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Systematics and Biogeography of the Clusioid Clade (Malpighiales)

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Systematics and biogeography of the clusioid clade (Malpighiales)

A dissertation presented
by

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ABSTRACT

The clusioids are a clade of flowering plants in the diverse rosid order Malpighiales. It includes five families (i.e., Bonnetiaceae, Calophyllaceae, Clusiaceae *sensu stricto*, Hypericaceae, and Podostemaceae) that form a conspicuous element of tropical forests worldwide and are economically important. Their phylogenetic and biogeographical history has remained uncertain, however, which has hindered our understanding of their evolution. I conducted the first taxon-rich multigene analysis of this important clade to clarify their phylogenetic relationships (Chapter 1). Plastid (cp: *matK*, *ndhF*, and *rbcL*) and mitochondrial (mt: *matR*) nucleotide sequence data from nearly 200 taxa produced a well-resolved clusioid phylogeny and indicate that several traditionally recognized genera are not monophyletic. These results provide a strong framework for improving the classification of the group. To further determine the placement of several key taxa lacking molecular data, especially the ancient fossil rosid *Paleoclusia* (~90 Myr), I assembled a morphological data set that I analyzed in combination with the cp and mt data (Chapter 2). My results support previous hypotheses of phylogenetic relationships for extant taxa and indicate that *Paleoclusia* is weakly placed as a member of the clusioid subclade Clusiaceae *sensu stricto*. Finally, I inferred molecular divergence estimates and ancestral ranges for the clade to test the hypothesis

that the pantropical distribution of many clusioid subclades is attributable to ancient Gondwanan vicariance (Chapter 3). The clusioids are ideal for examining this topic due to their well-sampled and strongly supported phylogeny, pantropical distribution, and ancient fossil record. Our results suggest a single Gondwanan vicariant event early in the history of the clade, followed by prevalent dispersal throughout the Cenozoic, most of which occurred after the mid-Eocene. These results are consistent with a growing body of literature that suggests that many traditionally recognized angiosperm clades are far too young for their distributions to have been influenced strictly by Gondwanan vicariance. Instead, it appears that dispersal is a more likely explanation for many Gondwanan distributions in angiosperms including the clusioids.

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CHAPTER 1:

Phylogeny of the clusioid clade (Malpighiales): evidence from the plastid and mitochondrial genomes

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PHYLOGENY OF THE CLUSIOID CLADE (MALPIGHIALES): EVIDENCE FROM THE PLASTID AND MITOCHONDRIAL GENOMES[1]

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- *Premise of the study* The clusioid clade includes five families (i e , Bonnetiaceae, Calophyllaceae, Clusiaceae s s , Hypericaceae, and Podostemaceae) represented by 94 genera and ~1900 species Species in this clade form a conspicuous element of tropical forests worldwide and are important in horticulture, timber production, and pharmacology We conducted a taxon-rich multigene phylogenetic analysis of the clusioids to clarify phylogenetic relationships in this clade
- *Methods* We analyzed plastid (*matK*, *ndhF*, and *rbcL*) and mitochondrial (*matR*) nucleotide sequence data using parsimony, maximum likelihood, and Bayesian inference Our combined data set included 194 species representing all major clusioid subclades, plus numerous species spanning the taxonomic, morphological, and biogeographic breadth of the clusioid clade
- *Key results* Our results indicate that *Tovomita* (Clusiaceae s s), *Harungana* and *Hypericum* (Hypericaceae), and *Ledermanniella* s s and *Zeylandium* (Podostemaceae) are not monophyletic In addition, we place four genera that have not been included in any previous molecular study *Ceratolacis*, *Diamantina*, and *Griffithella* (Podostemaceae), and *Santomasia* (Hypericaceae) Finally, our results indicate that *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* can be safely merged into *Hypericum* (Hypericaceae)
- *Conclusions* We present the first well-resolved, taxon-rich phylogeny of the clusioid clade Taxon sampling and resolution within the clade are greatly improved compared to previous studies and provide a strong basis for improving the classification of the group In addition, our phylogeny will form the foundation for our future work investigating the biogeography of tropical angiosperms that exhibit Gondwanan distributions

Key words: *Garcinia mangostana*, Guttiferae, *Hypericum perforatum*, mangosteen, *matK*, *matR*, morphology, *ndhF*, *rbcL*, St John's wort

The clusioids are a clade of flowering plants in the large rosid order Malpighiales (Savolainen et al , 2000, Soltis et al , 2000, Wurdack and Davis, 2009) Species in this clade are morphologically heterogeneous and ecologically diverse Growth forms include large tropical rainforest trees, temperate and high altitude tropical herbs and shrubs, and aquatic plants of swift-flowing rivers and streams Although their distribution is nearly cosmopolitan, their greatest species diversity is in the tropics This well-supported clade contains five families (APG III, 2009, Wurdack and Davis, 2009) representing 94 genera and ~1900 species (Kato, 2006, Cook and Rutishauser, 2007, Stevens, 2007a, b, Weitzman et al , 2007, Thiv et al , 2009, Koi and Kato, 2010, Tippery et al , in press) Bonnetiaceae, Calophyllaceae, Clusiaceae s s , Hypericaceae, and Podostemaceae The clusioids, excluding Podostemaceae, are an important component of tropical forests and comprise ~3% of the total species diversity in the Center for Tropical Forest Science's global network of tropical forest research plots (CTFS, 2009) Podostemaceae, the largest strictly aquatic flowering plant family, play a key role in river systems—especially through their impact on the ecology and nutrition of fish and invertebrates (Allan, 1995, Machado-Allison et al , 2003) This family occupies a unique ecological niche for angiosperms growing firmly attached to solid substrates in swift-flowing, nutrient-poor rivers and waterfalls (Philbrick and Novelo, 2004) Their ability to attach to substrates in these harsh environments is facilitated by biofilms partially composed of cyanobacteria, which may function as an important source of nitrogen for the plants

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(Jager-Zurn and Grubert, 2000) The clusioid clade also contains problematic invasive species, such as *Hypericum perforatum* L , which has been shown to outcompete native species and is toxic to livestock (Huffaker, 1951, Giese, 1980, Mitich, 1994, Vandenbogaerde et al , 1998, Buckley et al , 2003)

Clusioids are also economically important Many species are cultivated in the horticultural trade (e g , *Hypericum* spp) or harvested for timber (e g , *Calophyllum brasiliense* Cambess , *Mesua ferrea* L) Several species have pharmacological activity and are potentially useful for the treatment of tumors, depression, and AIDS (Bennett and Lee, 1989, Burkhardt et al , 1994, McKee et al , 1998, Ernst, 2003) St John's wort (*H perforatum*), for example, is one of the best-selling herbal medicines worldwide, with annual sales in the United States of around $200 million (Ernst, 2003) Furthermore, members of this clade produce the important tropical fruits the mangosteen (*Garcinia mangostana* L) and the mammey apple (*Mammea americana* L)

The current circumscription of the clusioid clade differs from previous morphology-based classifications, and molecular data were required to detect its component families and their interrelationships (Savolainen et al , 2000, Soltis et al , 2000, Gustafsson et al , 2002, Wurdack and Davis, 2009) Cronquist (1981), for example, placed the clusioids in two distantly related orders, Theales and Podostemales, in his subclasses Dilleniidae and Rosidae, respectively Terrestrial members of this clade (i e , Bonnetiaceae, Calophyllaceae, Clusiaceae s s , and Hypericaceae) have long been considered closely related, and the name Clusiaceae (alternately called Guttiferae) has historically been applied to various combinations of taxa now found in these four families (e g , Cronquist, 1981, Takhtajan, 1997, Mabberley, 2008) The alternate-leaved clusioids, Bonnetiaceae, and some Calophyllaceae were considered closely related to Theaceae s l (e g , Baretta-Kuipers, 1976, Cronquist, 1981, Takhtajan, 1997, Weitzman and Stevens, 1997), but subsequent phylogenetic evidence placed Theaceae s l in the asterid order Ericales (Stevens, 2001 onward, APG III, 2009) The wholly aquatic Podostemaceae have been very difficult to place owing to their highly atypical morphology, but were never thought to be closely related to other clusioids (Stevens, 2007b) They have long been considered morphological misfits and are so unlike most angiosperms that some systematists suggested they be recognized as their own class, equal in rank to monocots and dicots (Cusset and Cusset, 1988)

These newly discovered relationships have led to a reexamination of morphological characteristics that revealed several putative synapomorphies for the clusioid clade and its major subclades All clusioid families share distinctive xanthones, and many members of the clade possess exotegmic seeds (Bonnetiaceae, some Calophyllaceae, some Clusiaceae, Hypericaceae, and Podostemaceae) Bonnetiaceae, Clusiaceae s s , and Hypericaceae share staminal fascicles opposite the petals, and Hypericaceae and Podostemaceae share tenuinucellate ovules Additionally, Bonnetiaceae, some members of Hypericaceae, and Podostemaceae have papillate stigmas, and Hypericaceae, Calophyllaceae, Clusiaceae s s , and some Podostemaceae share resin-containing glands or canals that are especially visible in the leaves (Cook and Rutishauser, 2007, Stevens, 2007a, b, Weitzman et al , 2007)

Several molecular phylogenetic studies have focused on individual clusioid families, subfamilies, or genera (Kita and Kato, 2001, 2004a, Abdul-Salim, 2002, Gustafsson and Bittrich, 2002, Gustafsson et al , 2002, 2007, Notis, 2004, Moline et al , 2006, 2007, Sweeney, 2008, Koi et al , 2009, Thiv et al , 2009, Wurdack and Davis, 2009, Tippery et al , in press), but only two of these studies have addressed relationships broadly within the clade Gustafsson et al (2002) provided evidence for several major clusioid subclades, most notably Podostemaceae + Hypericaceae Relationships within and between most subclades, however, were not well resolved This lack of resolution is likely due to their limited taxon sampling and the use of a single plastid gene, *rbcL* Wurdack and Davis (2009) analyzed 13 genes from three genomes and provided strong resolution among the major clusioid subclades In particular, their results included the unexpected finding that Clusiaceae s l , as traditionally circumscribed, were not monophyletic However, their taxon sampling was also narrow, including only 17 genera (of 94), each represented by a single placeholder taxon Despite these insights, many questions remain unanswered In particular, molecular results surprisingly suggest that the pantropical Symphonieae (Clusiaceae s s), with their unique stigmas, are not monophyletic (Gustafsson et al , 2002, Sweeney, 2008) Additionally, intergeneric relationships in most clusioid subclades are unknown, and it is thought that some genera are likely not monophyletic (e g , *Hypericum, Garcinia, Ledermanniella* s s , Stevens, 2007a, b, Sweeney, 2008, Thiv et al , 2009, Nurk and Blattner, 2010) The major goal of our study is to assemble the first well-supported multigene phylogeny of the clusioid clade with dense taxonomic sampling This will allow us to better assess the classification of the group, elucidate patterns of character evolution, establish synapomorphies for the major clusioid subclades, and pave the way for larger biogeographic analyses To achieve our goal, we sampled three plastid genes (*matK*, *ndhF*, and *rbcL*) and the mitochondrial gene *matR* from the broadest clusioid taxon sampling to date

MATERIALS AND METHODS

Taxon sampling—Our taxon sampling comprises 222 terminals including outgroups Of these, 194 are clusioid species representing 71 of the 94 currently recognized genera and ~10% of the species diversity in this clade (Cook and Rutishauser, 2007, Stevens, 2007a, b, Weitzman et al , 2007, Thiv et al , 2009, Koi and Kato, 2010, Tippery et al , in press) Voucher information and GenBank numbers for all sequences are provided in Appendix 1 Most missing genera were from Podostemaceae (19 of 23, see Table 1) Tippery et al (in press) have shown that several genera of Podostemaceae are not monophyletic The species of *Oserya* that were transferred to *Noveloa* by Tippery et al are represented here by *N coulteriana* (Tul) C T Philbrick In addition, Tippery et al found that the monotypic *Vanroyenella* was embedded within a Central American clade of *Marathrum* Accordingly, we have included this species as *Marathrum plumosum* (Novelo & C T Philbrick) C T Philbrick & C P Bove

Only four small genera outside Podostemaceae are missing from our analyses *Lebrunia* (monotypic, Africa, Calophyllaceae), *Lianthus* (monotypic, China, Hypericaceae), *Neotatea* (four species, South America, Calophyllaceae), and *Thysanostemon* (two species, South America, Clusiaceae s s) Despite several attempts, we were unable to obtain polymerase chain reaction (PCR) amplicons from these taxa, perhaps due to the difficulty of obtaining high quality clusioid DNA from herbarium vouchers (Gustafsson and Bittrich, 2002) Our sampling included four genera that have not been included in previous molecular studies *Ceratolacis, Diamantina*, and *Griffithella* (Podostemaceae), and *Santomasia* (Hypericaceae) We have also increased the taxon sampling across the biogeographical range of the clusioid clade and within numerous genera to begin assessing generic circumscriptions and infrageneric relationships In some instances, gene sequences from different vouchers of a single species were combined (see Appendix 1) The sister group of the clusioid clade is unclear, therefore, we included 26 taxa representing all major lineages of Malpighiales sensu Wurdack and Davis (2009) as outgroups Two taxa from the more distant outgroups Celastrales (Celastraceae) and Oxalidales (Oxalidaceae) were also included Celastraceae were used to root our trees based on the findings by Wang et al (2009)

TABLE 1 Updated classification of the clusioid clade reflecting the findings of this and other recent studies (see text) Taxa are listed in alphabetical order Genera marked with "*" are represented in this study Genera marked with "⊗" have been suggested to be nonmonophyletic with molecular data but taxonomic changes have yet to be made Recent taxonomic changes sensu Tippery et al (in press) are marked with "$"

I Family Bonnetiaceae L Beauvis ex Nakai
Archytaea Mart *
Bonnetia Mart *
Ploiarium Korth *
II Family Calophyllaceae J Agardh
A Tribe Calophylleae Choisy
Calophyllum L *
Caraipa Aubl *
Clusiella Planch & Triana *
Haploclathra Benth *
Kayea Wall *
Kielmeyera Mart & Zucc *
Mahurea Aubl *
Mammea L *
Marila Sw *
Mesua L *
Neotatea Maguire
Poeciloneuron Bedd *
B Tribe Endodesmieae Engl
Endodesmia Benth *
Lebrunia Staner
III Family Clusiaceae Lindl
A Tribe Clusieae Choisy
Chrysochlamys Poepp *
Clusia L *
Dystovomita (Engl) D'Arcy *, ⊗
Tovomita Aubl *, ⊗
Tovomitopsis Planch & Triana *
B Tribe Garcinieae Choisy
Garcinia L *
(including *Allanblackia* Oliv *)
C Tribe Symphonieae Choisy
Lorostemon Ducke *
Montrouziera Planch & Triana *
Moronobea Aubl *
Pentadesma Sabine *
Platonia Mart *
Symphonia L f *
Thysanostemon Maguire
IV Family Hypericaceae Juss
A Tribe Cratoxyleae Benth & Hook f
Cratoxylum Blume *
Eliea Cambess *
B Tribe Hypericeae Choisy
Hypericum L * (including *Lianthus* N Robson, *Santomasia* N Robson *, *Thornea* Breedlove & E M McClint *, and *Triadenum* Raf *)
C Tribe Vismieae Choisy
Harungana Lam *, ⊗
Vismia Vand *, ⊗
V Family Podostemaceae Rich ex Kunth
A Subfamily Podostemoideae Wedd
Angolaea Wedd
Autania C T Philbrick $
Apinagia Tul *, ⊗
Butumia G Taylor
A Subfamily Podostemoideae Wedd (continued)
Castelnavia Tul & Wedd *
Ceratolacis (Tul) Wedd *
Cipoia C T Philbrick, Novelo & Irgang
Cladopus H A Moller *
Diamantina Novelo, C T Philbrick & Irgang *
Dicraeanthus Engl *
Diplobryum C Cusset
Djinga C Cusset *
Endocaulos C Cusset *
Farmeria Willis
Griffithella (Tul) Warm *
Hansenniella C Cusset *
Hydrobryum Endl *
Hydrodiscus Koi & M Kato
Inversodicraea Engl ex R E Fr *
Jenmaniella Engl ⊗
Ledermanniella Engl *, ⊗
Leiothylax Warm *
Letestuella G Taylor *
Lophogyne Tul
Macarenia P Royen
Macropodiella Engl *
Marathrum Humb & Bonpl *, ⊗, $
(including *Vanroyenella* Novelo & C T Philbrick *)
Monandriella Engl *
Monostylis Tul *
Mourera Aubl *, $
(including *Lonchostephus* Tul and *Tulasneantha* P Royen)
Noveloa C T Philbrick *, $ (*Oserya* Tul & Wedd pro parte)
Oserya Tul & Wedd
Paleodicraea C Cusset
Paracladopus M Kato *
Podostemum Michx *
(including *Crenias* Spreng * and *Devillea* Tul & Wedd)
Polypleurum Warm *
Rhyncholacis Tul *
Saxicolella Engl
Sphaerothylax Bisch ex Krauss
Stonesia G Taylor *
Thawatchaia M Kato, Koi & Y Kita *
Thelethylax C Cusset *
Wettsteiniola Suess
Willisia Warm
Winklerella Engl
Zehnderia C Cusset
Zeylanidium (Tul) Engl *, ⊗
B Subfamily Tristichoideae Engler
Cussetia M Kato
Dalzellia Wight *
Indodalzellia Koi & M Kato *
Indotristicha P Royen *
Terniopsis H C Chao *
Tristicha Thouars *
C Subfamily Weddellinoideae Engler
Weddellina Tul *

Molecular methods—PCR amplification and automated sequencing mostly followed Wurdack and Davis (2009) When these protocols were unsuccessful, we used additional primers from the literature (*matK* trnk-710F, 1168R [Johnson and Soltis, 1995], pod2R, pod3F, pod7F [Kita and Kato, 2001], *ndhF* 536F, 1318F, 1318R, 1603R [Olmstead and Sweere, 1994] and 2153R [Wang et al , 2009], and *rbcL* 1204R [Zurawski et al , 1981]) plus several designed here (see Table 2) Primers were frequently optimized independently for each major clusioid subclade Primer mismatch was also addressed using a step-down PCR procedure (Korbie and Mattick, 2008) Depending on the quality of the DNA template and the presence of homopolymer regions (which were particularly common in Hypericaceae and Podostemaceae), gene regions were sometimes amplified and sequenced in smaller fragments and assembled into a larger contig PCR products were sequenced using the facilities and protocols at Functional Biosciences (Madison, Wisconsin, USA)

In addition, we included plastid data (*matK*, *ndhF*, and *rbcL*) from seven clusioid plastid genomes *Clusia rosea* Jacq and *Garcinia mangostana* L (Clusiaceae s s), *Hypericum kalmianum* L , *H perforatum* L , *Triadenum fraseri* (Spach) Gleason, and *Vismia guianensis* (Aubl) Choisy (Hypericaceae), and *Podostemum ceratophyllum* Michx (Podostemaceae) These data were

TABLE 2 Primer table

Gene	Primer	Sequence	Original publication	Clade/Use
matK	Afm	5′-ATCCACTTATCTTTCAGGAG-3′	(Ooi et al , 1995)	P
	400fm	5′-TCAGAATTTACGATCCATTCTTTCAAT-3′	(Cameron et al , 2001)	H
	1053Fm1	5′-CAATRTCATTTTWMTGTRTG-3′	(Wurdack and Davis, 2009)	B, C
	1053Fm2	5′-TCAATRKCATTTTTHTGTRTGG-3′	(Wurdack and Davis, 2009)	H, K
	1159Rm1	5′-TSTARYATTTGACTYCGKACCACBG-3′	(Wurdack and Davis, 2009)	B, C
	1159Rm2	5′-AGCATTTGACTTCGTAYCRCTG-3′	(Wurdack and Davis, 2009)	H, K
	EHypR	5′-AACTCTCGAKCAAGATGTGTAGG-3′	New to this study	H
ndhF	1098F	5′-AATGGAAGCTATTGTTGGTTATTCTC-3′	New to this study	All clades
	1676R	5′-GAATTGATTGAAAGGAATTCCKA-3′	K Wurdack, unpublished	Degraded templates
rbcL	cRm	5′-GCAGCAGCTARTTCMGGACTCCA-3′	(Hasebe et al , 1994)	All clades
	636Fm	5′-ATGCGWTGGAGRGAYCGNTT-3′	(Lledo et al , 1998)	All clades
	724Rm	5′-TCRCATGTACCNGCRGTWG-3′	(Lledo et al , 1998)	All clades
	1204Rm	5′-CAAGGATGNCCTAARGTTCC-3′	(Zurawski et al , 1981)	All clades
matR	879Fm	5′-AGTTATTMTCAKGTCAGAGA-3′	(Meng et al , 2002)	All clades
	1002Rm	5′-CACCKWHGATTCCYAGTAGT-3′	(Meng et al , 2002)	All clades

Notes Primers have the same name as in the publication listed followed by an "m" to indicate that they have been modified for use in the clusioid clade Bonnetiaceae (B), Calophyllaceae (K), Clusiaceae s s (C), Hypericaceae (H), and Podostemaceae (P)

collected as part of a larger study to use complete plastid genomes to resolve relationships of the major subclades of Malpighiales (Xi et al , 2010)

Sequence assembly and phylogenetic analyses—Chromatograms were assembled into contiguous sequences and checked for accuracy using the program Sequencher ver 4 9 (Gene Codes Corp , Ann Arbor, Michigan, USA) Primer regions were removed and sequences were aligned by eye as translated amino acids using the program MacClade ver 4 08 (Maddison and Maddison, 2005) The ragged ends of the alignments and ambiguous internal regions were trimmed prior to analysis Data matrices and trees are available in the database TreeBASE (http //www treebase org, accession S10995) and from the first author

Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) were conducted to infer the phylogeny of the clusioid clade We analyzed potential conflict between the individual and combined data sets using alternative topology testing (see below) Analyses of the combined data were conducted on reduced and expanded data sets The reduced data set contained fewer taxa, but greater character density (ntax = 169, missing data = 8 4%) The expanded data set contained more taxa, but some taxa were missing data from one or more gene regions (ntax = 222, missing data = 19 4%) The expanded data set was important for including the most morphological, taxonomic, and biogeographic diversity in the group Taxa with missing characters or characters lacking data from some taxa are often excluded from phylogenetic studies due to concerns surrounding the adverse effects of missing data on phylogenetic inference However, recent work suggests that including taxa with missing data can provide increased phylogenetic resolution (McMahon and Sanderson, 2006, Wiens, 2006, Wiens and Moen, 2008)

The MP analyses were conducted with the program PAUP* ver 4 0b10 (Swofford, 2003) using the parsimony ratchet (Nixon, 1999) as implemented in the program PAUPRat (Sikes and Lewis, 2001, distributed by D Sikes at http // users iab uaf edu/~derek_sikes/software2 htm) We conducted 10 replicates of 200 iterations each with 15% of characters reweighted per iteration Gaps were treated as missing data and included in the analyses Bootstrap percentage (BP) support (Felsenstein, 1985) for each clade was estimated from 1000 heuristic search replicates using PAUP* (10 random taxon addition replicates, tree-bisection-reconnection [TBR] swapping, option MULTREES = yes, and holding no more than 10 trees per replicate)

The ML analyses were implemented with the parallel versions of the program RAxML ver 7 2 5 or 7 2 6 (Stamatakis, 2006, distributed by A Stamatakis at http //wwwkramer in tum de/exelixis/software html) Two partitioning schemes for each data set were used unpartitioned and partitioned by gene region Each analysis was conducted five times with different starting trees to check for convergence in likelihood values We determined the optimal model of evolution for the unpartitioned and partitioned data sets by using the Akaike information criterion (AIC) as implemented in the program ModelTest ver 3 7 (Posada and Crandall, 1998, Posada and Buckley, 2004) However, because RAxML does not allow for the specification of the TVM+I+Γ model (Table 3), the GTR+Γ model of evolution was applied to each partition in the partitioned data sets with all parameters estimated from the data The TVM and GTR models differ only by a single parameter, TVM constrains transition rates to be equal while transition rates are allowed to vary in the GTR model (Posada and Buckley, 2004) We chose not to estimate the proportion of invariant sites in the ML and BI analyses as suggested in the RAxML manual The invariant sites model, in particular, can fail to find important patterns of variation in the data as discussed by Pagel and Meade (2005) For each analysis, the optimal ML tree and BP values were estimated in the same run using the default settings The ML BP values were obtained from 1000 bootstrap replicates using the rapid bootstrap algorithm implemented in RAxML (Stamatakis et al , 2008)

The BI analyses were conducted using the parallel version of the program BayesPhylogenies ver 1 1 (Pagel and Meade, 2004, distributed by M Pagel at http //www evolution rdg ac uk/BayesPhy html) using a reversible-jump implementation of the mixture model as described in Venditti et al (2008) This approach allows the fitting of multiple models of sequence evolution to the data without a priori partitioning Default settings were applied, and a GTR model was used with among-site rate variation estimated by a gamma distribution with four rate categories We performed three independent analyses on each data set (six total runs) to determine consistency of stationary-phase likelihood values and estimated parameter values between runs Each Markov chain Monte Carlo run consisted of 10 million generations, with sampling of trees and parameters every 1000 generations Convergence was assessed using the program Tracer ver 1 5 (distributed by A Rambaut at http //tree bio ed ac uk/software/tracer/) Posterior probabilities (PP) were determined by building a 50% majority rule consensus tree after discarding the burn-in generations (the first 20% of the topologies were excluded in the first five runs, 40% of the topologies were excluded in the sixth)

Alternative topology tests—Alternative topology tests were conducted in a ML framework using the approximately unbiased (AU) test (Shimodaira, 2002) as implemented in the R software package, scaleboot ver 0 3-2 (Shimodaira, 2008, distributed by CRAN at http //www r-project org) All constrained searches were conducted, as described above, using the reduced and expanded data sets We initially determined whether the combined data could reject any of the topologies produced by individual genes, thereby indicating potential problems for analyzing these genes simultaneously To achieve this goal, we conducted separate tree searches on single gene data sets (*matK*, *ndhF*, *rbcL*, and *matR*) We considered two topologies to be at odds if both contained conflicting clades supported by ≥80 BP As such, clades supported by ≥80 BP in these individual gene analyses were then used to constrain searches on the combined data In addition, we also tested the monophyly of several traditionally recognized taxa that were found to be nonmonophyletic in our analyses We separately enforced monophyly for Clusiaceae s l (Calophyllaceae + Clusiaceae s s), *Dystovomita*, *Garcinia*, *Harungana*, *Hypericum*, *Ledermanniella*, *Tovomita*, and *Zeylanidium* Testing the monophyly of *Dystovomita* and *Zeylanidium* using the reduced data set was not possible due to insufficient taxon sampling Finally, we assessed the alternative placement of *Mourera* as found in the MP analyses In the MP analyses of both combined data sets, *Mourera* was placed sister to the Podostemoideae excluding *Diamantina*, while in the ML and BI analyses it was placed sister to a clade containing *Apinagia*, *Castelnavia*, *Marathrum*, *Monostylis*, *Noveloa*, and *Rhyncholacis* The MP placement was enforced and tested against the unconstrained ML trees

TABLE 3 Data set characteristics Values listed for individual genes are for the alignments derived from the reduced / expanded data sets, respectively Percentage of missing data is calculated as the total number of ?'s in the analyzed matrix divided by the total number of characters including gaps Models of sequence evolution were chosen by the Akaike information criterion using ModelTest 3 7 pt, plastid, mt, mitochondrial

