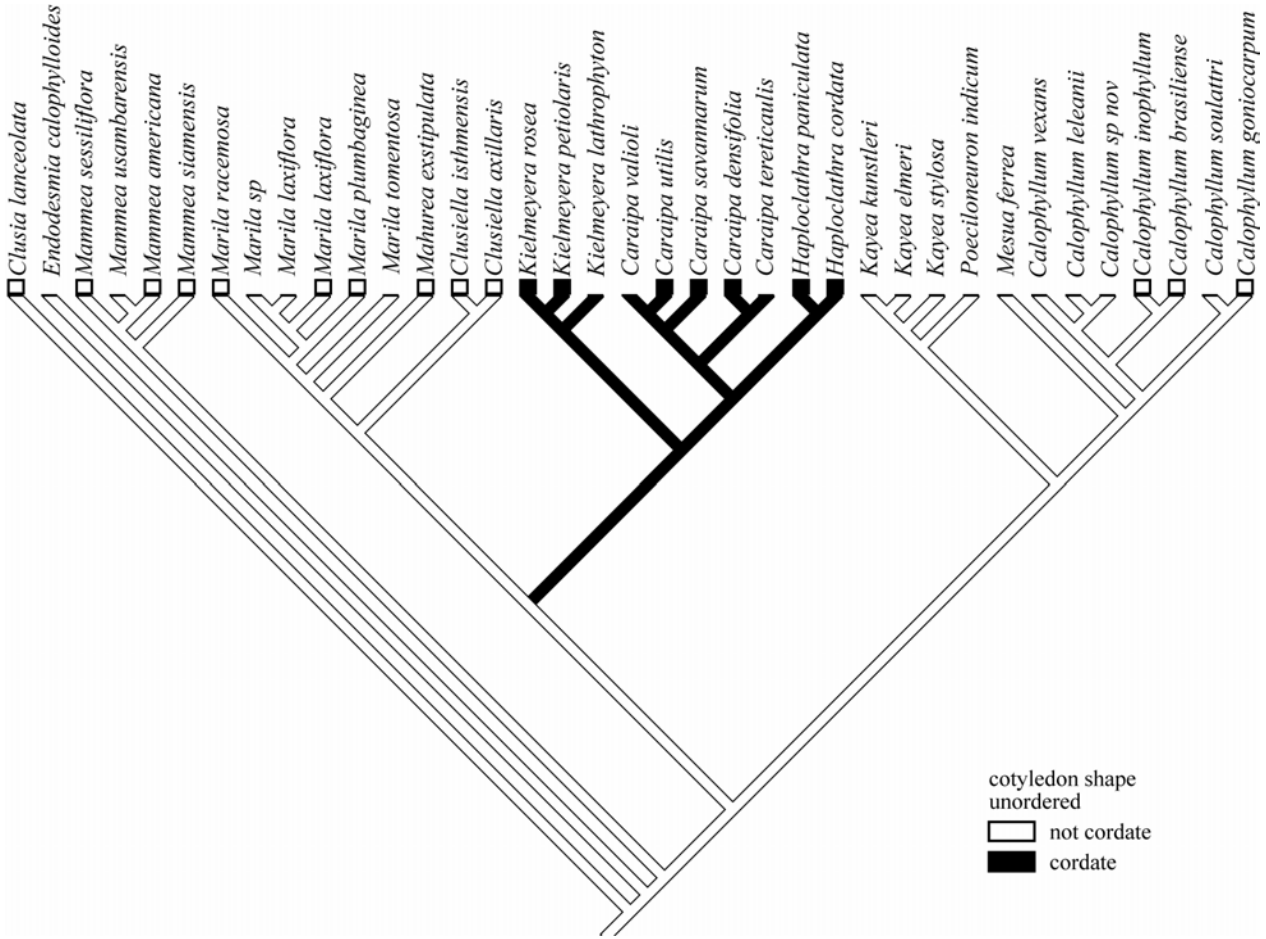

Figure 4-14. Character-state distribution for cordate cotyledons within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, has non-cordate cotyledons. MacClade was set to show all most parsimonious states at each node.