Characteristic	pt *matK*	pt *ndhF*	pt *rbcL*	mt *matR*	Reduced total	Expanded total
Terminals	169 / 209	169 / 204	169 / 201	169 /190	169	222
Characters analyzed	1455	1086	1296	2400	6237	6237
% missing data	8 4 / 10 9	15 4 / 17 6	5 1 / 5 1	6 9 / 9 6	8 4	19 4
% gaps plus missing data	31 4 / 32 7	28 0 / 29 8	5 4 / 8 1	35 4 / 36 0	26 9	35 6
Constant characters	555 / 528	403 / 400	781 / 770	1467 / 1450	3206	3148
Variable characters	900 / 927	683 / 686	515 / 526	933 / 950	3031	3089
Parsimony informative characters	732 / 766	550 / 560	371 / 382	586 / 606	2239	2314
% Parsimony informative characters	50 / 53	51 / 52	29 / 29	24 / 25	36	37
Model of sequence evolution	TVM+I+Γ / TVM+I+Γ	TVM+I+Γ / TVM+I+Γ	GTR+I+Γ / TVM+I+Γ	GTR+Γ / GTR+Γ	GTR+I+Γ	GTR+I+Γ

RESULTS

Sequences/matrices—Our combined alignment included 6237 nucleotide bases One hundred fifty-seven, 161, 125, and 144 sequences for *matK*, *ndhF*, *rbcL*, and *matR* were newly obtained for this study, respectively (Appendix 1, GenBank numbers HQ331542-HQ332128) These additions include the first published *ndhF* sequences for Podostemaceae Genes *matK* and *ndhF* were the most variable markers and had a nearly equal percentage of parsimony informative characters, *rbcL* was slightly more informative than *matR* Relevant characteristics for each gene region and data set are listed in Table 3

Phylogenetic analyses—Topologies derived from the combined data sets using MP, ML, and BI methods were largely congruent and contained no well-supported differences Additionally, ML topologies resulting from unpartitioned and partitioned data sets were also congruent within and between partitioning schemes The MP BP values were often lower than ML BP values, while BI support values were sometimes much higher (see Figs 1–4) Furthermore, artificially inflated support values in BI analyses have been previously noted (Suzuki et al , 2002, Douady et al , 2003, Simmons et al , 2004) For these reasons, we will focus our discussion below on the 50% ML majority-rule consensus tree of the partitioned expanded data set (Figs 1, 2) In addition, results from the partitioned reduced data set (Figs 3, 4) and BI support values from each figure will be mentioned where relevant

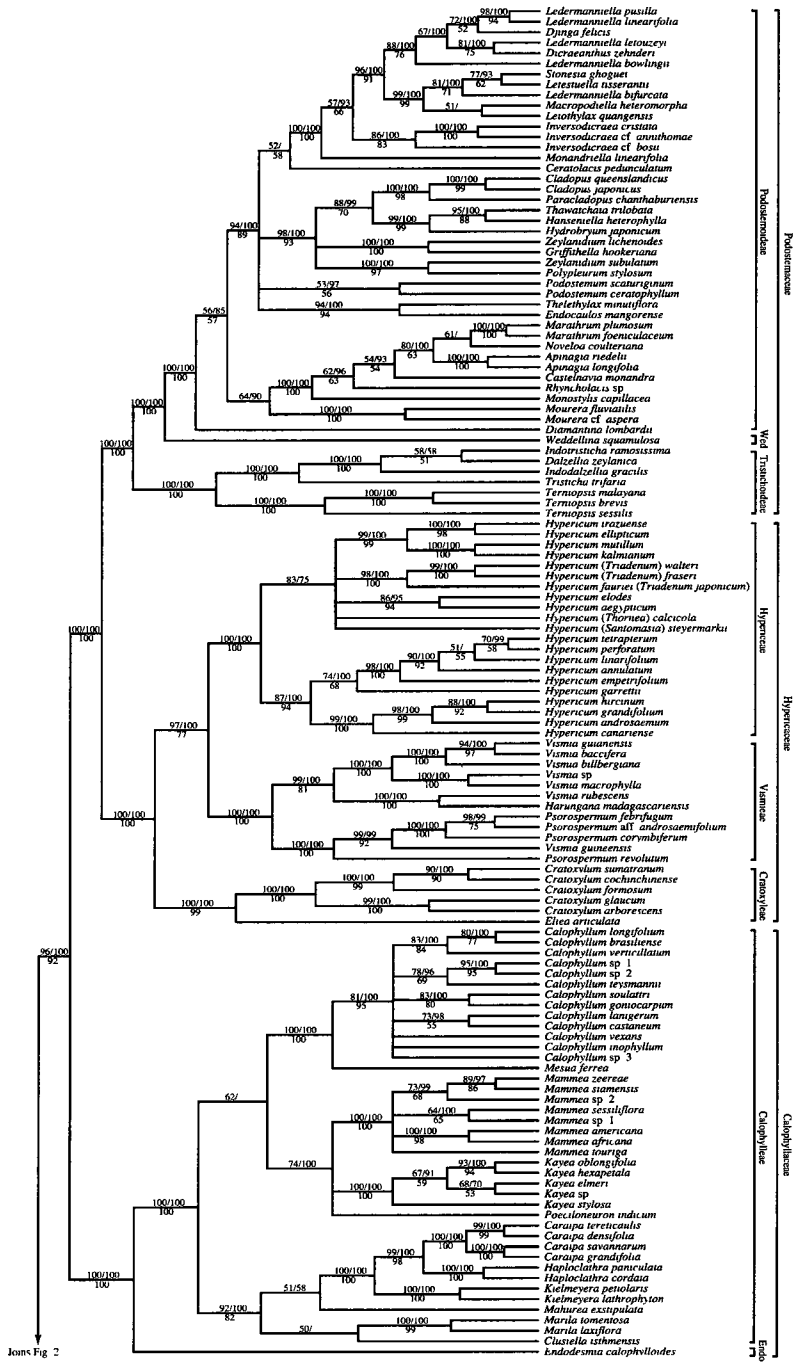
Outgroup relationships are generally in agreement with those reported in Wurdack and Davis (2009) Malpighiales are strongly supported (100 BP, data not shown) as monophyletic, but relationships between its major subclades are largely unresolved One difference in the Bayesian analyses relates to the placement of *Bruguiera* (Rhizophoraceae) as sister to *Cyrillopsis* (Ixonanthaceae) with 95 PP and 98 PP with the reduced and expanded data sets, respectively (data not shown) *Irvingia* (Irvingiaceae) is in turn sister to this clade with 98 PP and 90 PP with the reduced and expanded data sets, respectively (data not shown) The placements of Irvingiaceae and Ixonanthaceae were unresolved by Wurdack and Davis (2009), and Rhizophoraceae + Erythroxylaceae were instead placed as sister to Ctenolophonaceae The latter was unplaced in our results We advise caution when interpreting these results, however, because our sampling includes a relatively small representation of non-clusioid taxa and far fewer genes than in the study by Wurdack and Davis (2009)

The clusioid clade and each of its five families are strongly supported (100 BP) as monophyletic in all analyses Moreover, the interfamilial relationships reported here are the same as those in Wurdack and Davis (2009) Within the clusioid clade, Bonnetiaceae and Clusiaceae s s form a clade (88 BP, Fig 2) This clade is sister to a strongly supported (96 BP, Fig 1) clade containing the remaining three families Calophyllaceae, Hypericaceae, and Podostemaceae Calophyllaceae are sister to a strongly supported (100 BP) clade containing Hypericaceae and Podostemaceae

Alternative topology tests—No individual gene topologies from the expanded data set were rejected by the combined expanded data set The individual gene topologies of *ndhF* and *matR* derived from the reduced data set, however, were rejected by the reduced combined data (Table 4) In the *ndhF* topology, well-supported conflict was identified in Hypericaceae and Podostemaceae In Hypericaceae, conflict involved the placement of *Hypericum grandifolium* Choisy This taxon was sister to *Hypericum androsaemum* L in the *ndhF* topology (85 BP, data not shown), but sister to *Hypericum hircinum* L in the combined data topology (88 BP, Fig 3) Conflict in Podostemaceae involved the placement of *Dicraeanthus zehnderi* H E Hess, which was placed sister to *Ledermanniella bowlingii* (J B Hall) C Cusset in the *ndhF* topology (91 BP, data not shown) but sister to *Ledermanniella letouzeyi* C Cusset in the combined data topology (83 BP, Fig 3) In the *matR* topology, well-supported conflict was identified in the *Caraipa* (Calophyllaceae) and *Cratoxylum* (Hypericaceae) clades *Caraipa densifolia* Mart was placed sister to a well-supported (81 BP, data not shown) clade containing the remaining *Caraipa* species In the combined reduced topology, *C densifolia* was instead strongly placed

→

Fig 1 Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on the combined four-gene expanded data set (ntax = 222, missing data = 19 4%) Support values ≥ 50% are indicated Values above branches are ML bootstrap values (left) and Bayesian inference posterior probabilities converted to percentages (right) Maximum parsimony bootstrap values are given below each branch A hyphen indicates that the node was not present in a particular analysis Endo , Endodesmieae, Wed , Weddellinoideae Revised names for Hypericeae genera are given, former names are included in parentheses Tree continued in Fig 2


Ledermanniella pusilla
Ledermanniella lineariifolia
Djinga felicis
Ledermanniella letouzeyi
Dicraeanthus zehnderi
Ledermanniella bowlingii
Stonesia ghoguei
Letestuella usserantii
Ledermanniella bifurcata
Macropodiella heteromorpha
Letothylax guangensis
Inversodicraea cristata
Inversodicraea cf annithomae
Inversodicraea cf bosii
Monandriella linearifolia
Ceratolacis pedunculatum
Cladopus queenslandicus
Cladopus japonicus
Paracladopus chanthaburiensis
Thawatchaia trilobata
Hanseniella heterophylla
Hydrobryum japonicum
Zeylanidium lichenoides
Griffithella hookeriana
Zeylanidium subulatum
Polypleurum stylosum
Podostemum scaturiginum
Podostemum ceratophyllum
Thelethylax minutiflora
Endocaulos mangorense
Marathrum plumosum
Marathrum foeniculaceum
Noveloa coulteriana
Apinagia riedelii
Apinagia longifolia
Castelnavia monandra
Rhynchulacis sp
Monostylis capillacea
Mourera fluviatilis
Mourera cf aspera
Diamantina lombardii
Weddellina squamulosa
Indotristicha ramosissima
Dalzellia zeylanica
Indodalzellia gracilis
Tristicha trifaria
Terniopsis malayana
Terniopsis brevis
Terniopsis sessilis
Hypericum uralense
Hypericum ellipticum
Hypericum mutilum
Hypericum kalmianum
Hypericum (Triadenum) walteri
Hypericum (Triadenum) fraseri
Hypericum fauriei (Triadenum japonicum)
Hypericum elodes
Hypericum aegyptiacum
Hypericum (Thornea) calycicola
Hypericum (Santomasia) steyermarkii
Hypericum tetrapterum
Hypericum perforatum
Hypericum linarifolium
Hypericum annulatum
Hypericum empetrifolium
Hypericum garrettii
Hypericum hircinum
Hypericum grandifolium
Hypericum androsaemum
Hypericum canariense
Vismia guianensis
Vismia baccifera
Vismia billbergiana
Vismia sp
Vismia macrophylla
Vismia rubescens
Harungana madagascariensis
Psorospermum febrifugum
Psorospermum aff androsaemifolium
Psorospermum corymbiferum
Vismia guineensis
Psorospermum revolutum
Cratoxylum sumatranum
Cratoxylum cochinchinense
Cratoxylum formosum
Cratoxylum glaucum
Cratoxylum arborescens
Eliea articulata
Calophyllum longifolium
Calophyllum brasiliense
Calophyllum verticillatum
Calophyllum sp 1
Calophyllum sp 2
Calophyllum teysmannii
Calophyllum soulattri
Calophyllum gomiocarpum
Calophyllum lanigerum
Calophyllum castaneum
Calophyllum vexans
Calophyllum inophyllum
Calophyllum sp 3
Mesua ferrea
Mammea zeerae
Mammea siamensis
Mammea sp 2
Mammea sessiliflora
Mammea sp 1
Mammea americana
Mammea africana
Mammea touriga
Kayea oblongifolia
Kayea hexapetala
Kayea elmeri
Kayea sp
Kayea stylosa
Poeciloneuron indicum
Caraipa tereticaulis
Caraipa densifolia
Caraipa savannarum
Caraipa grandifolia
Haploclathra paniculata
Haploclathra cordata
Kielmeyera petiolaris
Kielmeyera lathrophyton
Mahurea exstipulata
Marila tomentosa
Marila laxiflora
Clusiella isthmensis
Endodesma calophylloides
Podostemaceae
Podostemoideae
Weddellinoideae
Tristichoideae
Hypericaceae
Hypericoideae
Vismioideae
Crateoxyleae
Clusioideae
Calophyllaceae
Calophylleae
Endo
Joins Fig. 2

(98 BP, Fig 3) as sister to *Caraipa tereticaulis* Tul Conflict within *Cratoxylum* involved the placement of *Cratoxylum formosum* (Jack) Dyer This taxon was sister to *Cratoxylum sumatranum* (Jack) Blume in the *matR* topology (88 BP, data not shown) but sister to the well-supported (89 BP, Fig 3) clade containing *Cratoxylum cochinchinense* (Lour) Blume and *C sumatranum* in the combined topology Upon further inspection, it appears that partially missing *matR* data for *Caraipa densifolia* and *Cratoxylum cochinchinense* may explain these incongruencies The *matR* sequences of the taxa within each of these clades are identical, and as a result, taxa with partial *matR* data may be spuriously placed

These topological conflicts suggest possible concerns with combining our data in the reduced data set for phylogenetic analyses However, topologies derived from the individual genes and the topology produced by the combined reduced data set are largely congruent, and where topological differences occur, very few of these are moderately to strongly supported Importantly, these differences are only near the tips and between closely related taxa, indicating that conflict in the backbone of the topology was not evident We advise readers to proceed cautiously when interpreting areas where conflict was discovered in the *ndhF* gene topology when compared to the combined reduced topology Nevertheless, none of these areas are the focus of our study, and as such their implications will not be discussed further

Finally, the monophyly of Clusiaceae s l (Calophyllaceae + Clusiaceae s s) could not be rejected (Table 4) Specific results concerning the topology and topological tests within each family are addressed in the Discussion

DISCUSSION

Our results have provided several new insights into the clusioid phylogeny We increased ingroup taxon sampling by at least a factor of 4 5 compared to previous studies (Gustafsson et al , 2002, Wurdack and Davis, 2009), and resolution within the clade is much greater than in previous studies over 60% of the clades in the ML tree were resolved with ≥80 BP (Figs 1, 2) We resolved the position of four genera that have not been included in previous molecular studies (i e , *Diamantina*, *Ceratolacis*, *Griffithella*, and *Santomasia*), and identified several genera that are not monophyletic as currently circumscribed (i e , *Harungana*, *Hypericum*, *Ledermanniella* s s , *Tovomita*, and *Zeylanidium*) This phylogeny provides a firm foundation for reassessing the current classification of the clusioid clade (see Table 1 for a summary of our proposed changes) We discuss important results for each family below

Bonnetiaceae—Bonnetiaceae are a small family of 35 species with a disjunct distribution between South America and Southeast Asia *Archytaea* and *Bonnetia* are distributed exclusively in the New World, while *Ploiarium* are found only in Southeast Asia Bonnetiaceae are split into two strongly supported (100 BP) subclades the first containing the genera *Archytaea* and *Ploiarium,* and the second containing *Bonnetia* These two subclades are well defined by anatomical, vegetative, and floral features (Baretta-Kuipers, 1976, Dickison and Weitzman, 1996, 1998, Weitzman and Stevens, 1997, Weitzman, 2005, Weitzman et al , 2007) *Archytaea* and *Ploiarium* share unilacunar nodes, vascularized disciform structures on leaves and/or bracts, and marginal setae of the leaves associated with vascular tissue Shared floral features between these two genera include a five-locular ovary that develops into a capsule that dehisces from the proximal end Additionally, their androecium is fasciculate with five staminodes In *Bonnetia*, nodes are trilacunar, no disciform structures are present on the leaves and/or bracts, and marginal setae are not associated with vascular tissue The ovary in *Bonnetia* is three- to four-locular and develops into a capsule that dehisces normally from the distal end The androecium is apparently not fasciculate (but see Steyermark, 1984), and staminodes are absent *Bonnetia* additionally have a mucilaginous epidermis, a foliar endodermis, and foliar sclereids, which are not present in the *Archytaea* + *Ploiarium* clade

All previous molecular studies that included *Bonnetia* sampled only a single species We include eight species representing the entire biogeographic range of the genus Within *Bonnetia*, *B roraimae* Oliv is placed sister to the remaining *Bonnetia* species This relationship is weakly supported by ML (53 BP), but strongly supported by BI (97 PP) *Bonnetia ahogadoi* (Steyerm) A L Weitzman & P F Stevens was placed by Steyermark (1984) in a separate genus, *Acopanea* Weitzman and Stevens (1997) transferred *Acopanea* into *Bonnetia* on the basis of anatomy and morphology, a conclusion which is supported by our analyses Only three *Bonnetia* species [i e , *B cubensis* (Britton) R A Howard, *B stricta* (Nees) Nees & Mart , and *B paniculata* Spruce] occur outside of the Guiana Shield region in adjacent areas in South America and Cuba These species are embedded within the *Bonnetia* clade (Fig 2) The phylogenetic distribution of *Bonnetia* species occurring in the Guiana Shield suggests that this region is not only the center of diversity for the genus, but may also be its center of origin

Calophyllaceae—All genera of Calophyllaceae are monophyletic in our analyses The monotypic genus *Endodesmia* is well supported (100 BP) as sister to the remaining Calophyllaceae This latter clade represents tribe Calophylleae, which contains three moderately to well-supported subclades, whose interrelationships are unclear The first is strongly supported (92 BP) and contains the strictly New World genera *Caraipa*, *Clusiella*, *Haploclathra*, *Kielmeyera*, *Mahurea*, and *Marila* The alternate-leaved genera *Caraipa*, *Kielmeyera*, and *Mahurea* occur together in a weakly supported clade (51 BP) with the opposite-leaved *Haploclathra*, which is sister to *Caraipa* (99 BP) In contrast to other Calophyllaceae, these four genera, as well as the unsampled *Neotatea*, possess winged seeds (Notis, 2004) Taxa with cordate cotyledons (*Caraipa*, *Haploclathra*, and *Kielmeyera*) form a strongly supported (100 BP) clade *Clusiella* and *Marila* are weakly supported (50 BP) as a clade in the expanded data set, but support for this relationship increases greatly in the reduced data set analysis (71 BP, Fig 3) This relationship has been suggested by Hammel (1999b) based on the shared features of small foveolate seeds and an embryo with well-developed cotyledons In addition, investigations of the cotyledon-to-hypocotyl ratio in Calophyllaceae indicate that *Clusiella*, *Marila*, *Neotatea*, and *Mahurea* possess ratios between 0 2 to 2, while all other Calophyllaceae have a ratio greater than 2 (P F Stevens, Missouri Botanical Garden and University of Missouri, St Louis, unpublished data)

The second and third subclades together form a poorly supported clade (62 BP) The second subclade is moderately supported (74 BP) and includes *Kayea*, *Mammea*, and *Poecilloneuron*, the third subclade is strongly supported (100 BP) and includes *Calophyllum* and *Mesua* Although molecular support for the sister-group relationship of these subclades is weak, a

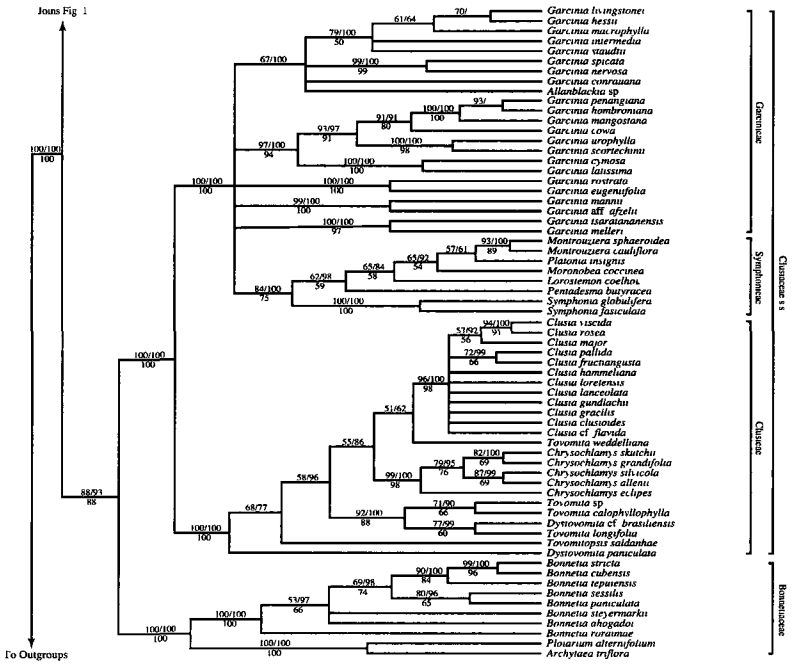


Fig 2 Continuation of Fig 1 Fifty percent maximum likelihood majority-rule consensus tree of the clusioid clade based on the combined four-gene expanded data set (ntax = 222, missing data = 19 4%) Outgroups removed to show only the clusioid clade

close relationship among these taxa has been suggested based on morphology (Engler, 1925, Stevens, 1980) Taxa in these clades possess ovules with basal placentation, and commonly two to four sepals, petals, and carpels Genera in these two subclades also share primarily Old World distributions The larger genera, *Calophyllum* and *Mammea,* additionally include a small number of New World species The New World species of these genera that we sampled are embedded within these principally Old World clades (*Calophyllum brasiliense* Cambess and *C longifolium* Willd , *Mammea americana*, Fig 1), suggesting a possible Old World origin for *Calophyllum* and *Mammea* In contrast to members of these principally Old World subclades, members of the strictly New World subclade described above tend to have axile or intruded parietal placentation, five sepals and petals, and three carpels (Notis, 2004, Stevens, 2007a) Our placement of *Mammea* differs strongly from Notis (2004), who found it to be sister to all other Calophylleae Relationships between *Kayea*, *Mammea*, and *Poeciloneuron* are unresolved in our trees Although *Kayea* and *Poeciloneuron* are poorly supported as sister taxa (<50 BP), this relationship is corroborated by Notis (2004) and by morphology These genera share a punctate stigma that differs from the expanded stigma of *Mammea* (Notis, 2004)

Our sampling within *Mammea* allowed us to partially examine the phylogenetic hypothesis of Dunthorn (2009) who proposed species groups based on variation in leaf and petiole anatomy Our results indicate that species of his "Americana group" (represented by *Mammea americana* L and *Mammea africana* G Don in Fig 1) are strongly monophyletic (100 BP), but members of his "Eugenioides group" (represented by *Mammea siamensis* (Miq) T Anderson, *M* sp 1, and *M* sp 2 in Fig 1) are not The position of the distinctive *Mammea touriga* (C T White & Francis) L S Sm , a species that lacks lamina fibers (Dunthorn, 2009), is not well supported in our ML analyses Nevertheless, in both ML trees (data not shown), this taxon is placed sister to a clade containing *M americana* and *M africana*, which also lack lamina fibers Finally, results within *Mammea* are interesting biogeographically because the Malagasy species (represented by *M* sp 1, *M sessiliflora* Planch & Triana, and *Mammea zeereae* P F Stevens in Fig 1) do not form a clade

We were unable to sample the genera *Lebrunia* (Endodesmieae) and *Neotatea* (Calophylleae) *Lebrunia* is considered to be a close relative of *Endodesmia* (Stevens 2007a), and these genera together constitute tribe Endodesmieae *Endodesmia* and *Lebrunia* are each monotypic and found in western tropical Africa They possess a single, apical ovule, which in Calophyllaceae, is found only in tribe Endodesmieae (Stevens, 2007a) *Neotatea* was originally described as a genus in Bonnetiaceae (Maguire, 1972) and was once considered a species of *Bonnetia* (Steyermark, 1984) However, the placement of this species was problematic due to its possession of unilacunar nodes, latex, an indumentum, smooth stigmatic surfaces, and anther glands (Weitzman and Stevens, 1997) More recently, it was transferred to Clusiaceae s l (including Hypericaceae, Weitzman and Stevens, 1997) and then placed in tribe Calophylleae (Stevens 2007a) *Neotatea* possesses alternate leaves and winged seeds, which as noted previously, appear in only one Calophyllaceae clade Thus, *Neotatea* is likely to be placed

somewhere among these taxa This hypothesis is supported by Notis (2004) who found *Neotatea* to be sister to *Mahurea*, based on the shared presence of intruded axile placentae bordered by in-curled carpel walls and seeds with a vascularized wing that does not completely surround the seed

Clusiaceae s.s.—Clusiaceae s s include two strongly supported (100 BP) subclades The first contains all genera of the strictly New World tribe Clusieae Clusieae are characterized by a lack of bud scales, prevalent dioecy, nonfasciculate androecia, and fleshy capsules with arillate seeds (Stevens, 2007a) Support for intergeneric relationships within Clusieae is generally weak Morphological characters indicating phylogenetic relationships are mostly lacking, but characters of the aril, leaf bases, and sepals seem promising for future study *Chrysochlamys* and *Clusia* are strongly supported as monophyletic (96 and 99 BP, respectively), *Dystovomita* and *Tovomita* are nonmonophyletic, but their monophyly could not be rejected (Fig 2, Table 4) *Dystovomita paniculata* (Donn Sm) Hammel is weakly placed as sister to all other Clusieae and *Dystovomita* cf *brasiliensis* D'Arcy is strongly (92 BP) embedded within a clade of *Tovomita* spp The nonmonophyly of *Dystovomita* should be interpreted cautiously, however, because the name *D brasiliensis* was applied to this taxon in the Flora Reserva Ducke (Ribeiro, 1999) with the hope of eventually comparing it to the type specimen Unfortunately, the type appears to have been lost Thus, we cannot validate the identification of our specimen and cannot know with certainty if *Dystovomita* sensu D'Arcy (1978) is nonmonophyletic However, we can say that the taxon labeled as *D* cf *brasiliensis* in our analyses and the taxon listed as *D brasiliensis* in the Flora Reserva Ducke are better attributed to *Tovomita*, a genus that may also be nonmonophyletic *Tovomita weddelliana* Planch & Triana is weakly placed (51 BP) as sister to *Clusia* rather than with the remaining *Tovomita* species (Fig 2) Interestingly, *T weddelliana* and species of *Clusia* are both found at relatively high altitudes in the Neotropics All other members of the tribe are generally found in lowland tropical forests (Gustafsson et al , 2007) It is surprising that *Tovomitopsis* is not placed near *Chrysochlamys* because the two are morphologically similar and have often been considered synonymous (Hammel, 1999a) It may be that biogeography is more helpful than morphology for separating these two genera *Chrysochlamys* occurs in Central America, the Caribbean, and northwestern South America, *Tovomitopsis* occurs in southeastern Brazil (Bittrich, 2010)