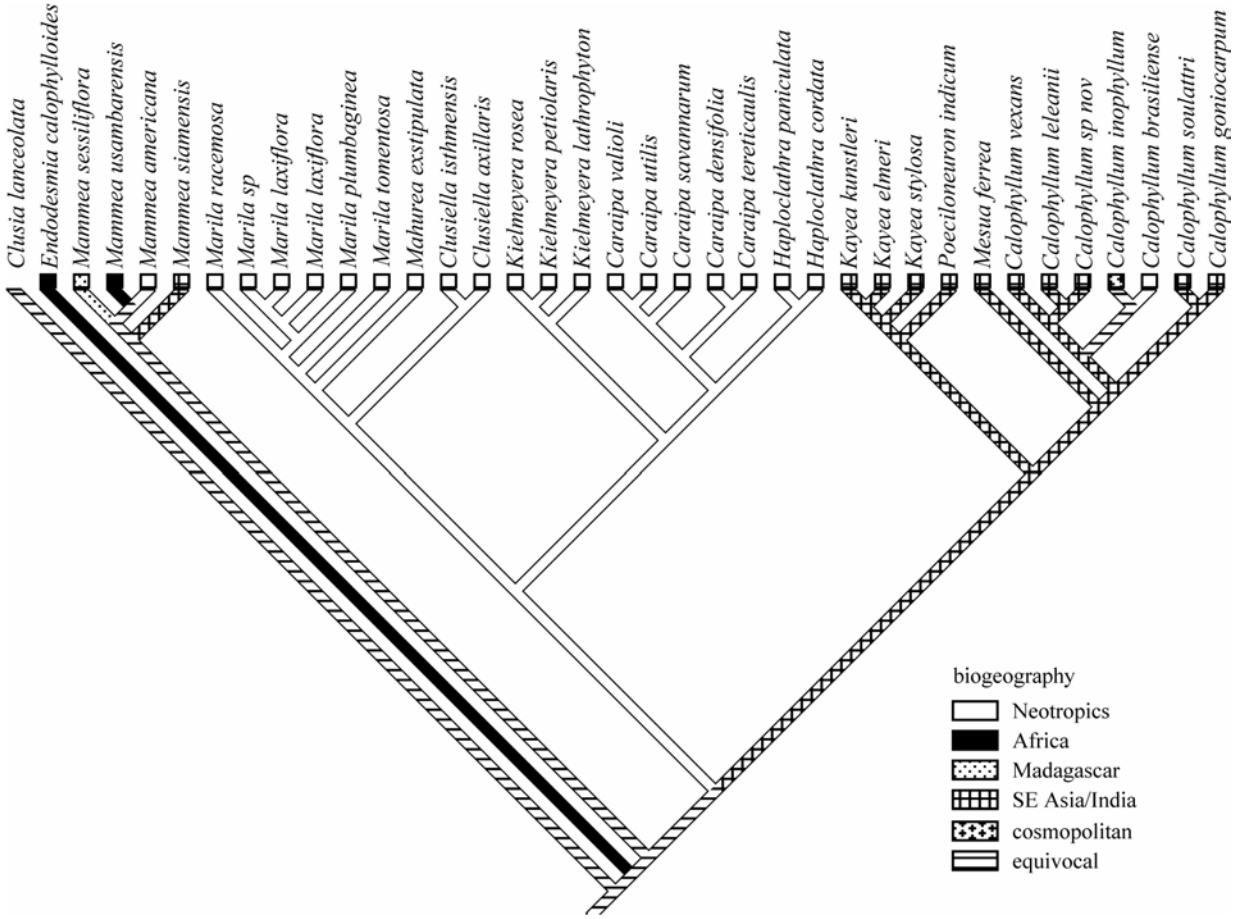

Figure 4-15. Biogeographical patterns within Kielmeyeroideae. Tree topology is one tree (Tree #2) from the four most parsimonious trees obtained in the unpruned combined molecular and morphological analysis (Analysis 7). *Neotatea*, which appears sister to *Mahurea* in Analysis 10, but was not included in this analysis, occurs in the Neotropics. Although *Clusia lanceolata* occurs in the Neotropics, Clusioideae are a pantropical group and therefore *C. lanceolata* is coded as equivocal. MacClade was set to show all most parsimonious states at each node.