The second subclade in Clusiaceae s s includes all Garcinieae and Symphonieae In contrast to Clusieae, this group is characterized by a fasiculate androecium (Stevens, 2007a, Sweeney, 2008) We provide the first strongly supported evidence that Symphonieae are monophyletic (84 BP, Fig 2) Previous results have suggested that they may not be monophyletic (Gustafsson et al , 2002, Sweeney, 2008), which was surprising based on morphology Members of this clade possess a branched style with each branch having no exposed stigmatic surface Instead, there is a small apical pore in the stigma through which pollen enters the stigmatic cavity, which is unique in Malpighiales (Bittrich and Amaral, 1996) Within Symphonieae, *Pentadesma* and *Symphonia* are genera with Old World origins (Dick et al , 2003, Stevens, 2007a, Dick and Heuertz, 2008) and are successive sister groups to a clade containing the New World taxa *Lorostemon*, *Moronobea*, and *Platonia* plus the New Caledonian genus *Montrouziera* The only genus in Symphonieae we were not able to include was the poorly known *Thysanostemon* from Guyana *Thysanostemon* is certainly a member of the tribe Symphonieae, based on both vegetative and floral characteristics, and may be closely related to *Lorostemon* These two genera have very elongated flower buds and pollen with supratectal elements, features not present in other Symphonieae (Maguire, 1964, Seetharam, 1985)

We found no support for a monophyletic Garcinieae In contrast, Sweeney (2008) found Garcinieae to be strongly monophyletic using nuclear data (ITS and GBSSI) Additionally, members of Garcinieae possess several characters that unite the group colleters, dioecy, capitate stigmas, eperulate buds (common), and introrse anthers (often) These features contrast with Symphonieae, which lack colleters, are hermaphroditic, and possess porose stigmas, perulate buds, and extrorse anthers (Stevens, 2007a, Sweeney, 2008) Relationships within *Garcinia* presented here are in agreement with Sweeney (2008) Importantly, we also find *Allanblackia* embedded within *Garcinia* (67 BP, Fig 2) Support for this placement increases in the analysis of the reduced data set (82 BP, Fig 4) and is strong in both BI analyses (100 PP) This corroborates the recommendation by Sweeney (2008) that *Allanblackia* be transferred to *Garcinia* Furthermore, floral characters also support this placement *Allanblackia* and all *Garcinia* species in this subclade have nectariferous appendages in the flower, unlike other members of *Garcinia* (Sweeney, 2008) However, a monophyletic *Garcinia* (excluding *Allanblackia*) could not be rejected by the combined data sets (Table 4)

Hypericaceae—Three strongly supported subclades (100 BP) are recovered in Hypericaceae corresponding to tribes Cratoxyleae, Hypericeae, and Vismieae (Stevens, 2007b, see also Wurdack and Davis, 2009) Cratoxyleae are sister to a strongly supported (97 BP) clade containing Hypericeae + Vismieae Within Cratoxyleae, *Cratoxylum* and the monotypic *Eliea* are sister taxa We sampled five of the six *Cratoxylum* species representing the three sections recognized by Gogelein (1967) This sampling allowed us to test his hypothesis of relationships in the group, which agreed with our results Species in section *Isopterygium* [*Cratoxylum arborescens* (Vahl) Blume and *Cratoxylum glaucum* Korth] are evergreen trees with straight secondary leaf venation and a wing that surrounds the seed This section is sister to a clade containing sections *Cratoxylum* [*Cratoxylum sumatranum* (Jack) Blume and *Cratoxylum cochinchinense* Blume] and *Tridesmos* [*Cratoxylum formosum* (Jack) Benth & Hook f ex Dyer and *Cratoxylum maingayi* Dyer (not sampled)], which are more or less deciduous trees with curved secondary leaf venation and a unilateral seed wing

Vismieae have been previously treated by Bamps (1966) and most recently by Stevens (2007b) Bamps recognized three genera *Harungana*, *Psorospermum*, and *Vismia* Bamps' *Harungana* and *Psorospermum* are found in Africa and Madagascar, while his *Vismia* is divided into two subgenera, *Vismia* and *Afrovismia,* found in the Americas and Africa, respectively More recently, Stevens (2007b) considered the tribe to have only two genera, *Harungana* and *Vismia*, distributed in the Old World (Africa and Madagascar) and New World (Central and South America), respectively Formal taxonomic changes however, were not made to reflect this viewpoint Morphological characteristics that Stevens used to separate these two genera included the fusion of bracts to the pedicels (unfused in *Vismia* vs fused in *Harungana*) and staminode pubescence (pubescent in *Vismia* vs glabrous in *Harungana*, Bamps, 1966, Stevens, 2007b)

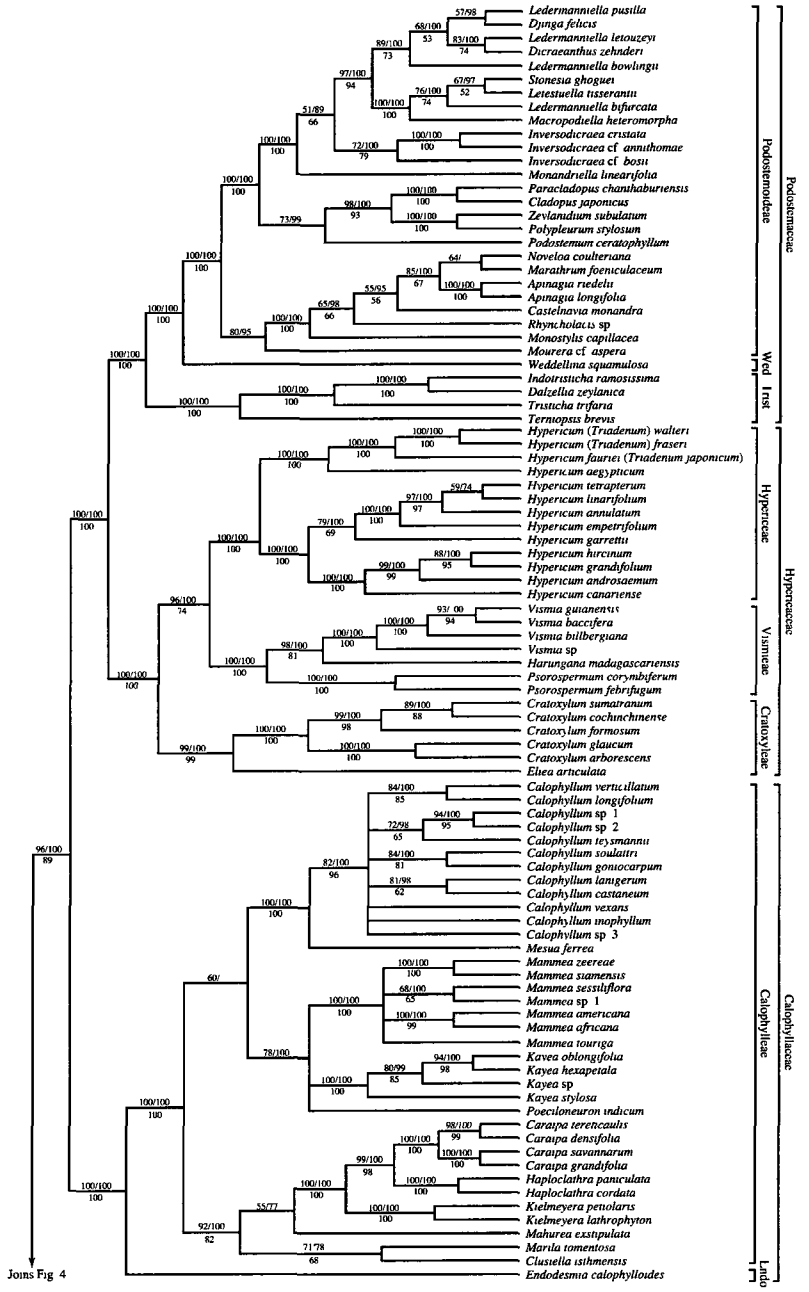


Fig 3 Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on the combined four-gene reduced data set (ntax = 169, missing data = 8 4%) Support values ≥ 50% are indicated Values above branches are ML bootstrap values (left) and Bayesian inference posterior probabilities converted to percentages (right) Maximum parsimony bootstrap values are given below each branch A hyphen indicates that the node was not present in a particular analysis Endo , Endodesmieae, Trist , Tristichoideae, Wed , Weddellinoideae Revised names for Hypericeae genera are given, former names are included in parentheses Tree continued in Fig 4

Our results indicate that neither of these classifications reflect phylogenetic relationships (Fig 1) *Harungana* sensu Stevens (i e , Old World Vismieae) is paraphyletic and includes American Vismieae *Vismia* subgenus *Afrovismia* sensu Bamps is also not monophyletic *Vismia guineensis* (L) Choisy is embedded in *Psorospermum*, and *Vismia rubescens* Oliv is sister to *Harungana madagascariensis* Poir We believe that the sampling here is too preliminary to propose taxonomic revisions However, restricting *Harungana* to include only *H madagascariensis* (the type species of the genus) and *Vismia rubescens*, and including all other African and Malagasy species in an extended *Psorospermum* is a reasonable solution if these relationships are further corroborated by additional data Morphological distinctions between these groups are lacking, but characters of the cotyledons and the position of the bracteoles on the inflorescence may be useful

Within the third subclade, Hypericeae, *Hypericum* sensu Robson (1977 onward) and Stevens (2007b) is not monophyletic (Fig 1, Table 4) These authors recognize four small genera (*Lianthus*, *Santomasia*, *Thornea*, and *Triadenum*) as separate from *Hypericum*, primarily based on the possession of staminodes, which are mostly absent in *Hypericum* (Robson, 1972, 1977, Stevens 2007b) White, pink, or reddish petals further separate *Lianthus*, *Thornea*, and *Triadenum* from *Hypericum*, which has yellow petals (Breedlove and McClintock, 1976, Robson, 1981, 2001, Stevens 2007b) However, in our analyses, *Santomasia*, *Thornea*, and *Triadenum* are well supported as members of a subclade of *Hypericum* (83 BP) This result does not agree with a recent morphological analysis of Hypericaceae where only *Santomasia* was found to be embedded within *Hypericum* (Nurk and Blattner 2010) The distribution of staminodes in the androecium of Hypericeae species offers additional support for our result As stated previously, staminodes are present in *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum*, as well as in all members of Cratoxyleae and Vismieae However, staminodes are largely absent in *Hypericum*, except in sections *Adenotrias* and *Elodes* (represented in our study by *H aegyptiacum* L and *H elodes* L , respectively [Robson, 1996, Fig 1]) All Hypericeae taxa with staminodes occur in the same *Hypericum* subclade We were unable to sample *Lianthus*, but it is very likely that this monotypic genus is also a member of this subclade because it possesses staminodes and shows strong affinities with *Thornea* and *Triadenum* (Robson, 2001) Given the embedded position of these smaller genera in *Hypericum*, we propose that *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* be reinstated as members of *Hypericum* (Table 1) These taxa have all previously been described as members of *Hypericum*, and as such, appropriate names are available (Table 5)

Podostemaceae—Our results generally agree with previous studies but include much denser character and taxon sampling (Kato et al , 2003, Kita and Kato, 2004a, b, Moline et al , 2006, 2007, Koi et al , 2008, 2009, Pfeifer et al , 2009, Thiv et al , 2009, Koi and Kato, 2010, Tippery et al , in press) We recognize the three subfamilies proposed by Engler (1930), which are each strongly supported (100 BP) as monophyletic here and elsewhere (Kita and Kato, 2001, Moline et al , 2007)

Tristichoideae are strongly supported as monophyletic (100 BP) and are sister to a clade containing subfamilies Podostemoideae + Weddellinoideae Tristichoideae have tricarpellate ovaries and pantoporate pollen, in contrast to Podostemoideae and Weddellinoideae, which have bicarpellate ovaries and mostly tricolporate or tricolpate pollen (Kita and Kato, 2001, Cook and Rutishauser, 2007) Within the Tristichoideae clade, bootstrap support for *Dalzellia* + *Indotristicha* is weak (58 BP, Fig 1), which is surprising because this clade has received strong support elsewhere (Koi et al , 2009) The *Dalzellia* + *Indotristicha* clade is also supported by morphology a leafy cupule surrounding the flower bud is a putative synapomorphy for this clade (Koi et al , 2009) The only genus in this subfamily we were unable to include was the recently described *Cussetia*, which shows affinities to *Terniopsis* and *Tristicha* (Kato, 2006, 2009, Koi et al , 2009)

Podostemoideae are strongly supported as monophyletic (100 BP) and are characterized by the presence of a spathella that encloses the flower bud prior to anthesis Its sister clade, Weddellinoideae, differs from Podostemoideae by the absence of a spathella and the presence of a distinct perianth, which are likely plesiomorphic characters shared with Tristichoideae (Kita and Kato, 2001) For the first time, we present evidence that the monotypic New World genus *Diamantina* is sister to the remaining Podostemoideae (Fig 1) Its position is poorly supported (56 BP), likely because we were only able to obtain a portion of *matK* for this taxon However, previous authors have hypothesized a similar phylogenetic placement of *Diamantina* (Philbrick et al , 2004b, Rutishauser et al , 2005, Koi et al , 2006) Among the remaining Podostemoideae, there are two subclades, an exclusively New World clade represented by *Apinagia*, *Castelnavia*, *Marathrum*, *Monostylis*, *Mourera*, *Noveloa*, and *Rhyncholacis* (Fig 1) and a primarily Old World clade containing all other genera sampled here The two New World genera, *Ceratolacis* and *Podostemum*, are an exception and are embedded within this primarily Old World clade Kita and Kato (2001) showed that *Podostemum* was more closely related to the Old World members of Podostemoideae, but our results are the first strong evidence that *Ceratolacis* belongs to the Old World clade (94 BP, Fig 1) This mostly Old World clade is loosely characterized by the possession of an androphodium, one or two stamens per flower, and pollen dyads (which are sometimes secondarily lost) The strictly New World clade is characterized by often having several free stamens per flower and pollen in monads (Cook and Rutishauser, 2007)

Much greater taxon sampling is needed in the New World Podostemoideae clade before evolutionary, taxonomic, and biogeographical patterns can be inferred (see also Tippery et al , in press) In particular, sampling in the genera *Apinagia*, *Marathrum*, and *Rhyncholacis* will need to be improved to further determine their limits Furthermore, the New World genera *Cipoia*, *Macarenia*, and *Wettsteiniola* have never been included in a molecular phylogenetic study *Macarenia* and *Wettsteiniola* are likely members of this clade based on morphological analysis (C T Philbrick, unpublished data) *Cipoia*, however, shares traits with members of the primarily Old World clade, such as pollen in dyads (Philbrick et al , 2004b, Bove et al , 2006) All New World taxa with dyad pollen sampled to date have been placed in the primarily Old Word clade (i e , *Ceratolacis* and *Podostemum*)

The mostly Old World Podostemoideae clade is composed of four subclades whose interrelationships are unresolved (1) the New World genus *Podostemum*, (2) the Malagasy genera *Endocaulos* and *Thelethylax*, (3) the Asian and Australian genera *Cladopus*, *Griffithella*, *Hansenuella*, *Hydrobryum*, *Paracladopus*, *Polypleurum*, *Thawatchaia*, and *Zeylanidium*, and (4) the Brazilian genus *Ceratolacis* plus the African genera *Dicraeanthus*, *Djinga*, *Inversodicraea*, *Ledermanniella*, *Leiothylax*, *Letestuella*, *Macropodiella*, *Monandriella*, and *Stonesia* *Podostemum* is a

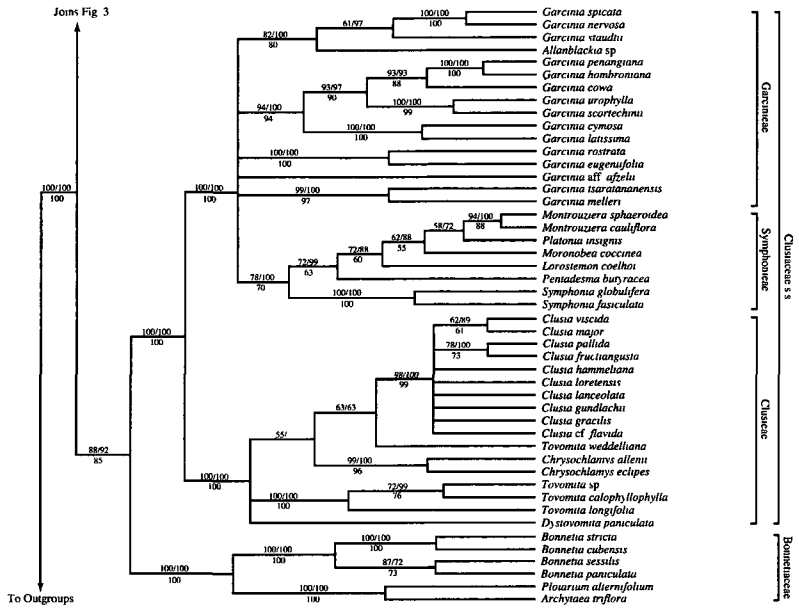


Fig 4 Continuation of Fig 3 Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on the combined four-gene reduced data set (ntax = 169, missing data = 8 4%) Outgroups removed to show only the clusioid clade

weakly supported (53 BP) clade in our ML expanded data set analysis, but is strongly supported by BI (97 PP) The latter results are corroborated by previous studies, which provide strong morphological and molecular evidence that *Podostemum* is monophyletic (Philbrick and Novelo, 2004, Moline et al , 2006) Although *Podostemum* forms a polytomy with the three other subclades in our expanded analysis (Fig 1), the reduced analysis provides moderate support (73 BP, Fig 3) for it being the sister group to the Asian and Australian taxa *Cladopus*, *Paracladopus*, *Polypleurum*, and *Zeylanidium* In contrast, Moline et al (2007) placed *Podostemum* sister to a clade of the African/Malagasy taxa, although with weak bootstrap support (Moline et al , 2007)

We were unable to obtain material of the Malagasy taxa *Endocaulos* and *Thelethylax* and were limited to available *matK* sequence data from GenBank (Appendix 1) Recent studies (Moline et al , 2007, Pfeifer et al , 2009) used these same sequences in their analyses and found a sister group relationship between these Malagasy taxa and the African Podostemoideae

TABLE 4 Log likelihoods of optimal tree, constraint trees, and results from AU topology tests

	Reduced data set		Expanded data set	
Topology	Likelihood	*P*	Likelihood	*P*
Optimal	−72673 656770	83 13	−78244 206942	83 76
80 BP ML constraints				
matK	−72690 359865	6 37	−78261 127486	13 43
ndhF	−72705 056806	2 98 *	−78270 805141	7 26
rbcL	−72673 656776	81 23	−78277 842951	7 52
matR	−72727 284331	0 04 *	−78259 285849	14 04
Monophyly constraints				
Clusiaceae s l	−72693 412489	5 91	−78265 061339	5 63
Dystovomita	—	—	−78257 353943	9 47
Garcinia	−72693 567356	12 26	−78263 982016	14 12
Harungana	−72686 106027	4 93 *	−78260 341692	3 25 *
Hypericum	−72710 968640	0 11 *	−78285 931353	0 66 *
Ledermanniella	−72754 773013	0 02 *	−78321 085180	0 19 *
Tovomita	−72677 789966	42 70	−78260 488872	5 11
Zeylanidium	—	—	−78336 063417	0 *
Alternate MP placement				
Mourera	−72676 969678	32 69	−78247 380007	55 47

Notes *P* values less than 5% (marked with a "*") indicate topologies that differ significantly from the best tree

TABLE 5 Proposed taxonomic changes for Hypericaceae

Synonym in use prior to this study	Proposed name
Lianthus ellipticifolius (H L Li) N Robson	*Hypericum ellipticifolium* H L Li
Santomasia steyermarkii (Standl) N Robson	*Hypericum steyermarkii* Standl
Thornea calcicola (Standl & Steyerm) Breedlove & E M McClint	*Hypericum calcicola* Standl & Steyerm
Thornea matudae (Lundell) Breedlove & E M McClint	*Hypericum matudae* Lundell
Triadenum breviflorum (Wall ex Dyer) Y Kimura	*Hypericum breviflorum* Wall ex Dyer
Triadenum fraseri (Spach) Gleason	*Hypericum fraseri* (Spach) Steudel
Triadenum japonicum (Blume) Makino	*Hypericum fauriei* R Keller
Triadenum tubulosum (Walter) Gleason	*Hypericum tubulosum* Walter
Triadenum virginicum (L) Raf	*Hypericum virginicum* L
Triadenum walteri (J F Gmel) Gleason	*Hypericum walteri* J F Gmel

clade They proposed that completely or partially inverted flower orientation in bud might be a synapomorphy for the African/Malagasy clade (Grob et al , 2007, Moline et al , 2007) However, we find that the New World *Ceratolacis*, rather than the Malagasy taxa, are sister to the African clade, albeit with poor support (52 BP) Although *Ceratolacis* shares two stamens, an andropodium, and dyad pollen with many members of the primarily Old World clade, it also shares an asymmetrically placed stipule and an andropodial tepal with some members of *Podostemum* (Philbrick et al , 2004a, b) and forms a clade with *Podostemum* in a morphological analysis of the family (C T Philbrick, unpublished data)

We present new relationships and increased support within the clade of African taxa recently studied by Thiv et al (2009) The monotypic *Monandriella* is weakly supported (57 BP) as sister to the remaining taxa from mainland Africa rather than embedded within the clade as in Thiv et al (2009) Thiv et al proposed that this genus might form a clade with other African taxa that shed their pollen in monads [their "*Ledermanniella*-monad" group, here represented by *Ledermanniella bifurcata* (Engler) C Cusset, *Leiothylax, Letestuella, Macropodiella and Stonesia*, Fig 1] Our data do not support this suggestion, although our placement of *Monandriella* does support maintaining it as a separate genus We also find strong support (86 BP) for a monophyletic *Inversodicraea* (*Ledermanniella* subgenus *Phyllosoma* sensu C Cussett), for which there was no previous molecular support, confirming the separation of *Inversodicraea* from *Ledermanniella* s l sensu Thiv et al The *Inversodicraea* clade is also supported by morphology these taxa possess stem scales (Cusset, 1983, Thiv et al , 2009) Two clades containing taxa whose pollen is shed primarily in monads (mentioned above, excluding *Monandriella*) or dyads [here represented by *Dicraeanthus, Djinga, Ledermanniella bowlingii* (J B Hall) C Cusset, *Ledermanniella letouzeyi* C Cusset, *Ledermanniella linearifolia* Engl , and *Ledermanniella pusilla* (Warm) C Cusset in Fig 1] are also moderately to strongly supported here but not in Thiv et al (2009) Pollen shed in monads appears only in a few subclades in the mostly Old World clade, particularly among the mainland African taxa, suggesting that other African members that possess monads not sampled here (e g , *Winklerella* and *Zehnderia*) belong among these taxa Furthermore, we find strong support that the genus *Ledermanniella* s s as proposed by Thiv et al (2009, former *Ledermanniella* subgenus *Ledermanniella* minus *Monandriella* sensu C Cusset) is not monophyletic (Fig 1, Table 4)

Within the Asian Podostemoideae clade, we show that *Zeylanidium* is not monophyletic (Fig 1, Table 4) *Zeylanidium subulatum* (Gardner) C Cusset is sister to *Polypleurum* (100 BP) and *Zeylanidium lichenoides* Engl is sister to *Griffithella* (100 BP) Koi and Kato (2010) also demonstrated the non-monophyly of *Zeylanidium*, but *Griffithella* was not included in their study We believe that the sampling here is too preliminary to consider taxonomic changes

Conclusions and future directions—The phylogeny of the clusioid clade presented here provides a greatly improved understanding of the evolutionary history of this morphologically and ecologically diverse clade Taxon sampling and resolution within the clade is greatly improved compared to previous studies, which has allowed us to propose a more refined classification of the group In the future, we will concentrate on two main areas of research using the clusioid clade as a study system

Increased taxon and character sampling—Many important clusioid taxa have not been sampled with molecular data, and key areas in our phylogeny remain unresolved or poorly supported To address these issues further, future taxon sampling should focus on unsampled genera, as well as on expanding sampling of distinct morphological or biogeographical groups within several larger genera (e g , *Apinagia, Calophyllum, Chrysochlamys, Clusia, Garcinia, Hypericum, Ledermanniella, Mammea*, and *Marathrum*) In several genera, such as *Chrysochlamys* and *Clusia*, particularly in Andean countries, the alpha taxonomy is poorly known, and many species are undescribed In these groups, revisionary taxonomic studies should be well integrated with phylogenetic investigations Additionally, obtaining well-sampled phylogenies of *Calophyllum, Hypericum*, and *Mammea* will be important for future biogeographic studies of the clusioid clade because the early biogeographic histories of these widely distributed genera are unknown and are critical to assessing ancestral areas within the clusioids (see below) Character sampling, in addition to taxon sampling, should also be increased to help provide better resolution and support in various areas of the tree Increased sampling of the plastid and mitochondrial genomes will be valuable, but nuclear markers should also be used in future studies to represent the evolutionary history of all three genomes A particularly useful marker may be the low-copy nuclear gene *PHYC*, which has been shown to be very informative at both the familial and ordinal levels in Malpighiales (Davis et al , 2002, Davis and Chase, 2004, Kathriarachchi et al , 2005, Samuel et al , 2005, Wurdack and Davis, 2009, B R Ruhfel, unpublished data)

Biogeography—The clusioids offer a unique opportunity to study the biogeography of tropical angiosperms with Gondwanan distributions because they are of ancient origin and possess

a pantropical distribution Fossil representatives of the clade are known from the Cretaceous (~90 Ma, Crepet and Nixon, 1998) and the Eocene (~45 Ma, Jan-du-Chene et al , 1978) and their stem group age dates to the mid Cretaceous (99–109 Ma, Davis et al , 2005) The clusioids are prominently featured in the classic work by Raven and Axelrod (1974), which integrated plate tectonics with angiosperm evolution and biogeography Raven and Axelrod hypothesized that various clusioid clades date back to Gondwanan times when Africa and South America were in close proximity to one another More recent analyses, however, have indicated that at least some intercontinental disjunctions within this group are far more recent and are more consistent with long-distance dispersal rather than ancient Gondwanan vicariance (Dick et al 2003 Kita and Kato, 2004b) Biogeographical studies of pantropical groups are few (see Clayton et al , 2009 and references therein) and are needed to increase our understanding of the relative roles of ancient vicariance and more recent dispersal in the assembly of the modern tropical biota (Pennington and Dick, 2004) Determining which of these two factors is most plausible for the many intercontinental disjunctions implied in our trees is testable and is a major focus of our future efforts

While many disjunctions involving former Gondwanan landmasses can now be localized in our topology an assessment of the influence of ancient vicariance vs more recent dispersal cannot be determined until we know where and when these events occurred This information can be gleaned from ancestral area reconstructions and divergence time estimation It is of utmost importance that these analyses include appropriately placed fossils *Paleoclusia chevalieri* Crepet & Nixon dates back to the Turonian (~90 Ma), is among the oldest rosid macrofossils, and has been attributed to Clusiaceae s s (Crepet and Nixon, 1998) Its exact phylogenetic placement within the clusioid clade however, remains to be determined Analysis of a data set containing both molecular and morphological data may allow us to place this and other critical clusioid taxa that lack molecular data (Wiens, 2009, B R Ruhfel, P F Stevens, and C C Davis, unpublished manuscript) The placement of this fossil will be important for estimating divergence times in the clusioid clade as well as in the broader rosid clade A further benefit of estimating divergences times within this clade concerns the response of tropical angiosperms to the Cretaceous–Tertiary (K-T) mass extinction event The ancient age of the clusioids makes this group amenable to examine what effect, if any, the K T mass extinction had on tropical rain forest diversity A biogeographical study of the clusioids (B R Ruhfel, C P Bove, C T Philbrick, and C C Davis, unpublished manuscript) will enable the exploration of these important topics and will help to clarify the origin and maintenance of diversity in modern tropical rain forests