CHAPTER 5
KEY TO THE GENERA OF KIELMEYEROIDEAE

In this key, all genera of Kielmeyeroideae are included except *Lebrunia* (Endodesmieae), which was omitted due to the lack of knowledge regarding its morphological variation.

1. Leaves alternate 2
1. Leaves opposite 5

2. Ovules 1-3 per carpel; seeds with narrow wing completely surrounding seed; stipuliform structures absent; colleters present; latex/resin cavities present in leaf blade ..... ***Caraipa***
2. Ovules more than 15 per carpel; winged seeds absent or present; stipuliform structures present or absent; colleters present or absent; latex/resin cavities or canals present in leaf blade 3

3. Anther glands deeply crateriform; stipuliform structures present; colleters present; tertiary venation percurrent to reticulate; latex/resin cavities present in leaf blade ..... ***Mahurea***
3. Anther glands spherical, subcrateriform, or absent; stipuliform structures absent; colleters present or absent; tertiary venation reticulate or not evident; latex/resin cavities or canals present in leaf blade 4

4. Anther glands spherical; colleters absent; seeds with elongate wing; tertiary venation not evident; latex/resin canals present in leaf blade ..... ***Neotatea***
4. Anther glands spherical to subcrateriform or absent; colleters present; seeds with broad wing; tertiary venation reticulate; latex/resin cavities present in leaf blade..... ***Kielmeyera***

5. Plant an epiphytic shrub or liana; dioecious; interpetiolar stipuliform structures present; staminate flowers with filaments fused into a tube for most of their length and resiniferous staminodes at the base of tube; carpellate flowers with an ovary with 5-20 expanded, sessile stigmas, the ovary surrounded at its base by resiniferous staminodes; fruit a many-seeded berry ..... ***Clusiella***
5. Plant a tree; monoecious or (andro)dioecious; stipuliform structures present or absent, but if present, then not interpetiolar; flowers without resiniferous staminodes; ovary with fewer than 5 stigma lobes; style(s) present; fruit a capsule or berry, but if berry, then with 4 or fewer seeds 6

6. Fruit indehiscent 7
6. Fruit dehiscent 9

7. Inflorescence a fascicle; plant (andro)dioecious; bracteoles present; anther glands spherical but sometimes absent; stipuliform structures absent..... ***Mammea***
7. Inflorescence a cyme or raceme- to panicle-like cyme; flowers bisexual; bracteoles present or absent; anther glands elongate or absent; stipuliform structures present or absent 8

8. Inflorescence a cyme; filaments fused into tube, with anthers arising from the apex and covering the entire inside surface of tube; anther glands elongate; stigma narrow (same thickness as style) and unlobed; placentation apical; leaf blade with latex/resin cavities and canals that cross secondary veins. ..... ***Endodesmia***
8. Inflorescence a raceme- or panicle-like cyme; filaments fused at base or appearing fasciculate, but not fused into a tube; anther glands absent; stigma expanded, with 2 or 3 poorly-developed lobes; placentation basal; leaf blade with latex/resin canals that alternate with secondary veins..... ***Calophyllum***

9. Inflorescence an axillary raceme (without terminal flower); ovules more than 15 per carpel; seeds plumose or not; placentation axile to intruded-parietal..... ***Marila***
9. Inflorescence a cyme, panicle-like cyme, or cyme of 1-3 flowers (and terminal flower present); ovules 1-4 per carpel; seeds not plumose; placentation basal or axile to intruded-parietal.....10

10. Inflorescence a panicle-like cyme; seeds with a narrow wing; placentation axile to intruded-parietal; ovary 3-loculate; stigmas 3, expanded..... ***Haploclathra***
10. Inflorescence a cyme or 1-3-flowered reduced cyme; seeds unwinged; placentation basal; ovary 1- or 2-loculate; stigmas 2 or 4, expanded or narrow.....11

11. Stigmas 2, expanded; inflorescence a reduced cyme of 1 or 2 flowers..... ***Mesua***
11. Stigmas 2 or 4, narrow (same thickness as style) 12

12. Style 4-parted; stamens more than 15; anthers open by longitudinal slits; calyx usually accrescent ..... ***Kayea***
12. Styles 2; stamens 15; anthers open by terminal pores; calyx not accrescent..... ..... ***Poeciloneuron***

APPENDIX A
TAXA USED FOR DNA ANALYSIS

Table A-1. Taxa used for DNA analysis.

Taxon	Collector and #	Herbarium with voucher	ITS	*matK*	*rbcL*
Calophyllum brasiliense Camp.	C. Notis 387	FLAS	AY625643	—	—
Calophyllum goniocarpum P.F. Stevens	F. Damon 318	MO, HUH	AY625638	—	—
Calophyllum inophyllum L.	C. Notis 391	FLAS	AY625640	AY625043	AY625020
Calophyllum leleanii P.F. Stevens	F. Damon 317	MO, HUH	AY625641	AY625045	AY625022
Calophyllum soulattri Burm. f.	F. Damon 320	MO, HUH	AY625639	AY625044	AY625021
Calophyllum sp. nov. P.F. Stevens	F. Damon 323	MO, HUH	AY625642	—	—
Calophyllum vexans P.F. Stevens	F. Damon 321	MO, HUH	AY625637	—	—
Caraipa densifolia Mart.	C. Grandez 16239	FLAS	AY625626	AY625035	AY625012
Caraipa savannarum Kubitzki	G. Aymard sn	PORT	AY625628	AY625034	—
Caraipa tereticaulis Tul.	Vormisto 578	AAU, AMAZ	AY625627	—	—
Caraipa utilis R. Vasquez	C. Grandez 16240	FLAS	AY625625	AY625036	AY625013
Caraipa valioli Paula	C. Grandez 16243	FLAS	AY625624	—	—
Clusia lanceolata Cambess.	C. Notis 389	FLAS	—	AY625054	—
Clusiella isthmensis Hammel	M. Whitten 2657	FLAS	AY625631	AY625042	AY625019
Endodesmia calophylloides Benth.	H. Ndoma & J. Ntui 899	MO	AY625610	AY625053	AY625030
Garcinia spicata Hook. f.	C. Notis 388	FLAS	—	AY625055	—
Haploclathra cordata R.Vásquez	C. Grandez 16237	FLAS	AY625630	AY625040	AY625017
Haploclathra paniculata Benth.	C. Grandez 16246	FLAS	AY625629	—	—
Hypericum tetrapetalum Lam.	C. Notis 444	FLAS	—	—	—
Kayea elmeri Merrill	A. Kalat *sn*	HUH	AY625633	—	—
Kayea kunstleri King	K. Larsen et al. 42186	MO	AY625632	—	—
Kayea stylosa Thw.	Kostermans 11106	HUH	AY625634	AY625048	AY625025
Kielmeyera lathrophyton Saddi	F. Feres *sn*	UEC	AY625623	AY625038	AY625015

Table A-1. Continued

Kielmeyera petiolaris Mart.	F. Feres 75	UEC	—	AY625039	AY625016
Kielmeyera rosea Mart.	Kubitzki et al. 97-5	HBG	AY625622	AY625037	AY625014
Mahurea exstipulata Benth.	Kubitzki et al. 97-27	HBG	AY625621	AY625041	AY625018
Mammea americana L.	C. Notis 392	FLAS	AY625613	AY625052	AY625029
Mammea sessiliflora Planch. & Triana	McPherson 18377	MO	AY625611	AY625050	AY625027
Mammea siamensis (Miq.) T. Anders.	P. Sweeney 1039	MO	AY625614	AY625051	AY625028
Mammea usambarensis B.Verdcourt		MO	AY625612	AY625049	AY625026
Marila laxiflora Rusby	M. Samaniego 124	STRI	AY625618	AY625031	AY625009
Marila laxiflora Rusby	van der Werff et al. 16246	MO	AY625619	AY625033	—
Marila plumbaginea P.F. Stevens	M. Nee 48390	MO	AY625617	—	—
Marila racemosa Sw.	B. Gunn 84	MO	AY625615	—	AY625008
Marila tomentosa Poepp. & Endl.	van der Werff et al. 16215	MO	AY625620	AY625032	AY625010
Marila sp.	van der Werff et al. 14476	MO	AY625616	—	—
Mesua ferrea L.	C. Notis 390	FLAS	AY625635	AY625047	AY625024
Poeciloneuron indicum Bedd.	U. Ghate *sn*	FLAS	AY625636	AY625046	AY625023