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APPENDIX 1 Voucher information and GenBank accessions for sequences used in this study New data have GenBank numbers beginning with HQ (HQ331542–HQ332128), and accessions in brackets are from a different voucher source A dash (—) indicates that the sequence was unavailable Herbaria acronyms follow Holmgren and Holmgren (1998 [continuously updated])

FAMILY. Species, *voucher* (herbarium), GenBank accessions *matK, ndhF, rbcL, matR*

ACHARIACEAE ***Acharia tragodes*** Thunb, *Cloete s n* (BOL), EF135500, AY425028, AF206728, AY674472

BALANOPACEAE. ***Balanops vieillardi*** Baill, *Chase 1816* (K), EF135505, AY425032, AF089760, AY674479

BONNETIACEAE. ***Archytaea triflora*** Mart, *Kubitzki & Feuerer 97-26* (HBG), HQ331545, AY425029, AY380342, AY674475, ***Bonnetia ahogadoi*** (Steyer) A L Weitzman & P F Stevens, *Weitzman et al 409* (K), HQ331546, AY425035, HQ332007, —, ***Bonnetia cubensis*** (Britton) R A Howard, *J Gutierrez et al HAJB 81795* (WIS), HQ331547, HQ331846, HQ332008, HQ331702, ***Bonnetia paniculata*** Spruce ex Benth, *P Berry 7789* (MICH), HQ331548, HQ331847, HQ332009, HQ331703, ***Bonnetia roraimae*** Oliv, *Weitzman et al 402* (K), —, HQ331848, AJ402930, —, ***Bonnetia sessilis*** Benth, *Berry s n 25 7 98* (MO), EF135509, HQ331849, HQ332010, EF135292, ***Bonnetia steyermarkii*** Kobuski, *Weitzman et al 403* (K), —, HQ331850, HQ332011, HQ331704, ***Bonnetia stricta*** (Nees) Nees & Mart, *Amorim 3958* (CEPEC), HQ331549, HQ331851, HQ332012, HQ331705, ***Bonnetia tepuiensis*** Kobuski & Steyerm, *P Berry 7788* (MICH), —, HQ331852, HQ332013, —, ***Ploiarium alternifolium*** Melchior, *Sugumaran 165* (US), FJ669999, FJ670063, FJ670161, FJ670352

CALOPHYLLACEAE. ***Calophyllum brasiliense*** Cambess, *C Notis 387* (FLAS), HQ331550, HQ331853, —, HQ331706, ***Calophyllum castaneum*** P F Stevens, *Ruhfel 111* (A), HQ331551, HQ331854, HQ332014, HQ331707, ***Calophyllum goniocarpum*** P F Stevens, *F Damon 318* (MO), HQ331552, HQ331855, HQ332015, HQ331708, ***Calophyllum inophyllum*** L, *Ruhfel 115* (A), HQ331553, HQ331856, HQ332016, HQ331709, ***Calophyllum lanigerum*** Miq, *Ruhfel 104* (A), HQ331554, HQ331857, HQ332017, HQ331710, ***Calophyllum longifolium*** Willd, *Aguilar 11657* (NY), HQ331555, HQ331858, HQ332018, HQ331711, ***Calophyllum soulattri*** Burm f, *Chase 1217* (K), HQ331556, AY425037, [*F Damon 320* (MO), AY625021], AY674484, ***Calophyllum sp 1***, *Ruhfel 108* (A), HQ331557, HQ331859, HQ332019, HQ331712, ***Calophyllum sp 2***, *Ruhfel 113* (A), HQ331558, HQ331860, HQ332020, HQ331713, ***Calophyllum sp 3***, *Ruhfel 114* (A), HQ331559, HQ331861, HQ332021, HQ331714, ***Calophyllum teysmannii*** Miq, *Ruhfel 112* (A), HQ331560, HQ331862, HQ332022, HQ331715, ***Calophyllum verticillatum*** P F Stevens, J *Rabenantoandro et al 733* (MO), HQ331561, HQ331863, HQ332023, HQ331716, ***Calophyllum vexans*** P F Stevens, *F Damon 321* (MO), HQ331562, HQ331864, HQ332024, HQ331717, ***Caraipa densifolia*** Mart, *C Grandez 16239* (FLAS), HQ331563, HQ331865, AY625012, HQ331718, ***Caraipa grandifolia*** Mart, *C Grandez 16244* (FLAS), HQ331564, HQ331866, HQ332025, HQ331719, ***Caraipa savannarum*** Kubitzki, *G Aymard s n* (PORT), HQ331565, HQ331867, HQ332026, HQ331720, ***Caraipa tereticaulis*** Tul, *Vormisto 578* (AAU), HQ331566, HQ331868, HQ332027, HQ331721, ***Clusiella isthmensis*** Hammel, *M Whitten 2657* (FLAS), HQ331585, HQ331889, AY625019, HQ331738, ***Endodesmia calophylloides*** Benth, *Burgt 762* (WAG), FJ670005, FJ670069, FJ670163, FJ670356, ***Haploclathra cordata*** R Vásquez, *C Grandez 16237* (FLAS), HQ331613, HQ331918, AY625017, HQ331764, ***Haploclathra paniculata*** Benth, *C Grandez 16246* (FLAS), HQ331614, HQ331919, HQ332068, HQ331765, ***Kayea elmeri*** Merr, *Ruhfel 110* (A), HQ331636, —, HQ332086, HQ331784, ***Kayea hexapetala*** Pierre, *Ruhfel 119* (A), HQ331637, HQ331939, HQ332087, HQ331785, ***Kayea oblongifolia*** Ridl, *Ruhfel 116* (A), HQ331638, HQ331940, HQ332088, HQ331786, ***Kayea sp***, *E Wood and G A Teck 5500* (A), HQ331639, HQ331941, HQ332089, HQ331787, ***Kayea stylosa*** Thw, *Kostermans 11106* (HUH), HQ331640, HQ331942, AY625025, HQ331788, ***Kielmeyera lathrophyton*** Saddi, *F Feres s n* (UEC), HQ331641, HQ331943, AY625015, HQ331789, ***Kielmeyera petiolaris*** Mart, *F Feres 75* (UEC), HQ331642, HQ331944, AY625016, HQ331790, ***Mahurea exstipulata*** Benth, *Kubitzki et al 97-*

27 (HBG), HQ331650, HQ331954, AY625018, HQ331799, ***Mammea africana*** Sabine, *D Kenfack 2055* (MO), HQ331651, HQ331955, HQ332098, HQ331800, ***Mammea americana*** L , *C Notis 392* (FLAS), HQ331652, HQ331956, AY625029, HQ331801, ***Mammea sessiliflora*** Planch & Triana, *McPherson 18377* (MO), HQ331653, HQ331957, AY625027, HQ331802, ***Mammea siamensis*** T Anderson, *Chase 1216* (K), FJ670006, FJ670070, AY625028, FJ670357, ***Mammea sp 1***, *P Sweeney 1305* (MO), HQ331654, HQ331958, HQ332099, HQ331803, ***Mammea sp 2***, *T G Laman et al TL 727* (A), HQ331655, HQ331959, HQ332100, —, ***Mammea touriga*** (C T White & W D Francis) L S Sm , *H van der Werff and B Gray 17055* (MO), HQ331656, HQ331960, HQ332101, HQ331804, ***Mammea zeereae*** P F Stevens, *P Sweeney 1273* (MO), HQ331657, HQ331961, HQ332102, HQ331805, ***Marila laxiflora*** Rusby, *van der Werff et al 16246* (MO), HQ331659, HQ331963, —, HQ331807, ***Marila tomentosa*** Poepp & Endl , *van der Werff et al 16215* (MO), HQ331660, HQ331964, AY625010, HQ331808, ***Mesua ferrea*** L , *M Sugumaran et al SM 120* (KLU), HQ331661, HQ331965, [*C Notis 390* (FLAS), AY625024], HQ331809, ***Poeciloneuron indicum*** Bedd , *U Ghate s n* (FLAS), HQ331673, HQ331977, AY625023, HQ331819

CARYOCARACEAE. ***Caryocar glabrum*** Pers , *Mori 22997* (NY), EF135515, AY425039, Z75671, AY674486

CELASTRACEAE. ***Celastrus orbiculatus*** Thunb , *Simmons 1773* (BH), EF135517, FJ670145, AY788194, EF135295

CENTROPLACACEAE. ***Centroplacus glaucinus*** Pierre, *White 128, ser 1* (MO), FJ670002, FJ670066, AY663646, FJ670355

CHRYSOBALANACEAE. ***Chrysobalanus icaco*** L , *Wurdack D711* (US), EF135519, FJ670067, L11178, AY674491

CLUSIACEAE S.S. ***Allanblackia sp*** , *E Ndive s n* (YU), HQ331542, HQ331843, HQ332004, HQ331699, ***Chrysochlamys allenii*** (Maguire) Hammel, *R Kriebel 2289* (INB), HQ331569, HQ331871, HQ332030, HQ331723, ***Chrysochlamys eclipes*** L O Williams, *BCI 158121* (STRI), HQ331570, HQ331872, HQ332031, HQ331724, ***Chrysochlamys grandifolia*** (L O Williams) Hammel, *R Aguilar ra12291* (NY), —, HQ331873, HQ332032, HQ331725, ***Chrysochlamys silvicola*** (Hammel) Hammel, *B Hammel 25293* (MO), HQ331571, HQ331874, —, HQ331726, ***Chrysochlamys skutchii*** Hammel, *R Aguilar ra12292* (NY), HQ331572, HQ331875, —, —, ***Clusia* cf. *flavida*** (Benth) Pipoly, *M H G Gustafsson 454* (AAU), HQ331575, HQ331878, HQ332035, HQ331728, ***Clusia clusioides*** (Griseb) D'Arcy, *M H G Gustafsson 272* (NY), —, HQ331879, AF518388, HQ331729, ***Clusia fructangusta*** Cuatrec , *M H G Gustafsson 485* (AAU), HQ331576, HQ331880, HQ332036, HQ331730, ***Clusia gracilis*** Standl , *Ruhfel 23* (A), HQ331577, HQ331881, HQ332037, HQ331731, ***Clusia gundlachii*** Stahl, *Chase 341* (NCU), EF135520, AY425041, Z75673, AY674493, ***Clusia hammeliana*** Pipoly, *M H G Gustafsson 451* (AAU), HQ331578, HQ331882, HQ332038, HQ331732, ***Clusia lanceolata*** Cambess , *C Notis 389* (FLAS), HQ331579, HQ331883, HQ332039, HQ331733, ***Clusia loretensis*** Engl , *M H G Gustafsson 500* (AAU), HQ331580, HQ331884, HQ332040, HQ331734, ***Clusia major*** L , *M H G Gustafsson 396* (AAU), HQ331581, HQ331885, HQ332041, HQ331735, ***Clusia pallida*** Engl , *M H G Gustafsson 464* (AAU), HQ331582, HQ331886, HQ332042, HQ331736, ***Clusia rosea*** Jacq , *Kent s n* (A), HQ331583, HQ331887, HQ332043, —, ***Clusia viscida*** Engl , *M H G Gustafsson 444* (AAU), HQ331584, HQ331888, HQ332044, HQ331737, ***Dystovomita* cf. *brasiliensis*** D'Arcy, *Sothers 452* (UEC), —, —, AF518387, —, ***Dystovomita paniculata*** (Donn Sm) Hammel, *B Hammel 25295* (MO), HQ331594, HQ331897, [*B Hammel 22728* (INB), HQ332051], HQ331746, ***Garcinia* aff *afzelii*** Engl , *P W Sweeney 1411* (MO), HQ331595, HQ331898, HQ332052, HQ331747, ***Garcinia conrauana*** Engl , *S Moses 961* (MO), —, HQ331899, HQ332053, —, ***Garcinia cowa*** Roxb , *M Sugumaran et al SM 146* (KLU), HQ331596, HQ331900, HQ332054, HQ331748, ***Garcinia cymosa*** (K Schum) I M Turner & P F Stevens, *P Sweeney 1000* (MO), HQ331597, HQ331901, [*T Motley s n* (AAU) AF518379], HQ331749, ***Garcinia eugenifolia*** Wall ex T Anderson, *P W Sweeney 985* (MO), HQ331598, HQ331902, HQ332055, HQ331750, ***Garcinia hessii*** (Britton) Alain, *Axelrod 4537* (UPR), EF135543, —, AJ402952, DQ110341, ***Garcinia hombroniana*** Pierre, *M Sugumaran et al SM 124* (KLU), HQ331599, HQ331903, HQ332056, HQ331751, ***Garcinia intermedia*** (Pittier) Hammel, *M J Balick 3570* (GH), HQ331600, HQ331904, —, HQ331752, ***Garcinia latissima*** Miq , *Chase 2100* (K), FJ670008, FJ670072, AF518386, FJ670359, ***Garcinia livingstonei*** T Anderson, *P Sweeney 1007* (MO), —, HQ331905, —, HQ331753, ***Garcinia macrophylla*** Mart , *Chase 1219* (K), —, FJ670073, FJ670165, FJ670360, ***Garcinia mangostana*** L , *Kent s n* (A), HQ331601, HQ331906, HQ332057, —, ***Garcinia mannii*** Oliver, *G Walters et al 604* (MO), HQ331602, HQ331907, —, HQ331754, ***Garcinia melleri*** Baker, *J Rabenantoandro and G McPherson 689* (MO), HQ331603, HQ331908, HQ332058, HQ331755, ***Garcinia nervosa*** Miq , *Ruhfel 106* (A), HQ331604, HQ331909, HQ332059, HQ331756, ***Garcinia penanguana*** Pierre, *Ruhfel 118* (A), HQ331605, HQ331910, HQ332060, HQ331757, ***Garcinia rostrata*** Hassk ex Hook f , *P W Sweeney 1071* (MO), HQ331606, HQ331911, HQ332061, HQ331758, ***Garcinia scortechinii*** King, *P W Sweeney 994* (MO), HQ331607, HQ331912, HQ332062, HQ331759, ***Garcinia spicata*** Hook f , *C Notis 388* (FLAS), HQ331608, HQ331913, HQ332063, HQ331760, ***Garcinia staudtii*** Engl , *P Sweeney et al 1445* (MO), HQ331609, HQ331914, HQ332064, HQ331761, ***Garcinia tsaratananensis*** (H Perrier) P Sweeney & Z S Rogers, *P Sweeney 1232* (MO), HQ331610, HQ331915, HQ332065, HQ331762, ***Garcinia urophylla*** Scort ex King, *P W Sweeney 1081* (MO), HQ331611, HQ331916, HQ332066, HQ331763, ***Lorostemon coelhoi*** Paula, *V Bittrich 95-170* (UEC), HQ331648, HQ331952, [*Assunção 492* (UEC), AF518401], HQ331797, ***Montrouziera cauliflora*** Planch & Triana, *Lowry 5601* (MO), FJ670007, FJ670071, FJ670164, FJ670358, ***Montrouziera sphaeroidea*** Planch ex Planch & Triana, *K Cameron 981* (NY), HQ331664, HQ331968, [*Cameron 981* (NY), AF518390], HQ331812, ***Moronobea coccinea*** Aubl , *SM 24698* (NY), HQ331665, HQ331969, AF518378, HQ331813, ***Pentadesma butyracea*** Sabine, *Kitjima s n* (A), HQ331669, HQ331973, [*Nagata 951*, (HLA), AF518383], HQ331817, ***Platonia insignis*** Mart , *V Bittrich s n 3 01 05* (INB), HQ331670, HQ331974, [*Mori 23699* (NY), AF518394], HQ331818, ***Symphonia fasciculata*** (Noronha ex Thouars) Vesque, *J S Miller et al 8836* (MO), HQ331679, HQ331984, HQ332117, HQ331825, ***Symphonia globulifera*** L f , *Ruhfel 21* (A), HQ331680, HQ331985, [*Mori 24792* (NY), AF518381], HQ331826, ***Tovomita calophyllophylla*** García-Villacorta & Hammel, *J Vormisto 579* (AAU), HQ331683, HQ331988, HQ332119, HQ331828, ***Tovomita longifolia*** (Rich) Hochr , *R Aguilar ra12290* (NY), HQ331684, HQ331989, HQ332120, HQ331829, ***Tovomita sp*** , *J Vormisto 562* (AAU), HQ331685, HQ331990, HQ332121, HQ331830, ***Tovomita weddelliana*** Planch & Triana, *M H G Gustafsson 478* (AAU), HQ331686, HQ331991, HQ332122, HQ331831, ***Tovomitopsis saldanhae*** Engl , *V Bittrich s n* (UEC), HQ331687, HQ331992, HQ332123, —

CTENOLOPHONACEAE. ***Ctenolophon englerianus*** Mildbr , *McPherson 16911* (MO), EF135524, FJ670074, AJ402940, AY674499

ELATINACEAE. ***Elatine triandra*** Schkuhr, *Burton et al 13384* (MICH), [EF135532], AY425049, [AY380349], AY674507

EUPHORBIACEAE. ***Ricinus communis*** L , *Wurdack D9* (US), EF135590, FJ670089, AY788188, AY674560

GOUPIACEAE. ***Goupia glabra*** Aubl , *Prevost 3031* (CAY), EF135544, AY425054, AJ235780, AY674516

HUMIRIACEAE. ***Humiria balsamifera*** Aubl , *Anderson 13654* (MICH), EF135549, AF351007, L01926, AY674523

HYPERICACEAE. ***Cratoxylum arborescens*** (Vahl) Blume, *Ruhfel 121* (A), HQ331586, HQ331890, HQ332045, HQ331739, ***Cratoxylum cochinchinense*** (Lour) Blume, *Church et al 2699* (A), HQ331587, HQ331891, HQ332046, HQ331740, ***Cratoxylum formosum*** (Jack) Dyer, *Ruhfel 107* (A), HQ331588, HQ331892, HQ332047, HQ331741, ***Cratoxylum glaucum*** Korth , *Ruhfel 102* (A), HQ331589, HQ331893, HQ332048, HQ331742, ***Cratoxylum sumatranum*** (Jack) Blume, *Chase 1218* (K), FJ670022, FJ670095, AF518395, FJ670373, ***Eliea articulata*** Cambess , *Razakamalala 295* (MO), FJ670023, FJ670096, FJ670167, FJ670374, ***Harungana madagascariensis*** Poir , *B Pettersson and L A Nilson 37* (UPS), HQ331615, HQ331920, [*Naugona 139* (NY), AF518396], HQ331766, ***Hypericum aegyptiacum*** L , *M Gustafsson MG 1148* (AAU), HQ331617, HQ331922, HQ332069, HQ331767, ***Hypericum androsaemum*** L , *J Christiansen s n* (AAU), HQ331618, HQ331923, HQ332070, HQ331768, ***Hypericum annulatum*** Moris, *J Christiansen s n* (AAU), HQ331619, HQ331924, HQ332071, HQ331769, ***Hypericum canariense*** L , *J Christiansen s n* (AAU), HQ331620, HQ331925, HQ332072, HQ331770, ***Hypericum ellipticum***

Hook, *C C Davis s n* (A), HQ331621, HQ331926, —, HQ331771, ***Hypericum elodes*** L, *Halliday s n, 6/7 1964* (AAU), HQ331622, —, HQ332073, HQ331772, ***Hypericum empetrifolium*** Willd, *Chase 837* (K), HQ331623, AY425060, HQ332074, AY674525, ***Hypericum garrettii*** Craib, *J Christiansen s n* (AAU), HQ331624, HQ331927, HQ332075, HQ331773, ***Hypericum grandifolium*** Choisy, *M Gustafsson MG1147* (AAU), HQ331625, HQ331928, HQ332076, HQ331774, ***Hypericum hircinum*** L, *J Christiansen s n* (AAU), HQ331626, HQ331929, HQ332077, HQ331775, ***Hypericum irazuense*** Kuntze ex N Robson, *Ruhfel 8* (A), —, —, HQ332078, HQ331776, ***Hypericum kalmianum*** L, *C C Davis s n* (A), HQ331627, HQ331930, HQ332079, —, ***Hypericum linarifolium*** Vahl, *J Christiansen s n* (AAU), HQ331628, HQ331931, HQ332080, HQ331777, ***Hypericum mutilum*** L, *C C Davis s n* (A), HQ331629, HQ331932, —, HQ331778, ***Hypericum perforatum*** L, *Ruhfel s n* (A), HQ331630, HQ331933, HQ332081, —, ***Hypericum tetrapterum*** Fr, *J Christiansen s n* (AAU), HQ331631, HQ331934, HQ332082, HQ331779, ***Psorospermum* aff. *androsaemifolium*** Baker, *R Randrianaivo et al 145* (UPS), HQ331675, —, HQ332111, —, ***Psorospermum corymbiferum*** Hochr, *J E Lawesson and Goudiaby 7578* (AAU), HQ331676, HQ331979, HQ332112, HQ331821, ***Psorospermum febrifugum*** Spach, *M Hedren et al 394* (UPS), HQ331677, HQ331980, HQ332113, HQ331822, ***Psorospermum revolutum*** (Choisy) Hochr, *M Thulin, P Kornhall, and M Popp 10312* (UPS), HQ331678, —, HQ332114, HQ331823, ***Santomasia steyermarkii*** (Standl) N Robson, *E Matuda S-228* (A), —, HQ331982, —, —, ***Thornea calcicola*** (Standl & Steyerm) Breedlove & E M McClint, *D E Breedlove 37070* (MO), HQ331682, [*J A Steyermark 48946* (A), HQ331987], —, —, ***Triadenum fraseri*** (Spach) Gleason, *C C Davis s n* (A), HQ331688, HQ331993, HQ332124, [*C C Davis s n* (A), HQ331832], ***Triadenum japonicum*** (Blume) Makino, *S Kobayashi 2713* (A), HQ331689, HQ331994, HQ332125, HQ331833, ***Triadenum walteri*** (J F Gmel) Gleason, *Brant 4792* (MO), HQ331690, FJ670097, FJ670168, FJ670375, ***Vismia baccifera*** (L) Triana & Planch, *Ruhfel 20* (A), HQ331692, HQ331996, [*Gustafsson 302* (NY), AF518382], HQ331835, ***Vismia bilbergiana*** Beurl, *B Hammel 25285* (MO), HQ331693, HQ331997, [*STRI BCI 734543* (STRI), GQ981917], HQ331836, ***Vismia guianensis*** (Aubl) Choisy, *Amorim 7659* (CEPC), HQ331694, HQ331998, HQ332126, [*Amorim 3978* (CEPC), HQ331837], ***Vismia guineensis*** (L) Choisy, *M Merello et al 1149* (UPS), HQ331695, HQ331999, —, HQ331838, ***Vismia macrophylla*** Kunth, *Amorim 3972* (CEPC), HQ331696, HQ332000, —, HQ331839, ***Vismia rubescens*** Oliv, *R Niangadouma et al 374* (MO), —, HQ332001, HQ332127, HQ331840, ***Vismia sp***, *Miller et al 9313* (MO), EF135601, FJ670098, FJ670169, AY674571

IRVINGIACEAE. ***Irvingia malayana*** Oliv, *Simpson 2638* (K), EF135553, AY425061, AF123278, EF135300

IXONANTHACEAE. ***Cyrillopsis paraensis*** Kuhl, *Hentrich 68* (NY), FJ670024, FJ670100, FJ670170, FJ670376

LACISTEMATACEAE. ***Lacistema aggregatum*** Rusby, *Pennington et al 583* (K), FJ670025, AY425064, AF206787, AY674529

LINACEAE ***Reinwardtia indica*** Dumort, *Chase 230* (NCU), AB048380, FJ670104, L13188, AY674559

LOPHOPYXIDACEAE. ***Lophopyxis maingayi*** Hook f, *Adelbai P-10203* (US), EF135560, FJ670105, AY663643, AY674534

MALPIGHIACEAE. ***Acridocarpus natalitius*** Adr Juss, *Goldblatt s n* (PRE), AF344525, AF351016, AF344455, EF135290

OCHNACEAE. ***Ochna multiflora*** DC, Chase 229 (NCU), EF135572, AY425072, Z75273, EF135302

OXALIDACEAE ***Averrhoa carambola*** L, *Chase 214* (NCU), FJ670048, FJ670141, FJ670180, AY674478

PANDACEAE. ***Panda oleosa*** Pierre, *Schmidt et al 2048* (MO), FJ670032, FJ670111, AY663644, FJ670383

PASSIFLORACEAE. ***Paropsia madagascariensis*** (Baill) H Perrier, *Zyhra 949* (WIS), EF135576, AY757164, AF206802, AY674547

PERACEAE. ***Pera bicolor*** (Klotzsch) Mull Arg, *Gillespie 4300* (US), EF135578, AY425075, AY794968, AY674549

PHYLLANTHACEAE ***Phyllanthus epiphyllanthus*** L, *Wurdack D56* (US), EF135581, AY425078, AY663604, AY674552

PICRODENDRACEAE. ***Podocalyx loranthoides*** Klotzsch, *Berry & Aymard 7226* (MO), EF135583, FJ670117, AY663647, AY674553

PODOSTEMACEAE. ***Apinagia longifolia*** (Tul) P Royen, *C T Philbrick 6023* (WCSU), HQ331543, HQ331844, HQ332005, HQ331700, ***Apinagia riedelii*** Tul, *C T Philbrick 5960* (WCSU), HQ331544, HQ331845, HQ332006, HQ331701, ***Castelnavia monandra*** Tul & Wedd, *C T Philbrick 5982* (WCSU), HQ331567, HQ331869, HQ332028, HQ331722, ***Ceratolacis pedunculatum*** C Philbrick, *Novelo & Irgang, C T Philbrick 5761* (MO), HQ331568, HQ331870, HQ332029, —, ***Cladopus japonicus*** Imamura, *S Koi and N Katayama JP-404* (TNS), HQ331573, HQ331876, HQ332033, HQ331727, ***Cladopus queenslandicus*** (Domin) C D K Cook & Rutish, *J J Bruhl and I R Telford 2542* (MO), HQ331574, HQ331877, HQ332034, —, ***Dalzellia zeylanica*** Wight, *M Kato and N Katayama SL-101* (TNS), HQ331590, HQ331894, [SL-04 (TNS), AB113760], HQ331743, ***Diamantina lombardii*** Novelo, *C Philbrick & Irgang, C T Philbrick 5783* (WCSU), HQ331591, —, —, —, ***Dicraeanthus zehnderi*** H E Hess, *Ghogue GHO-1650* (Z/ZT), HQ331592, HQ331895, HQ332049, HQ331744, ***Djinga felicis*** C Cusset, *Ghogue et al GAR-09* (Z/ZT), HQ331593, HQ331896, HQ332050, HQ331745, ***Endocaulos mangorense*** (H Perrier) C Cusset, *Kato et al MD-02* (TI), AB038191, —, —, —, ***Griffithela hookeriana*** (Tul) Warm, *C T Philbrick 4683* (WCSU), HQ331612, HQ331917, HQ332067, —, ***Hanseniella heterophylla*** C Cusset, *Kato et al TL-311* (TI), AB104562, —, —, —, ***Hydrobryum japonicum*** Imamura, *S Koi and N Katayama JP-401* (TNS), HQ331616, HQ331921, —, —, ***Indodalzellia gracilis*** (C J Mathew, Jager-Zurn, & Nileena) Koi & M Kato, *KI-115* (TNS), AB450015, —, —, —, ***Indotristicha ramosissima*** (Wight) Royen, *M Kato et al KI-210* (TNS), HQ331632, HQ331935, [*KI-26* (TNS), AB124844], HQ331780, ***Inversodicraea* cf. *annithomae*** (C Cusset) R Rutish and Thiv, *Ghogue et al GAHR-23* (Z/ZT), HQ331633, HQ331936, HQ332083, HQ331781, ***Inversodicraea* cf. *bosu*** (C Cusset) R Rutish & Thiv, *Ghogue et al GAR-01* (Z/ZT), HQ331634, HQ331937, HQ332084, HQ331782, ***Inversodicraea cristata*** Engler, *Ghogue GHO-1664* (Z/ZT), HQ331635, HQ331938, HQ332085, HQ331783, ***Ledermanniella bifurcata*** (Engler) C Cusset, *Ghogue GHO-1597* (Z/ZT), HQ331643, HQ331945, HQ332090, HQ331791, ***Ledermanniella bowlingii*** (J B Hall) C Cusset, *Ameka and Rutishauser AR-021010* (Z/ZT), HQ331644, HQ331946, HQ332091, HQ331792, ***Ledermanniella letouzeyi*** C Cusset, *Ghogue et al GAR-12* (Z/ZT), HQ331645, HQ331947, HQ332092, HQ331793, ***Ledermanniella linearifolia*** Engler, *Ghogue et al GAHR-41* (Z/ZT), —, HQ331948, HQ332093, HQ331794, ***Ledermanniella pusilla*** (Warming) C Cusset, *Ghogue et al GAHR-17* (Z/ZT), HQ331646, HQ331949, HQ332094, HQ331795, ***Leiothylax quangensis*** (Engler) Warming, *Ghogue GHO-1667* (Z/ZT), FM877842, HQ331950, HQ332095, —, ***Letestuella tisserantii*** G Taylor, *Ghogue GHO-1660* (Z/ZT), HQ331647, HQ331951, HQ332096, HQ331796, ***Macropodiella heteromorpha*** (Baillon) C Cusset, *Ghogue et al GAHR-24* (Z/ZT), HQ331649, HQ331953, HQ332097, HQ331798, ***Marathrum foeniculaceum*** Bonpl, *C T Philbrick 5958* (WCSU), HQ331658, HQ331962, HQ332103, HQ331806, ***Marathrum plumosum*** (Novelo & C T Philbrick) *C T Philbrick & C P Bove, MX-05* (TI), AB048378, —, [Les et al, U68090], —, ***Monandriella linearifolia*** Engler, *Ghogue GHO-1663* (Z/ZT), HQ331662, HQ331966, HQ332104, HQ331810, ***Monostylis capillacea*** Tul, *C T Philbrick 6076* (WCSU), HQ331663, HQ331967, HQ332105, HQ331811, ***Mourera* cf. *aspera*** (Bong) Tul, *C T Philbrick 6093* (WCSU), HQ331666, HQ331970, [Les et al, U68086], HQ331814, ***Mourera fluviatilis*** Aubl, *GU-24* (TI), AB038200, —, [not listed, AB113759], —, ***Noveloa coulteriana*** (Tul) C T Philbrick, *C T Philbrick 6270* (WCSU), HQ331667, HQ331971, HQ332106, HQ331815, ***Paracladopus chanthaburiensis*** Koi & M Kato, *S Koi et al TKF-24* (TNS), HQ331668, HQ331972, HQ332107, HQ331816, ***Podostemum ceratophyllum*** Michx, *Ruhfel s n* (A), HQ331671, HQ331975, HQ332108, [*Horn s n* (DUKE), EF135304], ***Podostemum scaturiginum*** (Mart) C Philbrick & Novelo, *C T Philbrick et al 5602* (MO), HQ331672, HQ331976, HQ332109, —, ***Polypleurum stylosum*** (Wight) J B Hall, *M Kato and N Katayama SL-103* (TNS), HQ331674, HQ331978, HQ332110, HQ331820, ***Rhyncholacis sp***, *Amaral s n* (INPA), EF135564, HQ331981, HQ332115, AY674537, ***Stonesia ghoguei*** E Pfeifer and Rutishauser, *Ghogue GHO-1665* (Z/ZT), FM877841, HQ331983, HQ332116, HQ331824, ***Terniopsis brevis*** M Kato, *S Koi et al TKF-25* (TNS), HQ331681, HQ331986, HQ332118, HQ331827, ***Terniopsis malayana*** (J Dransf & Whitmore) M Kato, *TL-106, 107* (TNS), AB048827, —, AB083098, —, ***Terniopsis sessilis***

HSIU C Chao, *CH-03* (TI), AB048377, —, AB083100, —, ***Thawatchaia trilobata*** M Kato, KOI & Y KITA, *Kato et al TL-419* (TI), AB104563, —, —, —, ***Thelethylax minutiflora*** (Tul) C Cusset, *Kato et al MD 01* (TI), AB038196, —, —, —, ***Tristicha trifaria*** (Bory ex Willd) Spreng , *C T Philbrick 6090* (WCSU), HQ331691, HQ331995, [*BR-01*, AB113746], HQ331834, ***Weddellina squamulosa*** Tul , *C T Philbrick 5827* (WCSU), HQ331697, HQ332002, [not listed, AB113758], HQ331841, ***Zeylanidium lichenoides*** Engl , *Kato et al KI-35* (TI), AB048828, —, —, —, ***Zeylanidium subulatum*** (Gardner) C Cusset, *M Kato and N Katayama SL-102* (TNS), HQ331698, HQ332003, HQ332128, HQ331842

PUTRANJIVACEAE. ***Putranjiva roxburghii*** Wall (as *Drypetes roxburghii* [Wall] Hurus), *Wurdack D57* (US), EF135530, [AY425048], [M95757], [AY674505]

RHIZOPHORACEAE. ***Bruguiera gymnorhiza*** Lam , *Chase 12838* (K), EF135511, AY425036, [AF127693], AY674483

SALICACEAE. ***Populus maximowiczii*** Henry, *Chase 996* (K), EF135587, AY425080, AJ418836, AY674556

VIOLACEAE ***Hybanthus concolor*** Spreng , *Alford 3056* (BH), EF135550, AY757141, AY788178, AY674524

CHAPTER 2:

Combined morphological and molecular phylogeny of the clusioid clade (Malpighiales) and the placement of the Cretaceous macrofossil *Paleoclusia*

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ABSTRACT

The clusioid clade is a member of the large rosid order Malpighiales and contains approximately 1900 species distributed among five families: Bonnetiaceae, Calophyllaceae, Clusiaceae *sensu stricto* (s.s.), Hypericaceae, and Podostemaceae. Despite a recent successful effort to clarify their phylogenetic relationships using molecular data, no molecular data are available for several critical taxa. Among others, these include *Hypericum ellipticifolium* (previously placed in the monotypic genus *Lianthus*), *Lebrunia*, *Neotatea*, *Thysanostemon*, and the extinct taxon from the Turonian (~90 Ma), *Paleoclusia chevalieri*. We constructed a morphological data set including 69 characters and 81 clusioid species/species groups and analyzed these data using parsimony, maximum likelihood, and Bayesian inference to determine the placement of these taxa. The phylogeny inferred from the morphological data was poorly resolved, but largely in agreement with the phylogeny inferred from molecular data alone. Our combined analyses of the molecular and morphological data largely confirm earlier hypotheses of relationships for the 22 included extant taxa that were scored only for morphology. Furthermore, these results suggest that *Paleoclusia* is weakly placed as a member of Clusiaceae s.s. Our ancestral character state reconstructions further corroborate this placement and shed light on the evolution of traits that have been historically important for circumscribing clusioid taxa.

Key words: Clusiaceae, combined analysis, Guttiferae, morphology, *Paleoclusia*, rosids

INTRODUCTION

The clusioid clade belongs to the large angiosperm order Malpighiales (Savolainen et al., 2000). It includes five families [Bonnetiaceae, Calophyllaceae, Clusiaceae *sensu stricto* (s.s.), Hypericaceae, and Podostemaceae (APG III, 2009; Wurdack and Davis, 2009)] representing 89 genera (Ruhfel et al., 2011) and ~1900 species (Stevens, 2001 onwards). Habitats and growth forms in the clusioid clade show extreme variation, from large tropical rainforest trees to temperate herbs and shrubs to diminutive aquatic plants of swift-flowing rivers and waterfalls. Their distribution is nearly cosmopolitan, but species diversity is greatest in the tropics. The clade is important ecologically and economically. Terrestrial members of the clade (i.e., all but Podostemaceae) are an important component of tropical rainforests worldwide (CTFS, 2009). Podostemaceae, on the other hand, are the largest strictly aquatic plant family (Philbrick and Novelo, 1995; Cook, 1996) and play a key ecological role in river systems via their interactions with fish and invertebrates (Allan, 1995; Machado-Allison et al., 2003). Species from Calophyllaceae, Clusiaceae s.s., and Hypericaceae are variously used in horticulture, tropical fruit production, timber production, and the pharmaceutical industry (Ernst, 2003; Stevens, 2007a, b; Ruhfel et al., 2011).

Recent molecular studies have strived to clarify relationships within the clusioid clade (Gustafsson et al., 2002; Wurdack and Davis, 2009; Ruhfel et al., 2011). Most recently, Ruhfel et al. (2011) produced the first well-resolved, taxon-rich phylogeny of the group. This study greatly improved our understanding of intrafamilial relationships within the clusioid families and indicated that several genera were not monophyletic as traditionally circumscribed. However, several important taxa representing a broad range

of morphological diversity within the group were excluded from these analyses. This is either because no specimens were available, extractions of genomic DNA from available herbarium material were unsuccessful, or the taxon is a fossil. Among others, these taxa include *Hypericum ellipticifolium* (H.L. Li) N. Robson (previously placed in the monotypic genus *Lianthus* [China; Hypericaceae]), *Lebrunia* (monotypic, Africa; Calophyllaceae), *Neotatea* (four species, South America; Calophyllaceae), *Thysanostemon* (two species, South America; Clusiaceae s.s.), and the extinct taxon from the Turonian (~90 Ma), *Paleoclusia chevalieri* Crepet & Nixon. A companion morphological data set of the clusioid clade can provide an independent assessment of the molecular phylogeny, and when analyzed in combination with molecular data should allow us to place these critical taxa.

Several recent studies have indicated that a combined analysis of morphological and molecular data can greatly clarify the phylogenetic relationships of taxa for which molecular data are unavailable. This is especially true when morphological data are informative, do not exhibit strong conflict with molecular data, and the overall number of characters scored is large (Wiens, 2003; Wiens and Moen, 2008; Wiens, 2009). A morphological data set will also allow us to conduct ancestral state reconstructions (ASRs) to understand patterns of morphological evolution in the clusioids. This will shed light on the evolution of morphological traits that have been historically important for circumscribing taxa in the clusioid clade. Furthermore, the placement of taxa lacking molecular data, especially the fossil taxon *Paleoclusia*, will be critical for our efforts to infer the biogeographic history of the clusioid clade. The inclusion of fossils in phylogenetic analyses is especially important because they can greatly influence the

phylogeny, increase our understanding of character evolution, and inform estimates of clade ages (Donoghue et al., 1989; Pennington et al., 2004; Olmstead and Scotland, 2005).

Paleoclusia chevalieri (Crepet and Nixon, 1998) is one of the oldest (~90 Ma) macrofossils that is readily assigned to an extant rosid clade (Crepet et al., 2004; Schönenberger and von Balthazar, 2006). As such, it has been used as a fossil constraint in studies aimed at estimating the divergence times of major angiosperm clades (Crepet et al., 2004; Davis et al., 2005; Magallón and Castillo, 2009; Wang et al., 2009; Bell et al., 2010). In their phylogenetic analysis of *Paleoclusia*, Crepet and Nixon (1998) placed it as sister to *Clusia* + *Garcinia* (Clusiaceae s.s.). Since their discovery, however, there have been major advances in our understanding of angiosperm phylogeny. Of particular relevance is that Clusiaceae *sensu lato* (s.l.) are not monophyletic: Clusiaceae s.l. included members of Calophyllaceae, Clusiaceae s.s., and Hypericaceae (Wurdack and Davis, 2009; Ruhfel et al., 2011). Additionally, the enigmatic aquatic Podostemaceae are now included within the clusioid clade (Gustafsson et al., 2002; APG III, 2009; Wurdack and Davis, 2009; Ruhfel et al., 2011). Earlier efforts to resolve the placement of *Paleoclusia* did not include many of these newly discovered clusioid subclades (i.e., Bonnetiaceae, Calophyllaceae, and Podostemaceae). Finally, the sampling by Crepet and Nixon (1998) included many ingroup taxa now known to be distantly related to Malpighiales. For example, they included several members of the asterid clade (e.g., Ericaceae and Theaceae s.l.).

Given the importance of *Paleoclusia* as a major reference point for understanding the timing of angiosperm diversification, determining an accurate phylogenetic placement

of this fossil is essential. *Paleoclusia* is especially important for understanding the evolution of rosids, which contain greater than one-fourth of all angiosperm species and represent most lineages of forest trees in temperate and tropical areas worldwide (Wang et al., 2009). Many of our most important crops are also members of the rosid clade, including legumes (Fabaceae) and numerous fruit crops (e.g., Rosaceae). Furthermore, the rosids have received intensive genomic investigation: whole draft genomes are now available for *Arabidopsis* (Arabidopsis Genome Initiative, 2000), *Carica* (Ming et al., 2008), *Cucumis* (Huang et al., 2009), *Glycine* (Schmutz et al., 2010), *Lotus* (Sato et al., 2008), *Malus* (Velasco et al., 2010), *Fragaria* (Shulaev et al., 2011), *Populus* (Tuskan et al., 2006), *Ricinus* (Chan et al., 2010), and *Theobroma* (Argout et al., 2011). Thus, determining the placement of *Paleoclusia* is a critical component in understanding many aspects of angiosperm evolution including biome and genome evolution.

In this study we present a phylogenetic hypothesis of the clusioid clade derived from morphological and molecular data. Importantly, the analyses we conduct here allow us to include taxa for which no molecular data are available. Our goals for this study are to: i) assess congruence of topologies inferred from morphological and molecular data, ii) analyze the morphological data simultaneously with molecular data to better place clusioid taxa for which molecular data are unavailable, and iii) use ASRs to examine the evolution of traits that have been important for circumscribing clusioid taxa and to further explore the placement of *Paleoclusia*.

MATERIALS AND METHODS

Taxon sampling–Taxa scored for morphology were selected to represent all extant genera of Bonnetiaceae, Calophyllaceae, Clusiaceae s.s., and Hypericaceae following Ruhfel et al. (2011) plus *Paleoclusia chevalieri* (Crepet and Nixon, 1998). In many cases we included more than one representative of morphologically diverse genera (e.g., *Clusia*, *Garcinia*, *Hypericum;* see Table 2.1). The molecular phylogeny of Ruhfel et al. (2011) revealed that *Hypericum* was not monophyletic because the genera *Santomasia*, *Thornea*, and *Triadenum* were well-supported as embedded with the genus. It is very likely that *Lianthus,* a genus for which molecular data are unavailable, is also nested within *Hypericum*. *Lianthus* shows strong morphological affinities with *Thornea* and *Triadenum* (Robson, 2001; Ruhfel et al., 2011). Species of these four genera have all previously been described as members of *Hypericum*, and we treat them here as such following Ruhfel et al. (2011; see Table 2.1). Within Podostemaceae three representative clades were included to represent the subfamilies Podostemoideae, Weddellinoideae, and Tristichoideae. Each of these subfamilies is well supported as a clade (Kita and Kato, 2001; Moline et al., 2007; Ruhfel et al., 2011).

Taxa scored for morphology included a mixture of single species and composite placeholder taxa (see Table 2.1). Composite taxa encompass several species and were mostly defined based on well-supported clades identified by Ruhfel et al. (2011). Some composite *Hypericum* taxa (*Hypericum* Ascyreia s.l., *Hypericum* Euhypericum, *Hypericum* sect. Adenotrias, *Hypericum* sects. Brathys + Trignobrathys, *Hypericum* sect. Elodes, and *Hypericum* sect. Myriandra) were defined based on the molecular results of (Nürk et al., 2010). Clade names for the composite taxa *Hypericum* Ascyreia s.l. and

Table 2.1. Taxonomic sampling scheme for morphological and molecular data (*matK*, *ndhF*, *rbcL*, and *matR*). Composite morphological taxa are marked with an asterisk (*). A dash (–) indicates that molecular data was not available for that taxon. The clade names for the morphological taxa *Hypericum* Ascyreia s.l. and *Hypericum* Euhypericum are based on informal clade names given to well-supported clades in Nürk et al. (2010). Names of former segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum* are indicated in parentheses.

Morphological data	Molecular data
Paleoclusia chevalieri Crepet & Nixon	–
Bonnetiaceae	
Archytaea *	*Archytaea triflora* Mart.
Bonnetia *	*Bonnetia sessilis* Benth.
Ploiarium *	*Ploiarium alternifolium* Melchior
Calophyllaceae	
Calophyllum *	*Calophyllum inophyllum* L.
Caraipa *	*Caraipa savannarum* Kubitzki
Clusiella *	*Clusiella isthmensis* Hammel
Endodesmia calophylloides Benth.	*Endodesmia calophylloides* Benth.
Hapoclathra *	*Haploclathra paniculata* Benth.
Kayea *	*Kayea oblongifolia* Ridl.
Kielmeyera *	*Kielmeyera petiolaris* Mart.
Lebrunia bushaie Staner	–
Mahurea *	*Mahurea exstipulata* Benth.
Mammea americana group *	*Mammea americana* L.
Mammea bongo (R. Vig. & Humbert) Kosterm.	–
Mammea siamensis group *	*Mammea siamensis* T. Anderson
Mammea touriga (C.T. White & W.D. Francis) L.S. Sm.	*Mammea touriga* (C.T. White & W.D. Francis) L.S. Sm.
Marila grandiflora group *	–
Marila tomentosa group *	*Marila tomentosa* Poepp. & Endl.
Mesua ferrea L.	*Mesua ferrea* L
Mesua thwaitesii group *	–
Neotatea columbiana Maguire	–
Poeciloneuron indicum Bedd.	*Poeciloneuron indicum* Bedd.
Poeciloneuron pauciflorum Bedd.	–
Clusiaceae s.s.	
Allanblackia *	*Allanblackia sp*
Chrysochlamys *	*Chrysochlamys allenii* (Maguire) Hammel
Clusia alata Planch. & Triana	–
Clusia caudatum (Planch. & Triana) Pipoly (synomym *Pilosperma caudatum* Planch. & Triana)	–
Clusia columbiana Pipoly (synonym *Havetia laurifolia* Kunth)	–
Clusia gundlachii Stahl	*Clusia gundlachii* Stahl
Clusia major L.	*Clusia major* L
Clusia panapanari (Aubl.) Choisy	–
Clusia p.p. (*Havetiopsis*) *	*Clusia* cf *flavida* (Benth.) Pipoly
Clusia p.p. (*Oedomatopus* spp.) *	–
Clusia p.p. (*Quapoya spp*) *	*Clusia hammeliana* Pipoly
Clusia p.p. (*Renggeria*) *	–
Decaphalangium peruvianum Melch	–
Dystovomita *	*Dystovomita paniculata* (Donn. Sm.) Hammel

Table 2.1 (Continued).

Morphological data	Molecular data
Clusiaceae s.s. (cont.)	
Garcinia cymosa (K. Schum.) I.M.Turner & P.F.Stevens	*Garcinia cymosa* (K. Schum.) I.M.Turner & P.F.Stevens
Garcinia dulcis (Roxb.) Kurz	*Garcinia spicata* Hook. f.
Garcinia morella Desr.	*Garcinia urophylla* Scort. ex King
Gacinia p.p. (*Pentaphalangium* spp.) *	*Garcinia latissima* Miq.
Garcinia p.p. (*Rheedia* spp.) *	*Garcinia macrophylla* Mart.
Lorostemon bombacıflorum Ducke	–
Lorostemon coelhoi Paula	*Lorostemon coelhoi* Paula
Montrouzeria *	*Montrouziera cauliflora* Planch. & Triana
Moronobea *	*Moronobea coccinea* Aubl.
Pentadesma *	*Pentadesma butyracea* Sabine
Platonia insignis Mart.	*Platonia insignis* Mart.
Septogarcinia sumbawaensis Kosterm	*Garcinia cowa* Roxb.
Symphonia *	*Symphonia globulifera* L. f.
Thysanostemon pakaraimae Maguire	–
Tovomita *	*Tovomita calophyllophylla* García-Villacorta & Hammel
Tovomita weddelliana Planch. & Triana	*Tovomita weddeliana* Planch. & Triana
Tovomitopsis *	*Tovomitopsis saldanhae* Engl.
Hypericaceae	
Cratoxylum sects Cratoxylum and Tridesmos *	*Cratoxylum cochinchinense* (Lour.) Blume
Cratoxylum sect Isopterygium *	*Cratoxylum arborescens* (Vahl) Blume
Eliea articulata	*Eliea articulata* Cambess.
Harungana madagascariensis Poir.	*Harungana madagascariensis* Poir.
Hypericum Ascyreia s.l *	–
Hypericum Euhypericum *	*Hypericum perforatum* L.
Hypericum ellipticifolium H.L. Li (synonym *Lianthus ellipticifolius* [H.L. Li] N. Robson)	–
Hypericum p.p. (*Thornea* spp.) *	*Hypericum* (*Thornea*) *calcicola* Standl. & Steyerm.
Hypericum p.p. (*Triadenum* spp.) *	*Hypericum* (*Triadenum*) *fraseri* (Spach) Steudel
Hypericum (*Santomasia*) *steyermarkii* Standl.	*Hypericum* (*Santomasia*) *steymarkii* Standl.
Hypericum sect *Adenotrias* *	*Hypericum aegypticum* L.
Hypericum sects *Brathys* and *Trignobrathys* *	*Hypericum irazuense* Kuntze ex N. Robson
Hypericum sect. *Elodes* *	*Hypericum elodes* L.
Hypericum sect *Myriandra* *	*Hypericum kalmianum* L.
Psorospermum lamianum H Perrier	–
Psorospermum cerasifolium group *	–
Psorospermum febrifugum Spach	*Psorospermum febrifugum* Spach
Psorospermum staudtii group *	–
Vismia affinis Oliv.	–
Vismia cayennensis (Jacq.) Pers.	*Vismia billbergiana* Beurl.
Vismia laurentii De Wild.	*Vismia guineensis* (L.) Choisy
Vismia orientalis Engl.	–
Vismia rubescens Oliv	*Vismia rubescens* Oliv.
Podostemaceae	
Podostemoideae *	*Podostemum ceratophyllum* Michx.
Weddellinoideae *	*Weddellina squamulosa* Tul.
Tristichoideae *	*Tristicha trifaria* (Bory ex Willd) Spreng

Hypericum Euhypericum are based on informal names given to well-supported clades in the later study. Those composite taxa that have not previously been identified in a molecular phylogenetic analysis were based on recent taxonomic circumscriptions by Stevens (2007a; b, unpublished). Molecular data from Ruhfel et al. (2011) were selected to match our morphological sampling. Each species scored for morphology was analyzed in combination with molecular data from the same species, except for three Clusiaceae s.s. taxa (*Garcinia dulcis* [Roxb.] Kurz, *G. morella* Desr., and *Septogarcinia sumbawaensis* Kosterm.) and two Hypericaceae taxa (*Vismia cayennensis* [Jacq.] Pers. and *V. laurentii* De Wild.). Morphological data from these Clusiaceae s.s. species were paired with molecular data from species that are closely related based on morphology or molecular data (Sweeney, 2008; P. Sweeney, pers. comm.). Morphological data from the two *Vismia* taxa were similarly paired with close relatives (Bamps, 1966; Ruhfel et al., 2011). For composite taxa we included molecular data from a single representative species that is known to be included in that clade (Table 2.1). For example, the genus *Bonnetia* is scored as a morphological composite taxon. Thus, in the combined analyses we paired morphological data from the composite taxon *Bonnetia* with molecular data from *Bonnetia sessilis* Benth.

A preliminary analysis using complete plastid genomes to resolve broad Malpighiales relationships (Xi et al., 2010) has identified a strongly supported clade containing the clusioids plus Ochnaceae s.l. (including Medusagynaceae and Quiinaceae), Ctenolophonaceae + Erythroxylaceae + Rhizophoraceae, and Pandaceae + Irvingiaeae. Family designations follow APG III (2009). We have included three of these taxa as outgroups in our molecular and combined analyses: *Ctenolophon englarianus* Mildbr.

(Ctenolophonaceae), *Ochna multiflora* DC. (Ochnaceae s.l.), and *Panda oleosa* Pierre (Pandaceae). *Ctenolophon* was used to root our trees. Outgroups were not scored for morphology. In order to infer directionality in our morphological topologies, we rooted these trees in a position similar to the ingroup rooting inferred using molecular data (i.e., along the branch connecting Bonnetiaceae + Clusiaceae s.s with Calophyllaceae + Hypericaceae + Podostemaceae; Ruhfel et al., 2011).

Finally, we further verified the placement of *Paleoclusia* as a member of the clusioid clade using two interactive keys: one by Watson and Dallwitz (1992 onwards) and a second by Nixon (http://www.plantsystematics.org). Both keys identified *Paleoclusia* as a member of Clusiaceae s.l. (i.e., including Calophyllaceae, Clusiaceae s.s., and Hypericaceae). For the purposes of this exercise we considered resin/latex as present in *Paleoclusia* due to the secretory canals observed in the ovary (Crepet and Nixon, 1998), but we did not score the presence of an aril (see discussion below). The Watson and Dallwitz key included all five clusioid families; Bonnetiaceae and Podostemaceae were absent from the Nixon key.

Morphological data–Sixty-nine discrete (binary or multistate) morphological characters (Appendix 2.1) representing vegetative and reproductive structures were scored for 81 clusioid taxa, including *Paleoclusia* (see Tables 2.1, 2.2; Appendices 2.1, 2.2, and 2.3). No molecular data are available for 23 of these taxa for the genes used in our study. Morphological data for the composite *Hypericum* taxa defined in Nürk et al., (2010; see above) were taken from Nürk and Blattner (2010). *Tovomitopsis*, and the subfamilies of Podostemaceae were also scored from the literature (Engler, 1888; Wanderly et al., 2001; Cook and Rutishauser, 2007).

Table 2.2. Number of state changes, consistency index (CI), retention index (RI), and rescaled consistency index (RC) for each morphological character scored in this study. *Paleoclusia* was not included in the calculation of these values.

	Character	% Missing	States	Changes	Steps	CI	RI	RC
1	Obvious root/stem/leaf construction	0	2	1	1	1	1	1
2	Phellogen initiation in root	71.3	2	1	1	1	1	1
3	Phellogen initiation in stem	20	2	1	2	1	1	1
4	Cortical sclereids in stem	15	3	14	17	0.29	0 63	0 18
5	Functional terminal buds	11.3	2	5	6	0.33	0.5	0.17
6	Terminal buds with scales	3.8	2	7	13	0 54	0 71	0 38
7	Axillary buds immersed	3.8	2	1	1	1	1	1
8	Branching from axils of leaves of current flush	12.5	2	4	4	0 25	0 63	0 16
9	Leaf insertion	3 8	2	4	5	0 4	0.5	0.2
10	Colleters present	6 3	2	6	7	0.29	0.85	0 24
11	Stipuliform structures	3 8	2	5	6	0 33	0.56	0 19
12	Secondary veins arising from the length of the midrib	3 8	2	1	6	1	0	0
13	Intersecondary veins modified as canals	11 3	2	2	2	0 5	0	0
14	Tertiary veins parallel at right angles to secondaries	3 8	2	1	4	1	1	1
15	Exudate in plant body	0	2	2	3	0 67	0 67	0 44
16	Shape of exudate containing structures in mesophyll	3 8	3	3-4	16	0 88	0 94	0 82
17	Fibers in mesophyll of lamina	6 3	2	1	1	1	1	1
18	Lamina with lignified margin	7 5	2	4	6	0.5	0 82	0 41
19	Midrib structure	6 3	4	8	10	0 5	0 81	0 41
20	Lateral bundles in leaf transcurrent	15	2	7	7	0 14	0 77	0 11
21	Abaxial palisade tissue present	12 5	2	3	5	0 6	0 33	0 2
22	Stomatal type	6 3	2	1	1	1	1	1
23	Indumentum of unbranched unicellular hairs	3 8	2	6	11	0 55	0 38	0 2
24	Indumentum of multicellular hairs	3 8	3	4-5	11	0 73	0 75	0 55
25	Marginal setae present	3 8	2	1	1	1	1	1
26	Marginal disciform glands present	3 8	2	1	1	1	1	1
27	Xylem parenchyma present	37 5	2	1	2	1	1	1
28	Prenylated anthranoids	48 8	2	1	1	1	1	1
29	Inflorescence or flower position	5	2	9	15	0 47	0 5	0 23
30	Inflorescence type	3.8	3	9	10	0 3	0 59	0 18
31	Patern of inflorescence internode elongation	8 8	2	1	1	1	1	1
32	Terminal flowers present on inflorescence	5	2	2	3	0 67	0 5	0 33
33	Bracteoles	6 3	3	3	5	0 8	0 89	0 71
34	Flower buds	0	2	1	2	1	1	1
35	Sepal number	3 8	4	12	15	0 4	0 55	0.22
36	Hairs on adaxial surface of petals	3 8	2	1	1	1	1	1
37	Androgynophore present	0	2	1	1	1	1	1

Table 2.2 (Continued).