APPENDIX B
SPECIMENS EXAMINED

***Calophyllum brasiliense* Camp.**: Puerto Rico, Municipio de Loiza, Barrio Torrecilla Baja, 2.2-2.6 km due WSW of Punta Vacia Talega, elev. near sea level, *G.R. Proctor 50003* (FTG); USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, *C. Notis 387* (FLAS).

***Calophyllum fibrosum* P.F. Stevens**: Madagascar, Toamasina, Nosy Mangabe, 5 km S of Maroantsetra, 15°30'S, 49°46'E, elev. 0-330 m, *G.E. Schatz & E. Carlson 2852* (MO); Madagascar, Toamasina, Masoala Peninsula, "South trail," S of Androka River, 15°38'S, 49°59'E, elev. 150-450 m, *G.E. Schatz & G. Modeste 3049* (MO).

***Calophyllum inophyllum* L.**: USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, *W.T. Gillis 11103* (FTG); USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, *C. Notis 391* (FLAS).

***Caraipa densifolia* Mart.**: Peru, Dpto. Loreto, Prov. Maynas, Puerto Almendras, 73°25'W, 03°48'S, elev. 122 m, *R. Vasquez & N. Jaramillo 7659* (MO); Peru, Dpto. Loreto, Prov. Maynas, Quebrada Sucusari, afluente iquierdo del Rio Napo, 03°20'S, 72°55'W, elev. 130 m, *R. Vasquez & N. Jaramillo 11750* (MO).

***Caraipa savannarum* Kubitzki**: Venezuela, Amazonas, Rio Sipapo, entre Barranco Tonina y Cano Veneno, 04°54'-05°3'N, 67°34'-67°46'W, *A. Castillo 6980* (MO).

Caraipa sp.: Venezuela, Amazonas, Dpto. Atures, Serrania Paru, planicie central, SW sector, 04°25'N, 65°32'W, elev. 1200-1250 m, *O. Huber & J. Rosales 4993* (MO).

***Clusia lanceolata* Cambess.**: Brazil, Guanabara, Restinga de Jacarepagua, Recreio dos Bandeirantes, *B. Maguire, C.K. Maguire, & J. Murca Pires 44586*; USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, *C. Notis 389* (FLAS).

***Clusiella axillaris* (Engl.) Cuatr.**: Venezuela, Amazonas, 3 km NE of San Carlos de Rio Negro, ca. 20 km S of confluence of Rio Negro and Brazo Casiquiare, 01°56'N, 67°03'W, elev. 120 m, *R.L. Liesner 6777* (MO); Brasil, Amazonas, Reserva Ducke, 02°53'S, 59°58'W, *M.T.V. Campos 548* (MO); Brasil, Amazonas, Sao Paulo de Olivenca, Ducke 1626 (HUH); Colombia, Amazonas, Vaupes, Rio Apaporis, Soratama (above mouth of Rio Kananari) and vicinity, 05°N, 70°40'W, elev. 900 ft, *R.E. Schultes & I. Cabrera 16089* (HUH).

***Clusiella isthmensis* B. Hammel**: Costa Rica, Limon, Canton de Talamanca, Bratsi, Amubri, Alto Lari, Kivut., alfluente innominado del Rio Lari, margen izquierda, 09°22'55''N, 83°05'10''W, elev. 1500m, *G. Herrera 5422* (MO); Panama, Comarca de San Blas, along El Llano-Carti Rd., W of road 13.8 km to 15.8 km from Interamerican Hwy, 09°19'N, 78°55'W, elev. 325 m, G. de Nevers, *C. de Leon, & M. Marcus 3747* (MO); Panama, Prov. Panama, along El Llano-Carti Rd., 09°15'N, 79°00'W, elev. 400 m, *G. McPherson 7585* (MO); Panama, Comarca de San Blas, El Llano-Carti Rd., 19.1 km from Interamerican Hwy, 09°19'N, 78°55'W, elev. 350 m, *G. de Nevers 4300* (MO).

***Endodesmia calophylloides* Benth.**: Gabon, Estuaire, S of Estuaire du Gabon along Remboue River, British gas site, 00°12'S, 10°01'E, elev. 10 m, *G. McPherson 15100* (HUH); Gabon, 12-15 km NE of Asok, elev. 660 m, *F.J. Breteler & J.J.F.E. de Wilde 123* (MO); Cameroun, 16 km on the road from Ebolowa to Minkok and 2 km to the left

along a forest exploitation track, 02°58'N, 11°17'E, elev. 650 m, *J.J.F.E. de Wilde 7926* (MO).

***Garcinia spicata* Hook. f.**: USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, *C. Notis 388* (FLAS).

***Haploclathra leiantha* (Benth.) Benth.**: Brasil, Igapo, Foz do rio Marie, afluente do rio Negro, 00°25'S, 66°24'W, *M.L. Kawasaki 360* (MO).

***Haploclathra paniculata* (Mart.) Benth.**: Brasil, Amazonas, Reserva Ducke, Igarape do Tinga, Floersta de Baixio, Arvore, 02°53'S, 59°58'W, *A. Vicentini 1144* (MO); Brasil, Amazonas, Mun. de Maues, basin of Rio Apoquitaua, lower Rio Pacoval, 03°48'S, 58°04'W, *J.L. Zarucchi et al. 3181* (MO); Brasil, Secus Rio Negro, inter Barcellos et San Isabel, *R. Spruce sn* (HUH).

***Hypericum tetrapetalum* Lam.**: USA, Florida, Levy Co., FL 24, 1/8 mile west of Bronson city limit, *C. Notis 444* (FLAS).

***Kayea borneensis* P.F. Stevens**: Sarawak, Sabal Kruim, Sabal F.R., 74th Mile Kuching/Simanggang Rd., 1st Division, *Y.P. Ching 41133* (MO).

***Kayea elmeri* Merrill**: Kalimantan, 7 km along side road at km 57 from Sangai (S. Mentaya), Kab. Kotawaringin Timur, 01°29'S, 112°31'E, *P. Wilkie sn* (HUH).

***Kayea kunstleri* (King) Kosterm.**: Malaya, Gunung Jerai, Kedah, road side, elev. 2100 ft, *K.M. Kochummen sn* (HUH); Malaya, Sungkop VJR Kedah, elev. 300 ft, *Y.C. Chan sn* (HUH); Thailand, Prov. Songkla, Koh Hong, 100°20'E, 07°00'N, elev. 350-450 m, *K. Larson et al. 42652* (MO); Thailand, Prov. Songkla, Khao Mot Daeng, Ko Hong, Hat Yai, 100°20'E, 07°00'N, elev. 100 m, *K. Larson et al. 42186* (MO).

***Kielmeyera coriacea* Mart.**: Bolivia, Dpto. Santa Cruz, Prov. Velasco, Estancia Flor de Oro, 7 km S of buildings, 13°36'S, 61°00'W, elev. 190 m, *M. Nee 41338* (MO); Brasil, Planalto do Brasil, Distrito Federal, ca. 10 km S of Brasilia, on road to Belo Horizonte, elev. 1200 m, *H.S. Irwin, R. Souza, R. Reis dos Santos 8576* (HUH); Brasil, Planalto do Brasil, Serra do Rio Preto, Estado de Goias, 16°S, 47°W, elev. 1000 m, *H.S. Irwin, R. Souza, R. Reis dos Santos 10395* (MO).