	Character	% Missing	States	Changes	Steps	CI	RI	RC
38	Androecium arrangement	0	2	7	10	0.4	0 83	0 33
39	Androecium adnate to petals	3 8	2	1	1	1	1	1
40	Fasciclodia present in staminate or perfect flowers	1 3	2	5	7	0 43	0 84	0 36
41	Filament attachment	1.3	2	1	5	1	1	1
42	Filament much thinner than anthers	1 3	2	3	3	0.33	0 92	0 31
43	Filaments papillate	0	2	2	3	0.67	0 67	0 44
44	Anther orientation	7 5	2	3	3	0.33	0 82	0 27
45	Anthers locellate	0	2	5	6	0.33	0 2	0 07
46	Anther length	0	2	3	3	0 33	0 75	0 25
47	Anthers with craterıform glands	1.3	2	2	6	0.83	0	0
48	Anthers with porose dehiscence	0	2	2	2	0.5	0 5	0 25
49	Pollen aperture number	10	2	4	11	0.73	0.67	0 48
50	Pollen with supratectal elements	12 5	2	5	6	0.33	0 5	0 17
51	Carpel number	0	6	12-14	50	0 82	0 68	0 56
52	Ovary septate	7.5	2	2	2	0 5	0	0
53	Ovules per carpel	0	2	7	12	0 5	0 57	0 29
54	Style length	6 3	2	6	13	0.62	0 82	0 51
55	Stylar fusion	10	2	5	8	0.5	0 86	0 43
56	Stigma type	1 3	2	1	1	1	1	1
57	Stigma width	1 3	3	8	12	0 5	0 6	0 3
58	Stigma surface	2 5	3	6	7	0 43	0 76	0 33
59	Fruit type	0	3	8	12	0 5	0 81	0 4
60	Seeds with aril	0	2	1	1	1	1	1
61	Seeds winged	0	2	3	4	0 5	0 67	0.33
62	Seeds with surface glands	3 8	2	2	3	0 67	0 75	0 5
63	Testa complex	5	2	5	6	0 33	0 88	0 29
64	Lignified exotegmen	7 5	2	10	11	0 18	0 64	0 12
65	Cotyledon hypocotyl radicle ratio	8 8	3	4	4	0 5	0 95	0 48
66	Cotyledons cordate at the base	27 5	2	1	1	1	1	1
67	Germination type	65	2	5	6	0 33	0 6	0 2
68	Seedling with accessory roots	63 8	2	5	5	0 2	0 2	0.04
69	Dioecy	0	2	5	5	0 2	0 86	0 17

Paleoclusia was scored for ~45% of the total morphological characters (i.e., 31 of 69). Because the fossil is a flower, vegetative and anatomical characters were largely unscored. Our character scoring was similar to that of Crepet and Nixon (1998) in those characters that were overlapping with one exception. We scored *Paloeclusia* as lacking an aril. If the structure in the fossil is indeed an aril, it is unlike that of extant Clusieae, which is the only clusioid clade with arillate seeds. In Clusieae the aril surrounds the seed (e.g., Fig. 2.1), but in the fossil it appears to be adjacent to the seed (Figs. 28 and 29 in Crepet and Nixon, 1998). In addition, the "aril" in *Paleoclusia* has a cell wall pattern that is very similar to the seeds (Figs. 28 and 30 in Crepet and Nixon, 1998). Thus, in our opinion it seems more likely that this structure is an aborted seed rather than an aril (Stevens 2001 [onwards], published online Aug. 2010).

Dioecy is known to occur in *Calophyllum*, Clusieae, *Clusiella*, Garcinieae, and *Mammea*. Dioecy may have evolved several times in *Calophyllum* (Stevens, 1980; Vamosi, 2006; Vela, 2010) and our scoring of *Calophyllum* as dioecious thus provides a minimum bound on the number of origins of dioecy in this clade. The presence/absence of dioecy was scored and used for ASRs, but not in phylogenetic reconstruction. This decision was made for two main reasons. First, despite the fact many clusioids are known to be dioecious (Dunthorn, 2004; Martins et al., 2007; Sweeney, 2008), it is likely that dioecy is not homologous across the clusioids. *Mammea,* for example is cryptically dioecious (Dunthorn, 2004): pollen from bisexual flowers, but not staminate flowers, are inaperaturate and likely not functional. This kind of dioecy is otherwise unknown in the clusioids. The second reason is related to the uncertainty of dioecy in *Paleoclusia.* Stamens of *Paleoclusia* mostly lack pollen, but in some anthers, pollen is present (Crepet

Figure 2.1. A seed of *Tovomitopsis saldanhae* Engl. (Clusieae, Clusiaceae s.s.); note the brightly colored aril surrounding the seed. Photograph by Dr. Volker Bittrich.

and Nixon, 1998). Given the uncertainty of this trait, combined with the relatively small number of traits scored for *Paleoclusia*, we felt that its inclusion might have an unnecessarily large effect on the placement of *Paleoclusia*.

The vegetative morphology of Podostemaceae has been difficult to interpret. This has made their comparison to other angiosperms difficult (Cusset and Cusset, 1988; Cook and Rutishauser, 2007; Stevens, 2007b). Recent developmental studies support this complexity and suggest that vegetative organs in some Podostemaceae may be a mixture of leaf and shoot identity (Katayama et al., 2010), and thus may not be easily comparable to vegetative organs in other clusioids. Because it is unclear which vegetative characters can be reliably scored as homologous with other clusioids (C.T. Philbrick, unpublished data; Katayama et al., 2008), very few vegetative characters were scored for Podostemaceae.

Phylogenetic analyses of morphological data– All phylogenetic analyses of the morphological data were conducted with and without *Paleoclusia*. Maximum-parsimony (MP) analyses were conducted with PAUP* ver. 4.0b10 (Swofford, 2003) using the parsimony ratchet (Nixon, 1999) as implemented in PAUPRat (Sikes and Lewis, 2001; distributed by D. Sikes at http://users.iab.uaf.edu/~derek_sikes/software2.htm). We conducted 100 replicates of 200 iterations each with 20% of characters reweighted per iteration. Morphological characters were equally weighted and character states were unordered. Gaps were treated as missing data and included in our analyses. Characters coded with multiple states for a single taxon were treated as polymorphic rather than uncertain. Bootstrap percentage (BP) support (Felsenstein, 1985) for each clade was estimated from 1,000 heuristic search replicates using PAUP* (10 random taxon addition

replicates, TBR branch swapping, MULTREES=yes, and holding no more than 10 trees per replicate). Maximum likelihood (ML) analyses of the morphological data were performed using the MK model of evolution (Lewis, 2001) with a GAMMA model of rate heterogeneity as implemented in RAxML ver. 7.2.6 (Stamatakis, 2006; available at http://wwwkramer.in.tum.de/exelixis/software.html). The optimal ML tree and BP values were estimated simultaneously using the default settings. The ML BP values were obtained from 1000 bootstrap replicates using the rapid bootstrap algorithm implemented in RAxML (Stamatakis et al., 2008).

Bayesian inference (BI) of the morphological data was conducted with Mr. Bayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001) using the MK model with a parameter for rate variation among characters ("rates=gama"). Our coding of morphological characters was biased because we included only variable characters ("coding=variable"). To determine the consistency of results from our Bayesian analyses we conducted two runs, each with two simultaneous replicate searches (four independent searches in total). Each of the replicate searches used eight chains and the temperature parameter for heating the chains was set to 0.05 to improve the acceptance rates of chain swapping. All searches ran for 30 million generations sampling every 1000 generations. Default priors were used. Convergence was assessed in three ways: i) using Tracer v1.5 (distributed by A. Rambaut at http://tree.bio.ed.ac.uk/software/tracer/) to determine stationarity of likelihood and other parameter values, ii) observing the average standard deviation of split frequencies between runs as reported by MrBayes, and iii) by using the "compare" and "cumulative" functions in AWTY (Wilgenbusch et al., 2004; Nylander et al., 2008). BI posterior probabilities (PP) were determined by building a 50% majority rule

consensus tree after discarding the burn-in generations (10%) and pooling the two replicates of the first run. Results of the two replicates from the second run were essentially identical to the results from the first run.

Molecular data and phylogenetic analysis–Our molecular data set included 58 clusioid taxa, plus three outgroups (Table 2.1; Appendix 2.4). These data were obtained from Ruhfel et al. (2011) and the alignment was unmodified except to remove indels that were no longer applicable following taxon removal. MP, ML, and BI analyses were conducted as above with the following differences. In the ML and BI analyses the data set was partitioned by gene region with all parameters estimated from the data. In the BI analyses each partition was allowed to have its own character state frequencies, substitution rates, and gamma shape parameter (i.e., these parameters were unlinked). We selected the best-fitting model for each gene partition using MrModelTest ver. 2.3 (distributed by J.A.A. Nylander at http://www.abc.se/~nylander/) using the Akaike information criterion (Table 2.3). We chose not to estimate the proportion of invariable sites following Ruhfel et al. (2011).

Phylogenetic analyses of combined data–To assess data set compatibility we first compared the morphological (Fig. 2.2) and molecular (Fig. 2.3) phylogenies for conflicting nodes, i.e., those nodes that disagreed with support greater than 70 BP or 95 PP. Two instances of strongly supported conflict were observed in the Garcinieae + Symphonieae clade in our ML analyses. The first involved the placement of *Garcinia macrophylla* Mart. and *G. urophylla* Scort. ex King in the molecular phylogeny, and the associated representatives of these species from the morphological data, *Garcinia* p.p. (*Rheedia* spp.) and *Garcinia morella*, respectively (Table 2.1). In the morphological

Table 2.3. Data set characteristics. Percent missing data were calculated as the total number of ?'s in the analyzed matrix divided by the total number of characters including gaps. Morphological and combined molecular + morphological data set totals include *Paleoclusia*. Numbers in parentheses are for the ML and Bayesian analyses. Models of sequence evolution were chosen by the AIC criterion using MrModelTest version 2.3.

Data set	*matK*	*ndhF*	*rbcL*	*matR*	Combined Molecular	Morphology	Combined morphology + molecular
Terminals	57	59	58	56	61	81	84
Characters Analyzed	1320	1041	1296	2331	5988	68	6056
% missing data	9.19	15 77	7 81	4 83	14.15	9 5 (12 07)	37 38 (37.42)
% gaps plus missing data	25 06	24 1	7 81	31 70	28 42	13 29	47 68
Constant Characters	592	498	928	1761	3779	0	3779
Variable Characters	728	543	368	570	2209	68	2277
Parsimony informative characters	526	374	243	269	1412	67	1479
% Parsimony informative characters	39 85	35 93	18 75	11 54	23.58	98.53	24 42
Model of sequence evolution	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ	GTR+Γ	NA	MK	NA

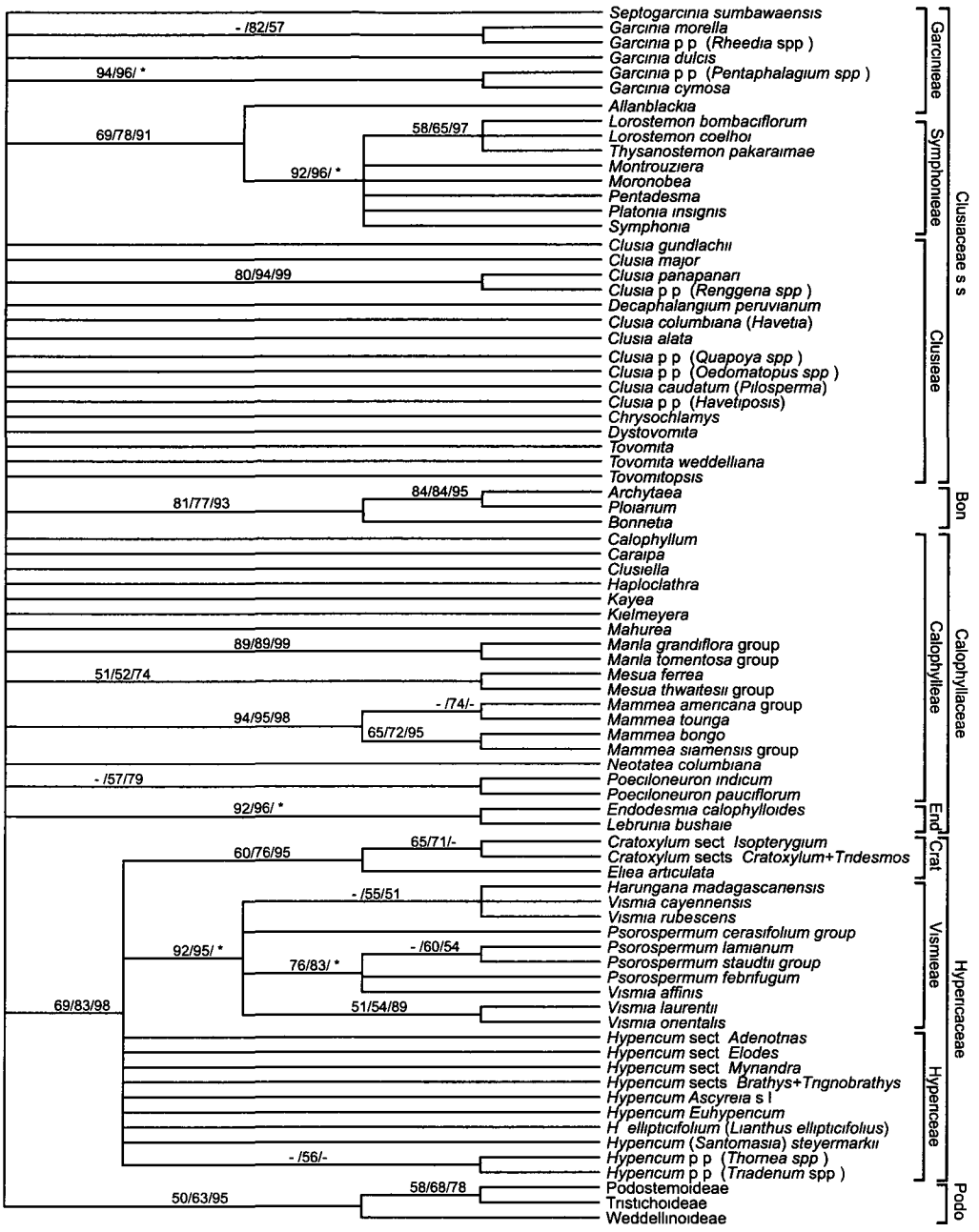

Figure 2.2. Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on the morphological data set. Support values ≥ 50% are indicated accordingly: maximum parsimony bootstrap percentages (BP; left), ML BP (center), and Bayesian posterior probabilities converted to percentages (right). An asterisk indicates maximum support (100 BP or 100 PP). A hyphen indicates that the node was not present in a particular analysis. Taxonomy in Hypericeae follows Ruhfel et al. (2011). Names of former segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum* are indicated in parentheses. Bon. = Bonnetiaceae, Crat. = Cratoxyleae, End. = Endodesmieae, Podo.= Podostemaceae.

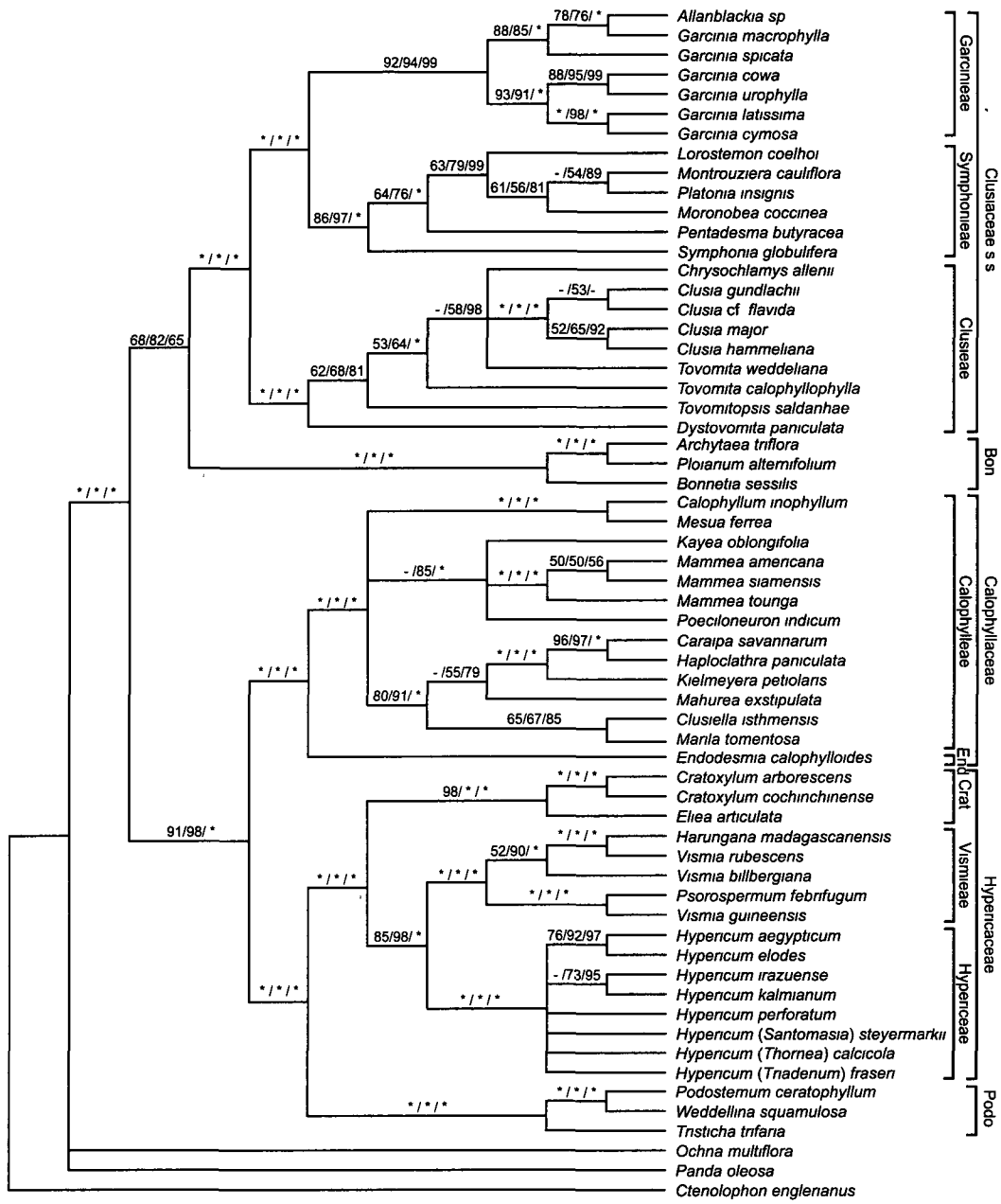

Figure 2.3. Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on a four-gene (*matK*, *ndhF*, *rbcL*, and *matR*) molecular data set. Support values ≥50% are indicated; maximum parsimony bootstrap percentages (BP; left), ML BP (center), and Bayesian posterior probabilities converted to percentages (right). An asterisk indicates maximum support (100 BP or 100 PP). A hyphen indicates that the node was not present in a particular analysis. Taxonomy in Hypericeae follows Ruhfel et al. (2011); former names are included in parentheses. Bon. = Bonnetiaceae, Crat. = Cratoxyleae, End. = Endodesmieae, Podo.= Podostemaceae.

topology (Fig. 2.2) these taxa are sisters with high support (82 ML BP). In the molecular topology (Fig. 2.3) *G. macrophylla* is instead sister to *Allanblackia sp.* with moderate support (76 ML BP). The second involved the placement of *Allanblackia.* In the morphological topology (Fig. 2.2), *Allanblackia* is sister to Symphonieae with moderate support (78 ML BP). In the molecular topology (Fig. 2.3) it is sister to *Garcinia macrophylla* with moderate support (76 ML BP).

To determine if data from the morphological and molecular data sets could reject the topology derived from the rival data set we performed alternative topology tests in an ML framework using the approximately unbiased test (AU; Shimodaira, 2002) as implemented in the R software package, scaleboot ver. 0.3-2 (Shimodaira, 2008; distributed by CRAN at http://www.r-project.org). Constrained searches using ML were conducted as above and did not include *Paleoclusia.* For the molecular data set we conducted two constraint searches. The first constrained *Allanblackia* to be a member of the Symphonieae clade, the second constrained *Garcinia macrophylla* and *G. urophylla* as sister taxa. The former constraint was not rejected by the molecular data ($p= 0.0697$) while the later was strongly rejected ($p = 0.0023$). Using the morphological data set we conducted two constraint searches. The first constrained *Allanblackia* to be sister to *Garcinia* p.p. (*Rheedia* spp.), the second constrained *Septogarcinia sumbawaensis* (the morphological taxon paired with *Garcinia cowa*) as sister to *Garcinia morella.* Each of these constrained topologies was strongly rejected by the morphological data ($p = 0.0216$ and 0.0468, respectively).

We further explored our data by analyzing several variations of our morphological and combined data sets with different taxon and morphological character

sampling. Analyses were conducted with and without *Paleoclusia* using MP, ML and BI as outlined above and below. Results of these analyses were largely consistent with those presented here, and additional conflicts were only evident when analyzing a reduced morphological data set (independently or in combination with molecular data) that included only those characters scored for *Paleoclusia.* For instance, some genera (e.g., *Mesua*) were no longer supported as monophyletic indicating that the characters removed (mostly vegetative and anatomical) were informative for inferring phylogenetic relationships. Because vegetative and anatomical characters appear to be important for placing taxa, we feel that the best estimate of the clusioid phylogeny is derived from the use of all characters and all taxa.

MP and ML analyses of the combined data were conducted as described above. ML and BI analyses each had five partitions, one for each gene and one for the morphological data. BI analyses of the combined data using the parameters listed above, however, did not reach convergence in many cases (especially when *Paleoclusia* was included). To achieve convergence we implemented two changes to our BI search strategy. First, for each MCMC search we supplied an optimal ML starting tree without branch lengths from the analysis of that data set. Since supplying a starting tree can inhibit the ability to detect problems with convergence using independent runs, we used the command "nperts=2", which introduces two random perturbations to the starting tree topology for each chain. Using this strategy, searches reached convergence in some instances, but not when *Paleoclusia* was included. Second, instead of allowing each partition to have its own rate ("ratepr=variable") we fixed the rate to the average rate across all partitions ("ratepr=fixed"). This allowed our BI analyses to achieve acceptable

levels of convergence. For consistency, these two changes were implemented in all BI analyses.

Ancestral state reconstructions–We used ML ASRs as implemented in Mesquite ver. 2.74 (Maddison and Maddison, 2010) to infer the evolution of the 69 morphological characters scored for this study. The ML method for ancestral state reconstruction was chosen over parsimony reconstruction for two reasons. First, ML reconstructions consider branch lengths, i.e., the longer a branch, the more likely it is that change may have occurred. Second, ML reconstructions estimate the relative probability of each state at a particular node (Cunningham et al., 1998). For the purpose of brevity only ASRs that fall into two categories will be reported. The first includes characters that have been historically important for determining relationships in the clusioid clade. These include leaf insertion, exudate presence/absence, shape of exudate containing structures in the leaf mesophyll, merosity (sepal number, in particular), stamen arrangement, fasciclodia presence/absence, carpel number, and breeding system (Cronquist, 1981; Stevens, 2007a, b; Weitzman et al., 2007). The second includes additional characters that are important in assessing the placement of *Paleoclusia*. These include: aril presence/absence, presence/absence of an indumentum of unbranched unicellular hairs, filament attachment, filament thickness, anther orientation, pollen aperture number, ovules per carpel, style length, stylar fusion, and stigma surface.

Data were analyzed using the Mk1 model with rate parameters estimated from the data. The likelihood decision threshold of two was used as suggested by Pagel (1999), to determine the optimal ASRs at each node. Characters were treated as unordered and reconstructed onto the ML topology derived from the combined data (Fig. 2.4). This

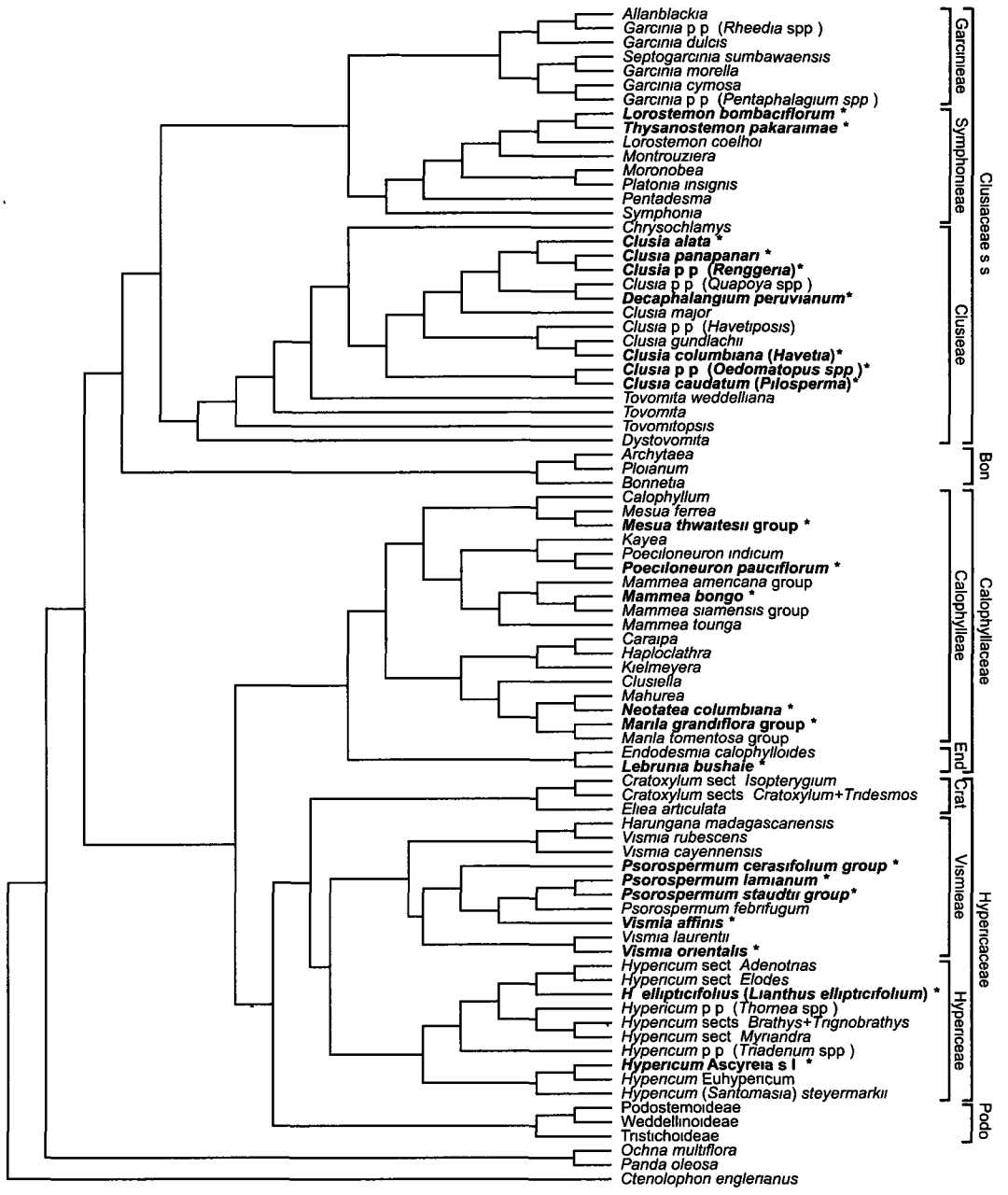

Figure 2.4. Optimal maximum likelihood (ML) topology of the clusioid clade based on the combined morphological and molecular data sets not including *Paleoclusia*. Taxa scored for morphology only are in bold and marked with an "*". Taxonomy in Hypericeae follows Ruhfel et al. (2011). Names of former segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum* are indicated in parentheses. Bon. = Bonnetiaceae, Crat. = Cratoxyleae, End. = Endodesmieae, Podo.= Podostemaceae.

allowed us to include the 22 extant taxa that were scored only for morphology. It is well known that taxon sampling is important for understanding character evolution (e.g., Crane et al., 2004; Manos et al., 2007). We chose to exclude *Paleoclusia* from ASRs given its phylogenetic uncertainty (Fig. 2.5; see below). Instead, we evaluated the alternate placements of this taxon based on ASRs using only extant taxa. We report the consistency index (CI; Kluge and Farris, 1969; Farris, 1989), retention index (RI; Archie, 1989a, b; Farris, 1989), and the rescaled consistency index (RC; Farris, 1989) for each character (Table 2.2) as calculated by the program MacClade ver. 4.08 (Maddison and Maddison, 2005).

RESULTS

Aside from the areas of conflict mentioned above, our analyses resulted in similar topologies with no strongly conflicting nodes. Furthermore, when including *Paleoclusia*, topologies were similar but the inclusion of the fossil resulted in a decline in support along the backbone of the tree (Fig. 2.5). Relevant characteristics for each gene region, the morphological data, and the combined data sets are listed in Table 2.3. The combined morphological and molecular matrix is available from the first author. We will focus our discussion of the results on the 50% ML majority rule consensus trees from i) the morphological data set (Fig. 2.2), ii) the molecular data set (Fig. 2.3), and iii) the combined morphological + molecular data (Fig. 2.6). We will also discuss the optimal ML topology derived from the combined analysis (Fig. 2.4). Unless otherwise noted, BP values given are from the ML analysis. MP BP and BI PP will be mentioned when relevant.

Figure 2.5. Summary of clusioid relationships from analyses of the combined morphology and molecular data sets including and excluding *Paleoclusia* (A: maximum parsimony (MP) and maximum likelihood (ML); B: Bayesian inference (BI). Values above and below branches are for analyses excluding and including the *Paleoclusia* fossil, respectively. Clade size is not drawn proportional to species number.

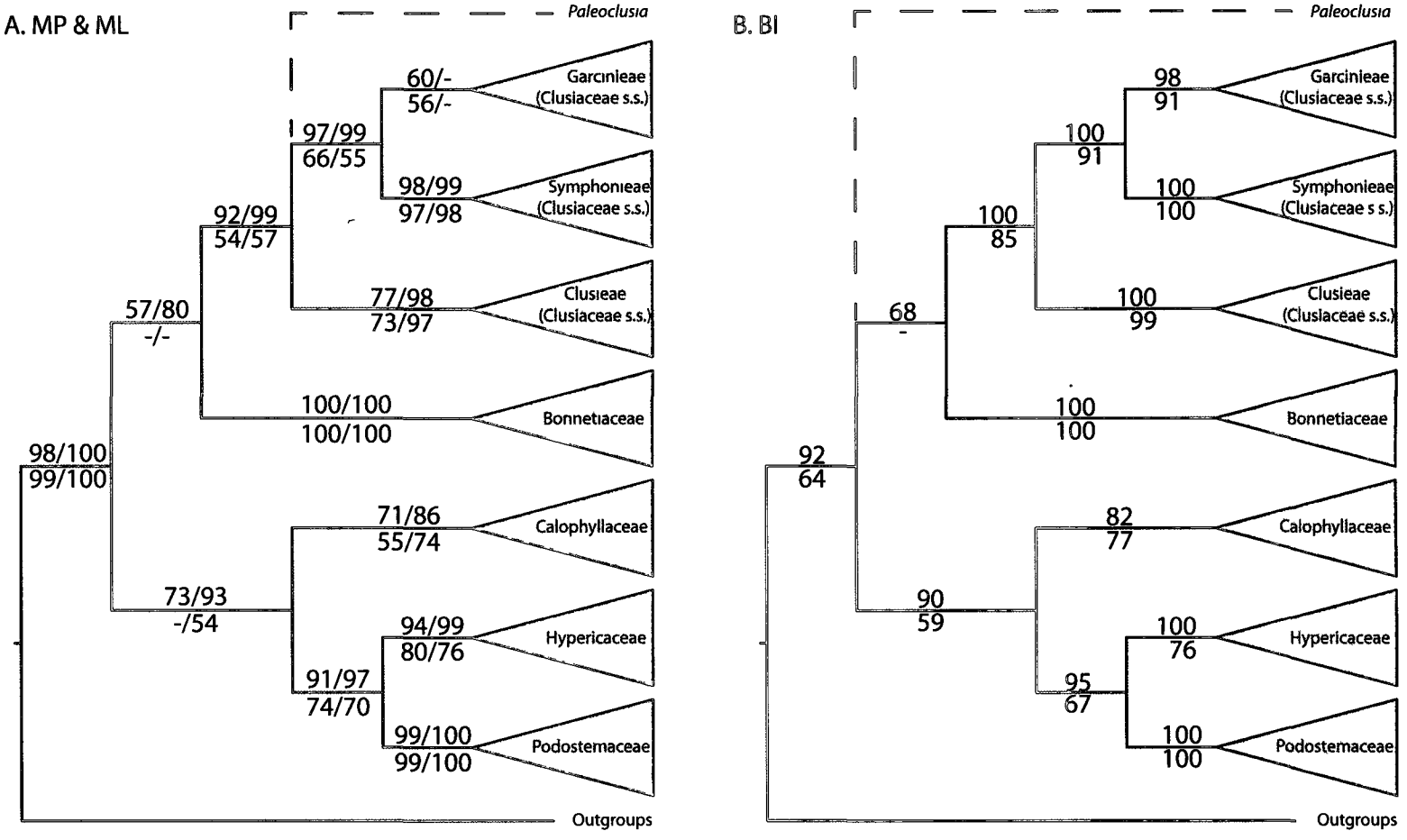

Fig. 2.5 (Continued)

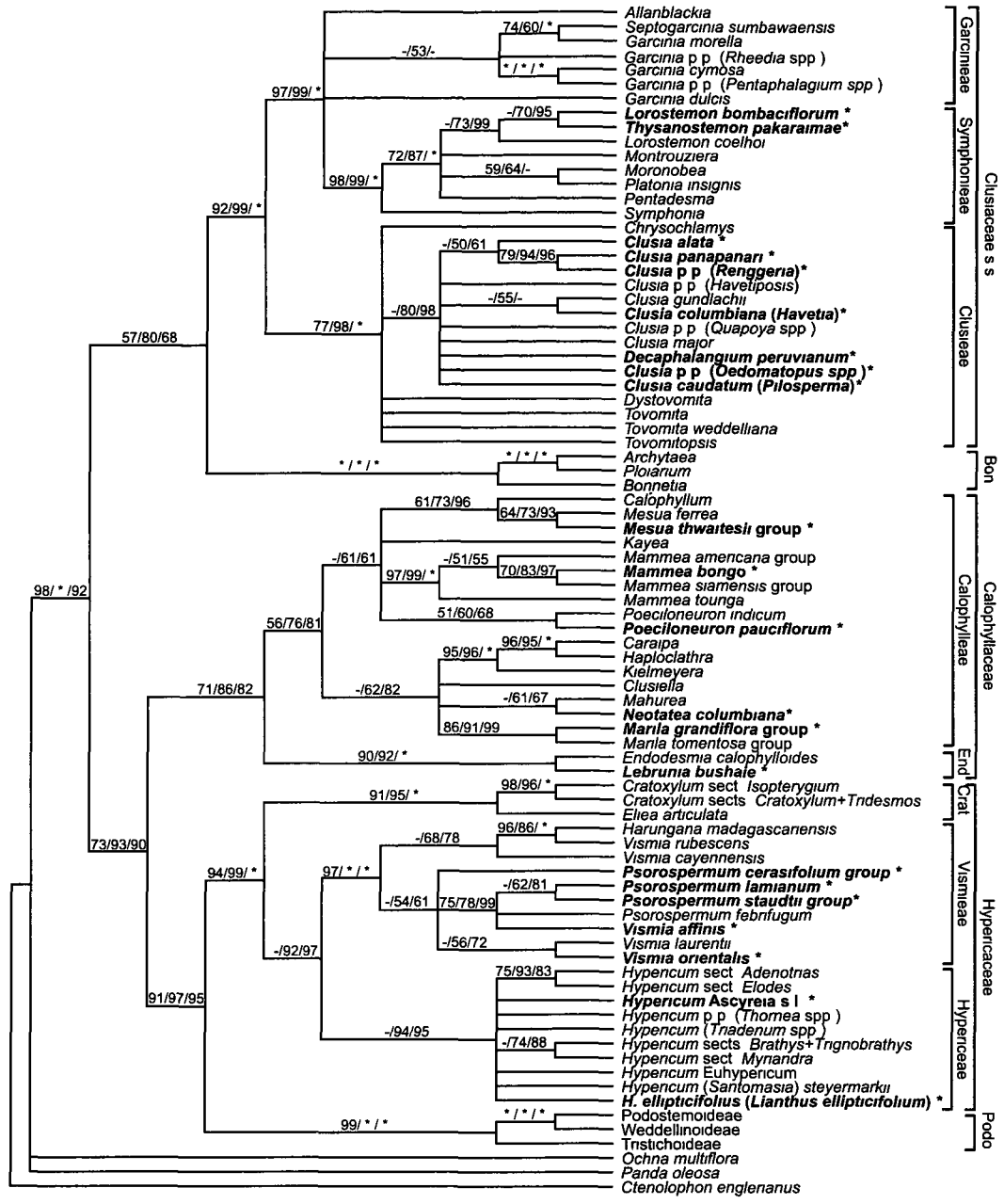

Figure 2.6. Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on the combined morphological and molecular data sets not including *Paleoclusia*. Support values ≥ 50% are indicated; maximum parsimony bootstrap percentages (BP; left), ML BP (center), and Bayesian posterior probabilities converted to percentages (right). An asterisk indicates maximum support (100 BP or 100 PP). A hyphen indicates that the node was not present in a particular analysis. Taxa scored only for morphology only are in bold and marked with an "*". Taxonomy in Hypericeae follows Ruhfel et al. (2011). Names of former segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum* are indicated in parentheses. Bon. = Bonnetiaceae, Crat. = Cratoxyleae, End. = Endodesmieae, Podo.= Podostemaceae.

Morphological data and phylogenetic analyses–Sixty-seven of the 68 characters used in our analyses were parsimony informative. Approximately 10% of the data were missing in the MP analyses and 12% in the ML and BI analyses (ML and BI treat polymorphisms as missing data, hence the discrepancy in missing data). Missing data for each character ranged from 0 to ~71% (Table 2.2).

The phylogeny inferred from our morphological data was less resolved, but uncovered many clades in common with phylogenies derived from molecular data (here, and elsewhere). When *Paleoclusia* was excluded, several clades were recovered that coincide with traditionally recognized taxa including Bonnetiaceae, Cratoxyleae, Endodesmieae, Hypericaceae, Podostemaceae, Symphonieae, and Vismieae (Fig. 2.2). MP tree searches resulted in 163 topologies of 398 steps (CI=0.60, RI=0.81, RC=0.49). Taxa that have not previously been included in molecular phylogenetic studies were placed with varying levels of support. The placement of *Neotatea* (Calophyllaceae) was unresolved, but was consistently placed within Calophyllaceae in the most parsimonious island of trees. *Lebrunia* (Calophyllaceae) was placed with strong support (96 BP) as sister to *Endodesmia*. *Hypericum ellipticifolium* (Hypericaceae), was well placed (83 BP) as a member of Hypericaceae, but its position within the family was unresolved. *Thysanostemon* (Clusiaceae s.s.) was strongly placed (96 BP) within Symphonieae in a poorly supported (65 BP) clade containing two *Lorostemon* species.

When *Paleoclusia* was included in these analyses, its placement was poorly supported (<50% BP; <95% PP, not shown). MP tree searches resulted in 39 equally parsimonious topologies of 400 steps (CI=0.60, RI=0.81, RC=0.49). In these trees, *Paleoclusia* was always placed within Clusiaceae s.s., either as sister to a clade

containing *Allanblackia* + Symphonieae or as sister to a clade containing Garcinieae + Symphonieae. In the optimal ML topology *Paleoclusia* was similarly placed within Clusiaceae s.s. but as sister to Symphonieae. BI analyses also placed *Paleoclusia* (63 PP) within Clusiaceae s.s. in a poorly supported (64 PP) clade with *Allanblackia* and Symphonieae. Resolved nodes and support values in Fig. 2.2 generally remained unchanged with the inclusion of the fossil except for two clades. Support for *Allanblackia* + Symphonieae dropped from 78 to 54 BP; support for Hypericaceae dropped from 83 to 75 BP.

Molecular data and phylogenetic analyses–The aligned molecular data set included 5988 nucleotide bases and 61 taxa including three outgroups. MP searches resulted in 289 topologies of 4978 steps (CI=0.64, RI=0.82, RC=0.52). The ML 50% majority rule topology is very similar to the analyses by Ruhfel et al. (2011). The clusioid clade and all five families received strong support (100 BP; Fig. 2.3). Interfamilial relationships were the same as reported previously (Wurdack and Davis, 2009; Ruhfel et al., 2011). There were areas in our topology where support improved from the Ruhfel et al. (2011) topology. In particular, we recovered a strongly supported (94 BP) Garcinieae and increased support along the backbone of Symphonieae. There were also areas of the phylogeny where support values declined, but only one area that declined dramatically. Relationships within *Hypericum* were well resolved by Ruhfel et al. (2011) but were generally unresolved here.

Combined morphological and molecular data and phylogenetic analyses–Our combined data matrix included 84 taxa and 6056 characters [~37% of which were missing (Table 2.3)]. Of the 84 taxa, 23 taxa (including *Paleoclusia*) were scored only for

morphology, 58 taxa were scored for morphology and molecular data, and three taxa (outgroups) were scored only for molecular data. When analyzing the combined data set without *Paleoclusia*, MP searches resulted in 187 topologies of 5408 steps (CI=0.63, RI=0.81, RC=0.51). Support for the clusioid clade and for its major subclades generally received strong support (>80 BP; Fig. 2.6) and results were largely consistent with the separate analyses (Figs. 2.2 and 2.3). The optimal ML topology can be seen in Fig. 2.4. The combined topology (Fig. 2.6) was less resolved than the molecular topology (Fig. 2.3) in several key areas, especially in Calophylleae, Clusieae, Garcinieae, and Symphonieae. This is perhaps due to conflicting signal in the morphological data set, even though very few of these conflicts were strongly supported (see Discussion).

All taxa scored only for morphology were placed in phylogenetic positions implied by earlier taxonomic accounts (Figs. 2.4 and 2.6). Within Clusiaceae s.s., *Lorostemon coelhoi* Paula, *L. bombaciflorum* Ducke and *Thysanostemon* formed a clade (73 BP) and were strongly placed (99 BP) within Symphonieae. Within this clade, *L. bombaciflorum* was more closely related to *Thysanostemon* (70 BP) indicating that *Lorostemon* may not be monophyletic. Within Clusieae, the many segregate genera that now belong in *Clusia* (Gustafsson et al., 2007) were well supported (80 BP) as monophyletic clade. Most of these segregate genera have been included in previous molecular studies, except *Pilosperma*. Our results indicate that *Pilosperma* is properly treated in *Clusia* as has been suggested by Jorgensen et al. (1999). Within Calophyllaceae, *Lebrunia* is placed sister to *Endodesmia* with strong support (92 BP). *Neotatea* is weakly placed (61 BP) as sister to *Mahurea,* a relationship also present in Notis (2004). Vismieae are monophyletic (100 BP). *Hypericum* Ascyreia s.l., *Hypericum*

ellipticifolium, *Mammea bongo* (R. Vig. & Humbert) Kosterm., the *Marila grandiflora* group, the *Mesua thwaitesii* group, and *Poeciloneuron pauciflorum* Bedd. are all placed in clades with their respective congeners. The placements of these taxa are well supported (>70 BP) except for the sister group relationship of *Poeciloneuron indicum* Bedd. with *P. pauciflorum* (60 BP).

Results of the analysis including *Paleoclusia* produces a dramatic drop in support along the backbone of the tree (Fig. 2.5), but the relationships among the extant taxa remain unchanged from that shown in Fig. 2.6. MP trees searches resulted in 132 topologies of 5411 steps (CI=0.63, RI=0.81, RC=0.51). In the MP trees *Paleoclusia* was placed in four positions near or within Clusiaceae s.s.: sister to Clusiaceae s.s., sister to Symphonieae + Garcinieae, sister to Symphonieae, and sister to Clusieae. In the optimal ML topology, *Paleoclusia* was placed within Garcineae sister to *Allanblackia* (<50 BP). Support was weak (57 BP) for an unresolved clade containing *Paleoclusia* and the two major lineages of Clusiaceae s.s. (Fig. 2.5). BI analyses differed in the placement of the fossil by weakly (64 PP) placing *Paleoclusia* in a trichotomy with the two major lineages of the clusioid clade (Fig. 2.5).

Ancestral state reconstructions–Results for the ASRs are shown in Appendix 2.5 (Figs. A2.1-A2.18). Care should be taken in interpreting our ASRs as taxa coded as polymorphic, missing, or inapplicable for a character were considered absent from the tree in the ML estimations of ancestral character states (Maddison and Maddison, 2010). Any implications of this limitation will be addressed in the discussion. Furthermore, variability for any particular character may be present in composite taxa, but may not be

reflected in our coding. This is only the case when there is evidence that this variation exists in a derived state in the composite taxon.

DISCUSSION

Comparison of the morphological and molecular phylogenies–The topology derived from morphological data (Fig. 2.2) was much less resolved than that derived from the molecular data (Fig. 2.3). Despite this reduced resolution, several clades were recovered when analyzing the morphological data that reflect our current understanding of relationships within the clusioids (Ruhfel et al. 2011). Bonnetiaceae, Hypericaceae, Podostemaceae, and the tribes Cratoxyleae, Endodesmieae, Symphonieae, Vismeae were all identified as clades. Calophyllaceae and Clusiaceae s.s., however, were surprisingly not monophyletic. This may be due to uncertainty in the placement of *Clusiella*, *Endodesmieae*, and Podostemaceae as judged by their alternative placements in the MP trees. Analyses of the full morphological data matrix excluding these three taxa, *Paleoclusia*, and the taxa involved in our strongly reported conflicts (see Methods) resulted in a monophyletic Clusiaceae s.s. and Calophyllaceae. However, when *Paleoclusia* is included, Calophyllaceae and Clusiaceae s.s. are once again not recovered as monophyletic.

Clusiella, Endodesmieae, and Podostemaceae are perhaps causing a loss of resolution in the topology inferred from morphological data due to instances of convergence and highly modified morphologies. *Clusiella* is very similar to *Clusia* and their similarity has been cited as an instance of convergent evolution (Hammel, 1999b; Gustafsson et al., 2002; Stevens, 2007a). *Clusia* and *Clusiella* share an epiphytic habit,

dioecy, a resiniferous, non-fasciculate androecium, and sessile stigmas. It is not surprising that the inclusion of Podostemaceae causes loss of resolution for two reasons. First, the family cannot be easily compared with other angiosperm families because of its highly modified morphology (Cusset and Cusset, 1988; Stevens, 2007b). Second, vegetative characters seem important in placing clusioid taxa: the decreased resolution in our topologies when these characters are excluded was dramatic (data not shown), and many vegetative characters cannot easily be scored for Podostemaceae (see Methods). Reasons for the conflicting placement of Endodesmieae are less clear, but may result from their vegetative similarity to Clusiaceae s.s. and their possession of fruits similar to Calophylleae (Notis, 2004; Stevens, 2007a). Endodesmieae were placed either within Calophylleae or sister to *Garcinia cymosa* (K. Schum.) I.M.Turner & P.F.Stevens + *Garcinia* p.p. (*Pentaphalangium* spp.) in the MP trees. Placement of *Endodesmieae* with these *Garcinia* taxa is likely due to the shared features of a fasciculate androecium and one ovule per carpel, which are features not found in Calophylleae (the sister group of Endodesmieae).

Combined morphological and molecular analyses: the placement of previously unsampled taxa–Analysis of the combined morphological and molecular data set produced a much better resolved topology than the morphological data alone, especially when *Paleoclusia* was excluded from these analyses (Fig. 2.6). However, the topology from the combined analysis is less resolved than the topology produced using molecular data alone. This reduction in resolution and support may result from conflicting phylogenetic signal in the two data sets (see above). Despite this reduction in overall support there are two reasons to have confidence in our combined results. First, there is a

high degree of topological similarity, especially along the backbone of the topology, between the combined results and the results derived only from molecular data. Second, our morphological data set appears to have sufficient signal to place taxa scored only for morphology, at least when analyzed in combination with the molecular data. This is evident as extant taxa scored only for morphology are generally well placed with their closest relatives as suggested by earlier taxonomic classifications (see below; Table 2.1, Fig. 2.6).

Taxa that were unplaced (<50 BP) in the morphological analysis are now placed confidently. In most cases support for the placement of these taxa increased in the combined analyses. Only the placement of *Poeciloneuron indicum* with *P. pauciflorum* did not increase in support. We will focus our discussion on the placements of four taxa that have received little previous phylogenetic attention (*Hypericum ellipticifolium, Neotatea*, *Lebrunia,* and *Thysanostemon*) and then briefly comment on relationships within Vismieae.

Neotatea was originally described as a genus of Bonnetiaceae (Maguire, 1972) and was subsequently treated within that family as a species of *Bonnetia* (Steyermark, 1984). However, these placements were problematic due to its possession of unilacunar nodes, exudate, indumentum, smooth stigmatic surfaces, and anther glands. More recently, it was transferred to Clusiaceae s.l. (including Calophyllaceae and Hypericaceae; Weitzman and Stevens, 1997) and subsequently placed in tribe Calophylleae (Stevens, 2007a). Our results are consistent with this later hypothesis of relationships. *Neotatea,* a strictly neotropical genus, is supported as a member of Calophylleae (76 BP) and is placed within a strictly neotropical clade including the

genera *Caraipa*, *Clusiella*, *Haploclathra*, *Kielmeyera*, *Mahurea*, and *Marila*. This clade is not well supported (62 BP; Fig. 2.6) in our combined analysis, but receives strong support in our molecular analyses (91 BP; Fig. 2.3). In addition to the biogeographic support for this placement, *Neotatea* is a good fit morphologically with members of this neotropical clade. It has alternate leaves and winged seeds, which is a combination of clusioid characters found only within this subclade of Calophyllaceae. Furthermore, the neotropical genera of Calophylleae tend to have terminal inflorescences, five sepals and petals and three carpels. In contrast the primarily Old World members of Calophylleae (*Calophyllum, Kayea, Mammea*, *Mesua*, and *Poeciloneuron*) possess primarily axillary inflorescences and two to four sepals, petals, and carpels. Within this neotropical clade, *Neotatea* is poorly supported (61 BP) as sister to *Mahurea*. This placement is also supported by Notis (2004). That study found *Neotatea* to be sister to *Mahurea* based on the shared presence of features such as seeds with a vascularized wing that does do not completely encircle the seed. In our optimal ML topology (Fig. 2.4) and the MP strict consensus tree (not shown) *Neotatea* is placed with weak support (< 50 BP) in a clade with *Clusiella*, *Marila*, and *Mahurea*. Two morphological characters unique in the Calophyllaceae define this clade: the presence of a lignified exotegmen and a ratio of cotyledon to hypocotyl + radicle between 0.2 and 2. All other Calophyllaceae lack a lignified exotegmen and have a ratio of cotyledon to hypocotyl + radicle greater than 2. The type of seed wing may also be relevant to understanding relationships in this clade. In our morphological data set we have scored winged seeds as equivalent, but it may be that the wing type of the *Kielmeyera* + *Haploclathra* + *Caraipa* clade (i.e., one in which the wing completely surrounds the seed and vascular tissue is absent) is independently

derived in the *Neotatea* + *Mahurea* clade (wing not completely surrounding the seed, vascular tissue present; Notis, 2004).

The second unplaced genus in Calophyllaceae, *Lebrunia,* is considered a close relative of *Endodesmia*, which together constitute Endodesmieae (Stevens, 2007a; Ruhfel et al., 2011). *Endodesmia* and *Lebrunia* are each monotypic and found in western tropical Africa. In the combined analyses, as in the morphology analyses, these taxa are strongly supported (92 BP) as sister clades. They each possess a single, apical ovule, and a one-carpelate gynoecium, the latter of which was found to be a synapormophy for this clade (Fig. A2.1).

Hypericum ellipticifolium (Hypericeae), which was previously recognized in the monotypic genus *Lianthus* from China, remains unplaced with molecular data. In our combined analyses *H. ellipticifolium* is strongly placed (94 BP; Fig. 2.6) in the largely unresolved subclade Hypericeae. In Hypericaceae, staminodes are present in all members of Cratoxyleae and Vismieae. However, staminodes are largely absent in Hypericeae, except in sections *Adenotrias* and *Elodes* (represented in our study by *H. aegypticum* L. and *H. elodes* L., respectively; Robson, 1996) and in the former generic segregates *Lianthus*, *Santomasia, Thornea*, and *Triadenum* (Ruhfel et al. 2011). All Hypericeae taxa with staminodes occurred in the same *Hypericum* subclade in Ruhfel et al. (2011). In contrast to our results, *H. ellipticifolium* (i.e., *Lianthus*) was found to be sister to *Hypericum* in a morphological analysis of the genus with much better taxon sampling (Nürk and Blattner, 2010). Future work should concentrate on gathering additional material of *H. ellipticifolium.* There are very few herbarium specimens of this species,

some details of its floral morphology are unclear, and efforts to extract DNA from available material have been unsuccessful (Ruhfel et al., 2011).