***Kielmeyera speciosa* St. Hil.**: Brasil, Minas Gerais, Municipio de Formoso, Parque Nacional Grande Sertão Veredas, ca. 23 km da Sede da FUNATURA, 15°19'07''S, 46°00'21''W, elev. 820 m, *R.C. Mendonça, M.L. Fonseca, F.C.A. Oliveira, & F. das Chagas e Silva 3473* (MO); Brasil, Goiás, Municipio de Niquelândia, Estrada para Colinas do Sul, ca. 41 km de Niquelândia, 14°23'53''S, 48°04'37''W, elev. 485 m, *M.L. Fonseca, M.A. da Silva, D. Alvarenga, & A.J.V. Santos 1783* (MO); Brasil, Distrito Federal, proximo ao portão principal da Reserva Biologica de Aguas Emendadas, ca. 40 km NE of Brasilia, *A.P. Silva 06* (MO).

***Mahurea exstipulata* Benth.**: Colombia, Caqueta, Araracuara, trocha a Yari, parcelas experimentales de regeneracion, 00°25'S, 72°20'W, elev. 200 m, *A. Gentry & M. Sanchez 64919* (MO); Venezuela, Estd. Bolivar, along road from Icabaru to Santa Elena, 16-17 km NE of Icabaru, ca. 04°28'N, 61°40'W, elev. 735-790 m, *T.B. Croat 54167* (MO); Venezuela, Municipio Gran Sabana, entre km 114 y 113 de la Carretera Santa Elena, Piedra de La Virgen, elev. 950 m, *C. Benitez & W.G. D'Arcy 5274* (MO).

***Mammea americana* L.**: USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, *C. Notis 392* (FLAS).

***Mammea siamensis* (Miq) T. Anders.**: Thailand, Chi Avg Mai, 76/1 Sei 5, Sutep Rd., Chisne Mai, base of Doi Sutep, east side, elev. 350 m, *J.F. Maxwell 92-70* (HUH); Thailand, Songkla, Maa Bee Yah Village, foothills of Klong Rhang Hill, elev. 50 m, *J.F. Maxwell 86-70* (HUH); Thailand, Chiang Mai, Biology Dept., Faculty of Science, Chiang Mai Univeristy, elev. 350 m, *Franco Paleo 30* (HUH).

***Mammea subsessifolia* (Hochr.) Kosterm.**: Toamasina, Masoala Peninsula, ca. 11 km S of Ambanizana, 15°43'S, 49°57-58'E, elev. 0-5 m, *G.E. Schatz & G. Modeste 3099* (HUH); Country Unknown, Est (Nord), Forêt d'Analamateza au Sud d'Antsirabe-Nord, *R. Capuron sn* (HUH).

***Marila laxiflora* Rusby**: Panama, Prov. of Panama, in high ridges of the Serrania de Maji, S of the Choco village of Ipeti, ca. 5 hours walk from the village, 08°45'N, 78°30'W, elev. 500-650 m, *S. Knapp, R. foster, J. Mallet, & M. Huft 4476*; Panama, Prov. of Panama, Cerro Azul, *R.A. Lopez 38*; Panama, San Blas-Panama border, on Kuna divide trail west of Llano-Carti Rd, 09°20'N, 79°00'W, elev. 250 m, *G. McPerson 11882*; Panama, Canal Zone, Barro Colorado Island, upper Allee creek, *R. Foster 2820*.

***Marila plumbaginea* P.F. Stevens**: Bolivia, Dpto. La Paz, Prov. Larecaja, Copacabana, ca. 10 km S of Mapiri, elev. 850-950 m, *B.A. Krukoff 11057*; Bolivia, Dpto. Santa Cruz, Prov. Ichilo, ca. 2 km W of Villa San German on highway from Buena Vista to Rio Ichilo, 17°21'S, 64°30'W, elev. 275 m, *M. Nee 48390*; Ecuador, Prov. Napo, Canton Tena, Estacion Biologica Jatun Sacha, Rio Napo, 8 km al E de Misahualli, 01°05'S, 77°39'W, elev. 450 m, *W. Palacios 3273*.

***Marila racemosa* Sw.**: Dominica, on road bordering Imperial Rd., Sylvania to Mahaut River, elev. 549 m, *W.H. Hodge 532*; Saint Lucia, Quarter of Castries, vicinity of

Piton Flore, elev. ca. 1500 ft, *G.L. Webster, J.R. Ellis, & K. Miller 9302*; Saint Lucia, Piton Flore, elev. 1200 ft, *V. Slane, L. JnPierre, C. Jimber 489*.

***Mesua ferrea* L.**: Malaya, Kepong F.R.I. area, Selangor, elev. 350 ft., *M.B. Sider 13223*; Malaya, Selangor, University of Malaya Campus, road to 5th Res. College, elev. 100 ft., *B.C. Stone 11039*; Puerto Rico, Villa leon, Bayamon, Toa Alta Rd., *L.R. Holdridge 244*; USA, Florida, Dade Co., Miami, Fairchild Tropical Garden, Plot 42, *W.B. Zomlefer 748*.

***Neotatea duidae* (Kobuski & Steyermark) A.L. Weitzman & P.F. Stevens**: Venezuela, Terr. Federal de Amazonas, Dpto. Atabapo cumber del Cerro Duida, 03°22'N, 65°39'W, elev. 1640 m, *O. Huber 13444*.

***Neotatea longifolia* (Gleason) Maguire**: Venezuela, Cerro Duida, Rio Cunucunuma, Amazonas, along North Escarpment above Culebra, elev. 1400 m, *B. Maguire, R.S. Cowan, & J.J. Wurdack 29585*; Venezuela, Dpto. Atures, Serrania Paru, planicie central, SW sector, 04°25'N, 65°32'W, elev. 1200-1250 m, *P.E. Berry, O. Huber, & J. Rosales 4976*.

***Neotatea neblinae* Maguire**: Venezuela, Cerro de la Neblina, Rio Yatua, south slope of Cumbre Camp Caño toward Caño Grande, elev. 1500 m, *B. Maguire, J.J. Wurdack, & G.S. Bunting 37321*; Venezuela, Territorio Federal Amazonas, Dpto. Rio Negro Cerro de Neblina, Puerto Chimo Camp on Rio Mawarinuma and up north slope of canyon, 5 km E of La Neblina Base Camp by air, 00°50'N, 66°07'W elev. 150-1800 m, *R. Liesner & C. Brewer 15874*.

***Poeciloneuron indicum* Bedd.**: India, Palghat District, Kerala State, Vattapparai Shola, Sirurani Slopes, *E. Vajrarelu 77764*; India, Shimoga District, Kashataka State, 13.3°N, 75.7°E, elev. 650 m, *U. Ghate sn*.

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BIOGRAPHICAL SKETCH

Christine H. Notis was born on June 13, 1979, in Des Moines, Iowa. Christine graduated from Johnston High School in 1997, after which she attended Iowa State University in Ames, Iowa. At Iowa State, Christine was interested in biology, and became fascinated with plants while taking an introductory biology class from Lynn G. Clark. She gained her first research experience in the lab of Jonathan F. Wendel, where she harvested ovules from *Gossypium* spp. as part of a larger project on the seed trichomes of these plants. Christine became interested in fern gametophyte ecology, and decided to complete her honors project on the longevity and swimming distance of fern sperm (under the direction of Donald R. Farrar). She received her Bachelor of Science degree (with honors) in botany from Iowa State in the spring of 2001.

In the fall of 2001, Christine came to the University of Florida to start a master's degree under the direction of Walter S. Judd and Douglas E. Soltis. Her project focused on a phylogeny of Kielmeyeroideae (Clusiaceae) using molecular and morphological data. Christine had first become intrigued by Clusiaceae when she left Iowa for a summer to take Walter Judd's Tropical Botany class at the National Tropical Botanical Garden-Kampong and Fairchild Tropical Botanic Garden in Miami. While a masters student at the University of Florida, Christine was a teaching assistant in several courses, including introductory biology, introductory botany, plant diversity, and plant taxonomy. Christine received her Master of Science degree in August, 2004.