The remaining unplaced genus in Clusiaceae s.s. is the poorly known *Thysanostemon* (Symphonieae) from Guyana. *Thysanostemon* is a member of the tribe Symphonieae, and has been suggested to be closely related to *Lorostemon* (Seetharam, 1985). Our results uncover a well-supported clade (73 BP) of *Lorostemon coelhoi*, *L. bombaciflorum*, and *Thysanostemon*. Furthermore, our results indicate that *Lorostemon* is not monophyletic: *Thysanostemon* is embedded within *Lorostemon* as sister to *L. bombaciflorum* (70 BP); both genera have pollen with supratectal elements, a feature not present in other Symphonieae (Seetharam, 1985). *Thysanostemon* is similar to other Symphonieae in having porose stigmas with no exposed stigmatic surface, which is an apparent syanpomorphy for the tribe. It is further supported as embedded within the Symphonieae by the presence of an androgynophore, a trait that all Symphonieae, except *Symphonia*, share. Members of this clade also possess anthers longer than 6 mm, a trait only otherwise observed in *Neotatea* and *Poeciloneuron pauciflorum* (Calophyllaceae). *Thysanostemon* also has papilate filaments, which is a trait found only in the Symphonieae taxa *Platonia*, *Moronobea*, *Montrouziera*, *Thysanostemon*, and *Lorostemon*. This character is not constant within these taxa however, *Lorostemon bombaciflorum* lacks papillate filaments and *Montrouziera* is polymorphic for this character. Elongated flower buds are found only in Symphonieae, where they occur in *Lorostemon, Thysanostemon,* and *Moronobea* (polymorphic). Relationships among these taxa are poorly supported so it remains to be seen if this character defines a clade. Any nomenclatural changes should be deferred until molecular data are available for the

poorly known taxon *Thysanostemon* (Stevens, 2007a). Previous attempts made to extract DNA from *Thysanostemon* using available herbarium vouchers have been unsuccessful (Ruhfel et al., 2011).

Vismia and *Psorospermum* are not monophyletic (Fig. 2.6), further stressing the need for phylogenetic and taxonomic work in Vismieae. Furthermore, our results suggest that the African and Malagasy members of Vismieae do not form a monophyletic group, and that neotropical *Vismia* (represented by *V. cayennensis* [Jacq.] Pers.) are embedded among these taxa. This result is similar to the topologies presented in Ruhfel et al. (2011) where neotropical representatives of *Vismia* were monophyletic and embedded within a clade of African and Malagasy taxa. Ruhfel et al. (2011) suggested that three genera of Vismieae could be recognized (i.e., *Harungana*, *Psorospermum*, and *Vismia*) but greatly revised compared to their present circumscriptions. *Vismia* should be restricted to neotropical *Vismia* species, *Harungana* should be expanded to include *Vismia rubescens*, and *Psorospermum* should be expanded to include all other African and Malagasy species of Vismieae. Our results further support these ideas, but the support for the clade representing the recircumscribed *Psorospermum* is weak (54 BP). A more detailed molecular and morphological study of Vismieae is necessary before any taxonomic changes are made.

Ancestral state reconstructions–Several characters have been historically important for determining relationships in the clusioid clade. Alternate leaf insertion was often thought to "link" Clusiaceae s.l. to the Theaceae s.l.
(e.g., Baretta-Kuipers, 1976; Cronquist, 1981; Takhtajan, 1997), but subsequent phylogenetic evidence placed Theaceae s.l. in the asterid order Ericales (see Stevens,

2001 onwards; APG III, 2009, and refs. therein). ASRs of this trait (Fig. A2.2) reveal that the clusioid clade is ancestrally opposite/whorled leaved and that alternate leaves evolved at least four times: in Bonnetiaceae, in two subclades of Calophyllaceae (*Mahurea* + *Neotatea* and *Caraipa* + *Haploclathra* + *Kielmeyera*), and in *Psorospermum febrifugum.* The ASR of the most recent common ancestor of the *Caraipa* + *Haploclathra* + *Kielmeyera* clade is ambiguous for this character (alternate= 0.51, opposite or whorled=0.49)—it is unclear whether there is one gain of alternate leaves at this node and a reversion to opposite leaves in *Haploclathra*, or two independent gains of alternate leaves, once in *Caraipa* and again in *Kielmeyera.* Podostemaceae were not scored for this character due to the uncertain homology of their vegetative structures. However, if Podostemaceae are indeed alternate as suggested by their gross morphology, this does not change the reconstruction of the ancestral condition of opposite/whorled leaves within the clade; alternate leaf insertion in Podostemaceae would represent another gain of alternate leaves. *Psorospermum febrifugum* is polymorphic for this character and this variation could not be included in the ML reconstructions due to limitations of the method. This species is however deeply embedded in a clade of opposite leaved taxa and thus represents an independent gain of alternate leaves.

Exudate (referred to as either latex or resin in the literature) is often considered a major identifying character of clusioid families, particularly Clusiaceae s.s., Calophyllaceae, and Hypericaceae. This is evident in the alternative name for Clusiaceae, Guttiferae, meaning gum-bearing. Our ASRs indicate that the presence of exudate is ancestral in the clusioid clade (Fig. A2.3), and that it has been lost independently in Bonnetiaceae, Podostemoideae, and Tristichoideae. Given the phylogenetic relationships

within the clusioid clade, anatomical studies of Bonnetiaceae are needed to clarify the apparent absence of secretory tissues in this family. We scored Bonnetiaceae as lacking exudate, but Takhtajan (1997) describes the pith of species in this family as having secretory canals like Clusiaceae (cf. Baretta-Kuipers, 1976). The presence of exudate in Podostemoideae is polymorphic and thus not applicable for our ASRs. A detailed study of the distribution of exudate in the plant body is also needed in Podostemoideae to determine the number of gains and losses within the subfamily. Exudate has only been reported in neotropical Podostemoideae to date (Cook and Rutishauser, 2007). We also suggest a detailed chemical analysis of exudate across the clusioid clade to determine the homology of these substances. In addition to the presence of exudate, the shape of exudate cavities in the mesophyll of the leaf (i.e., glands [spherical structures] vs. canals [elongated structures]) may be relevant for determining relationships in this clade. ASRs are equivocal (Fig. A2.4) for the reconstruction of this character at the crown node of the clusioid clade, but "glands" receives the majority of the proportional likelihood (glands = 0.60, canals = 0.20, and none = 0.20). Bonnetiaceae + Clusiaceae s.s. are also reconstructed as equivocal, but crown Clusiaceae s.s. are estimated to have canals ancestrally (> 0.99). Glands are estimated to be the ancestral state in the Calophyllaceae + Hypericaceae + Podostemaceae clade (glands = 0.99). Podostemaceae were not scored for this character. However, we explored the effect of all scorings for Podostemaceae. No matter which state is present, glands still receives > 80% of the proportional likelihood at the crown node containing these three families.

Merosity in the clusioid clade has also been used to distinguish major groups. We have only scored sepal number because petal number is often similar. ASRs indicate that

the clusioid clade is ancestrally five-merous (Fig. A2.5). Podostemaceae have not been scored for this character, and are thus not considered in the ASRs. No distinction can be made regarding sepals or petals in the family; perianth number in Tristichoideae is usually three, in Weddellinoideae five, and in Podostemoideae 2-20 (Cook and Rutishauser, 2007). When Tristichoideae and Weddellinoideae are scored as having three and five sepals, respectively, and Podostemoideae is left as unknown, the reconstructions of this character do not change elsewhere in the tree. Several independent shifts from five-merous to four-merous, or four-merous to two-merous flowers were detected in our data particularly within Calophyllaceae and Clusiaceae s.s. While not represented in our scoring, four-merous flowers also occur in *Hypericum*, which is reconstructed as being ancestrally five-merous.

The clusioid androecium shows variation in two potentially informative characters: stamen arrangement (fasciculate vs. not), and the presence of staminodes or fasciclodes in staminate or perfect flowers. The latter terms refer to sterile stamens or fascicles of stamens. There may be some association between these two characters: taxa with fasciculate androecia often have fasciclodes. Stamen arrangement is reconstructed as equivocal at the clusioid crown node (Fig. A2.6; not fasciculate = 0.53, fasciculate = 0.47), as well as at the other early diverging nodes within the clusioid clade. Only the following four nodes are confidently reconstructed as having fascicled stamens (>0.92): *Archytaea* + *Ploiarium,* Endodesmieae, Garcinieae + Symphonieae, and Hypericaceae. The arrangement of the androecium in *Bonnetia* needs further study. Steyermark (1984) reported *Bonnetia* as having fascicled stamens, but we did not observe them in bud or flower. Podostemoideae were scored as polymorphic for this character but the fused

stamens present in many members of the subfamily likely represent an at least one additional independent origin (Fig. A2.6). Fasciclodes or staminodes in staminate or perfect flowers appear to have arisen three times independently (Fig. A2.7): in Hypericaceae, a subclade of Symphonieae (all Symphonieae, minus *Symphonia*), and in a subclade of Bonnetiaceae. However, there are several points to keep in mind regarding the ASR of this character. Within Bonnetiaceae, *Archytaea* is scored as polymorphic so it is unclear whether staminodes arose in the common ancestor of *Archytaea* + *Ploiarium*, or independently within each genus. What we have scored as staminodes within Symphonieae are of uncertain origin but previous authors have interpreted them as staminodial (Robson, 1961). We have scored *Symphonia* as inapplicable for this character; a similar structure is present in *Symphonia*, but lies outside of the fused ring of fertile stamens. If this structure were staminal in origin, then the origin of this character state would be moved down one node to include all Symphonieae. Similar structures in Garcinieae were recently determined not to be of staminal origin (Sweeney, 2010), as such Garcinieae are not scored as having staminodes. Our ASRs suggest that these structures have arisen multiple times within the clusioid clade, but more work is needed to explore their developmental origins.

Carpel number is also of interest in the clusioid clade because it appears to define the two major subclades (Fig. A2.1). The crown node of the clusioid clade is reconstructed as either three-carpellate (0.35) or five-carpellate (0.55). The Clusiaceae s.s. + Bonnetiaceae clade is ancestrally five-carpellate, as are Clusiaceae s.s. Bonnetiaceae are also possibly ancestrally five-carpellate but *Bonnetia* is polymorphic for this character (three to five carpels) so the ancestral state at this node could not be

confidently determined. If the ancestral state in *Bonnetia* is either three or four-carpellate, the proportional likelihood still favors five carpels as the ancestral state for the family (> 0.71). The Calophyllaceae + Hypericaceae + Podostemaceae clade is reconstructed as being either three or five-carpellate with neither state preferred by the decision threshold (three = 0.71, five= 0.16). The crown node of Calophyllaceae is reconstructed as being one, two or three carpellate (one=0.17, two=0.09, and three=0.68). Many Calophyllaeae taxa are polymorphic for this character, which hinders our ASRs at this node. Three carpels are common in the New World clade and two carpels are common in the Old World clade (Notis, 2004; Stevens, 2007a); these states are reconstructed as the favored states at the crown nodes of these two clades.

Dioecy is prevalent in the clusioid clade, particularly in Clusiaceae s.s., but has also evolved in Calophyllaceae. Dioecy appears to have evolved at least four times within the clusioid clade (Fig. A2.8). It has arisen at least three times independently in Calophyllaceae (i.e., in *Clusiella*, *Calophyllum*, and *Mammea*). This is likely an underestimate: dioecious species of *Calophyllum* are not likely to be monophyletic (Stevens, 1974, 2007a). Reconstructions within Clusiaceae s.s. are less clear. Clusieae and Garcinieae are ancestrally dioecious (0.98 in each). However, the state at the crown node of Clusiaceae s.s. and the node subtending Garcinieae and Symphonieae are each equivocal (absent =0.49, present=0.51 in each case).

***Placement of* Paleoclusia**–Our analyses suggest that *Paleoclusia* is closely related to Clusiaceae s.s. Morphological data consistently place it within Clusiaceae s.s. near Garcinieae or Symphonieae, but support for this placement is poor (< 50 BP or PP). The combined analyses also place *Paleoclusia* with weak support (57 ML BP; Fig. 2.5)

as a member of the Clusiaceae s.s. and optimally as sister to *Allanblackia* (<50% BP). Similarly, the strict consensus of the most parsimonious trees placed *Paleoclusia* in a polytomy at the base of Clusiaceae s.s. but with weak support (54 MP BP; Fig. 2.5). In these respects our MP and ML results agree with Crepet and Nixon (1998) who placed *Paleoclusia* near Clusiaceae s.s. Bayesian analyses are consistent with this placement, but we have some reservations regarding the Bayesian results because studies suggest that missing data can be problematic for Bayesian analyses, at least in some cases (Lemmon et al., 2009; Wiens, 2009).

Characters that support the placement of *Paleoclusia* with Clusiaceae s.s. include extrorse anthers; a five-carpellate gynoecium; short, fused styles; and dioecy. Extrorse anthers (Fig. A2.9) occur only in Clusiaceae s.s., but have arisen multiple times within this clade (in *Allanblackia*, *Clusia* s.l., and Symphonieae). Garcinieae could not be reliably assessed for this character because scoring anther orientation is problematic in these taxa: anthers are tightly clumped and their orientation is unclear. A five-carpellate gynoecium is present in *Paleoclusia*, which is also reconstructed as the ancestral condition in the Bonnetiaceae + Clusiaceae s.s. clade (Fig. A2.1). Five carpels also occur in Hypericeae and Vismieae but these taxa are dissimilar to *Paleoclusia* in important ways. Hypericeae often have stigmas with rounded papillae (Fig. A2.18), and Vismieae have many characters not present in *Paleoclusia* including hairs on the adaxial surface of the petals, which is a synapomophy of the tribe. *Paleoclusia* also has very short, fused styles, which occurs in very few taxa outside of Clusiaceae s.s. (Figs. A2.11 and A2.10, respectively): *Bonnetia* (Bonnetiaceae), *Clusiella* (Calophyllaceae), and *Marathrum* and *Weddellina* (Podostemaceae). Finally, as mentioned above, dioecy (Fig. A2.8) occurs

only in Calophylleae (Calophyllaceae) and Clusieae and Garcinieae (Clusiaceae s.s.). If *Paleoclusia* were indeed dioecious, its fasciculate androecium, five carpels, and short styles would be very out of place in Calophylleae.

Two characters that we did not include in our analyses, resin production in the anthers and pollen shape, also support the close relationships of *Paleoclusia* to Clusiaceae s.s. The production of floral resin is a rare condition in angiosperms; outside of the clusioid clade this is known only from the distantly related *Dalechampia* (Euphorbiaceae; Armbruster, 1984; Gustafsson and Bittrich, 2002). Among the clusioids, resin production in the anthers is only known in *Clusiella* (Calophyllaceae), *Chrysochamys*, *Clusia* s.l., and *Tovomitopsis* (Clusiaceae s.s.; Hammel, 1999a; Gustafsson and Bittrich, 2002; Gustafsson et al., 2007). Within Clusieae it is likely that resin production has arisen at least five times independently: three times in *Clusia* and once each in *Chrysochlamys* and *Tovomitopsis* (Gustafsson and Bittrich, 2002; Gustafsson et al., 2007). Unfortunately, it may be difficult to confirm or refute the presence of resin in the anthers of *Paleoclusia* (Crepet and Nixon, 1996). The pollen of *Paleoclusia* also suggests a close relationship to extant Clusieae (Crepet and Nixon, 1998). Seetharam, who has conducted an extensive survey of pollen in the clusioid clade (excluding Podostemaceae; Seetharam, 1985; Seetharam and Maheshwari, 1986; Seetharam, 1989), considers the pollen of *Paleoclusia* to be most similar to the early diverging members of Clusieae (*Dystovomita*, *Tovomita*, and *Tovomitopsis*; Seetharam, pers. comm.).

Variation in other characters, however, does not support the placement of *Paleoclusia* with Clusiaceae s.s. *Paleoclusia* has dorsifixed anthers, which are absent in

Clusiaceae s.s.: this character otherwise only occurs in Bonnetiaceae, Calophyllaceae, and Hypericaceae (Fig. A2.12). *Paleoclusia* also possesses an indumentum of unicellular hairs on its pedicle and receptacle (Figs. 2 through 6 in Crepet and Nixon, 1998), which is uncommon in Clusiaceae s.s. Unicellular hairs in Clusiaceae s.s. occur only in two of our included taxa, *Lorostemon bombaciflorum* (Symphonieae) and *Garcinia dulcis* (Garcinieae; Fig. A2.13). Unicellular hairs arose independently in each of these groups and it is unlikely that *Paleoclusia* is embedded within Garcinieae or Symphonieae for reasons that are discussed below. An indumentum of unicellular hairs is common in Calophyllaceae, but *Paleoclusia* would be a bad fit here for the same reasons listed above.

Paleoclusia certainly seems to be a member of the clusioid clade. Its placement is perhaps along the stem leading to crown Clusiaceae s.s. or even to one of its major subclades (=tribes). Thus, we will now discuss the possible affinities of *Paleoclusia* to the three extant tribes of Clusiaceae s.s. Clusieae are defined by the synapomorphy of an arillate seed (Fig. A2.16). The original publication of this fossil indicates that the seed of *Paleoclusia* is arillate (Crepet and Nixon, 1998). Our interpretation of this structure is that it is most likely an aborted seed (Stevens 2001 [onwards], published online Aug. 2010). Without an aril, *Paleoclusia* would be a bad fit in Clusieae. In addition, its indumentum of unicellular hairs, fasciculate androecium, and filaments that are much thinner than their anthers (Fig. A2.17) make it a bad fit with this group. Clusieae, in contrast, are nearly always glabrous, their androecium is not fasciculate, and the filaments are approximately equal in thickness to the anthers.

Symphonieae are defined by the synapomophy of having stigmas enclosed in a cavity. In *Paleoclusia* the stigmas are exposed. Several other characters scored here define subclades of Symphonieae none of which are present in *Paleoclusia* (androgynophore, elongate flower buds, papillate filaments, fasciclodia, anthers greater than 6 mm long). The filaments of Symphonieae are also not thinner than the anthers as in *Paleoclusia.* Finally, Symphonieae possess perfect flowers. If *Paleoclusia* truly is dioecious as indicated by Crepet and Nixon (1998) this would also be out of place in the tribe.

Among the tribes of Clusiaceae s.s., Garcinieae is perhaps the best fit for *Paleoclusia.* The fossil shares many features with Garcinieae or one of its two major subclades: five sepals, fasciculate stamens, filaments thinner than the anthers, five carpels, and possibly dioecy. The pollen of *Paleoclusia* has three apertures in contrast to the ancestral condition of Garcinieae (>3 apertures; Fig. A2.14), however, reversals to three apertures occur in the tribe. The optimal ML topology placed *Paleoclusia* within Garcinieae, as sister to *Allanblackia.* Although *Allanblackia* has multiple ovules per carpel, as does *Paleoclusia,* the two otherwise have nothing substantive in common. Garcinieae usually possess one ovule per carpel, and this is the ancestral condition in the clade (Fig. A2.15). Despite the fact that *Paleoclusia* shares many features with Garcinieae, the fossil is quite distinct from the major subclades in this group. The Garcinieae subclade that includes *Garcinia dulcis* and *Allanblackia* is defined by having nectariferous floral structures (lineage A in Sweeney, 2008), which are not seen in *Paleoclusia.* Lineage B of Sweeney (2008) lacks these nectatriferous structures, but species in this clade usually have four (Fig. A2.5) sepals, not five as in *Paleoclusia.*

From our placement of extant morphology-only taxa in our combined analyses we have good reason to believe that our morphological characters are sufficient to place taxa with good support. The uncertainty in the placement of *Paleoclusia* could be due to the lack of better vegetative and anatomical data. Vegetative characters indeed do seem important in placing clusioid taxa scored only with morphology. When these characters are excluded from analysis (see Methods), the placements of some taxa changed dramatically and resolution was noticeably decreased. If more complete material of *Paleoclusia* is found, it will likely improve our ability to place this fossil. Characters that would be especially helpful in clarifying the placement of *Paleoclusia* would be the position of phellogen initiation in the stem and root, cortical sclereid presence and shape, shape of exudate containing structures in the mesophyll (i.e., glands or canals), stomata type, fruit type, testa complexity, and especially cotyledon to hypocotyl ratio. Determining the relationship of *Paleoclusia* to other clusioids is especially important in understanding the biogeographic history of the clade. At the time of deposition the fossil locality in New Jersey, USA was in Southern Laurasia in a subtropical to tropical environment (Crepet and Nixon, 1998). Most extant members of the clusioid clade are found in similar environments but in regions that are further south, mostly on former Gondwanan fragments.

***Placement of* Paleoclusia *for divergence time estimation*–**The use of fossils as age constraints in divergence time estimations studies is now commonplace. Fossil constraints are an important component to such studies, and when possible they should be based on a careful phylogenetic analysis of the fossil in question. We now have a much better understanding of phylogenetic relationships and morphological evolution within

the clusioid clade. *Paleoclusia* is consistently placed with Clusiacecae s.s. but not with strong support. Thus, we have two recommendations for the placement of *Paleoclusia* as a fossil age constraint. The first approach would be to consider *Paleoclusia* as a member of the Clusiaceae s.s. stem lineage (i.e., the constraint would be placed at the most recent common ancestor of Bonnetiaceae and Clusiaceae s.s.). In the second approach one would treat it as a member of the clusioid stem lineage (i.e., the constraint would be placed at the most recent common ancestor of Ochnaceae s.l. and the clusioid clade). The first approach would result in older age estimates for nodes within the clusioid clade; the second approach would result in younger ages. Preliminary divergence time estimates of the clusioid clade (B. Ruhfel, unpublished data) using a Bayesian approach (Drummond and Rambaut, 2007) with these alternate placements result in very different ages for the early history of the clusioid clade. For example, using the first approach, the crown node of the clusioid clade is estimated to be 102.9 Ma (min=92.3, max=113.7), but the second approach gives optimal estimates for this node that are ~20 Ma younger (min=78.0, mean=83.4, Ma max=88.7). Until *Paleoclusia* is placed more confidently, we suggest any future divergence time estimation studies explore these two alternate placements.

Conclusions and future directions–The results presented here have helped to resolve the clusioid phylogeny and provide a greatly improved understanding of morphological evolution in the group. We also provide additional support for the idea that with sufficient morphological data, taxa for which only morphological data are available can be placed with certainty using a combined analysis of molecules and morphology (Wiens, 2009; Wiens et al., 2010). The placement of *Paleoclusia* is uncertain, but the fossil does share many similarities with Clusiaceae s.s.

Further clarifying the number of origins of dioecy in the clusioid clade, particularly in *Calophyllum*, Clusieae, and Garcineae, will greatly aid our attempt to assess the correlates of shifts in diversification rates in the group. Although dioecious clades in general have been shown to be species poor in relation to sister clades with perfect flowers (Heilbuth, 2000), they tend to be more species rich when associated with other traits common in many clusioids such as fleshy fruits, tropical distributions, and woody growth form (Vamosi and Vamosi, 2004). Interestingly, some dioecious clades in Clusiaceae s.s. are quite species rich (e.g., Clusieae, ~387 spp. and Garcinieae, ~270 spp.) whereas those in Calophyllaceae are relatively species poor (e.g, Clusiella, 7 spp. and Mammea ~75 spp.; Stevens, 2007a). A comparative methods approach will assist in determining the evolutionary correlates of the seemingly dramatic rates of speciation observed in certain dioecious clades.

Finally, several important taxa in the clusioid clade remain to be sampled with molecular data and key areas in the topology remain unresolved or poorly supported. Future taxon sampling should focus on these unsampled taxa and on expanding sampling in several of the large clusioid genera. In addition to expanded taxon sampling, additional molecular characters should also be sought, particularly from the nuclear genome. Further work should also focus on improving the morphological data set for the clusioid clade. Ideally, taxa should be coded at the species level rather than as composite taxa, however, choosing appropriate representative species will require a much better understanding of relationships in many large clusioid subclades (e.g., Clusieae, *Hypericum*, and *Mammea*). A better understanding of phylogenetic relationships and morphological evolution in the clusioid sister group, Ochnaceae s.l., and more broadly in

Malpighiales, will help to polarize characters in the clusioid clade and aid in selecting appropriate outgroups for an expanded morphological analysis.

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CHAPTER 2 APPENDICES

Appendix 2.1. Morphological characters scored for clusioid taxa in this study.

1. Obvious root/stem/leaf construction: no (0); yes (1).

2. Phellogen initiation in root: superficial (0); deep-seated (1).

3. Phellogen initiation in stem: superficial (0); deep-seated (1).

4. Cortical sclerids in stem: absent (0); present, thickening of cell wall even (1); present, thickening of cell wall U-shaped (2).

5. Functional terminal buds: no (0); yes (1).

6. Terminal buds with scales: no (0); yes (1).

7. Axillary buds immersed: no (0); yes (1).

8. Branching from axils of leaves of current flush: no (0); yes (1).

9. Leaf insertion: alternate (0); opposite or whorled (1).

10. Colleters present: no (0); yes (1).

11. Stipuliform structures: none or colleter-like (0); small, paired, round or peltate (1).

12. Secondary veins arising from the length of the midrib: no (0); yes (1).

13. Intersecondary veins modified as canals: no (0); yes (1).

14. Tertiary veins parallel at right angles to secondaries: no (0); yes (1).

15. Exudate in plant body: absent (0); present (1).

16. Shape of exudate containing structures in mesophyll: none (0); glands (1); canals (2).

17. Fibers in mesophyll of lamina: no (0); yes (1).

18. Lamina with lignified margin: no (0); yes (1).

19. Midrib structure: one layer of tissue only (0); at least two layers, adaxial layer inverted (1); at least two layers, adaxial layer not inverted (2); at least two layers,

adaxial layer with no clear arrangement (3).

20. Lateral bundles in leaf transcurrent: no (0); yes (1).
21. Abaxial palisade tissue present: no (0); yes (1).
22. Stomatal type: paracytic (0); anomocytic (1).
23. Indumentum of unbranched unicellular hairs: no (0); yes (1).
24. Indumentum of multicellular hairs: no (0); stellate (1); other than stellate (2).
25. Marginal setae present: no (0); yes (1).
26. Marginal disciform glands present: no (0); yes (1).
27. Xylem parenchyma present: no (0); yes (1).
28. Prenylated anthranoids: absent (0); present(1).
29. Inflorescence or flower position: axillary (0); terminal (1).
30. Inflorescence type: at least some internodes developed (0); fasciculate (1); flower single (2).
31. Pattern of inflorescence internode elongation: at least basal internode developed (0); basal internode not developed at least some subsequent internodes developed (1).
32. Terminal flowers present: no (0); yes (1).
33. Bracteoles: absent (0); present normal (1); present displaced one internode (2).
34. Flower buds: round (0); strongly elongated (1).
35. Sepal number: five or multiples of five (0); four (1); two (2); three (3).
36. Hairs on adaxial surface of petals: no (0); yes (1).
37. Androgynophore present: no (0); yes (1).
38. Androecium arrangement: not fasciculate (0); fasciculate (1).
39. Androecium adnate to petals: no (0); yes (1).

40. Fasciclodia present in staminate or perfect flowers: no (0); yes (1).

41. Filament attachment: dorsifixed (0); basifixed (1).

42. Filament much thinner than anthers: no (0); yes (1).

43. Filaments papillate: no (0); yes (1).

44. Anther orientation: introrse (0); extrorse (1).

45. Anthers locellate: no (0); yes (1).

46. Anther length: less than 6mm (0); greater than 6mm (1).

47. Anthers with crateriform glands: no (0); yes (1).

48. Anthers with porose dehiscence: no (0); yes (1).

49. Pollen aperture number: three (0); at least four (1).

50. Pollen with supratectal elements: no (0); yes (1).

51. Carpel number: more than five (0); one (1); two (2); three (3); four (4); five (5).

52. Ovary septate: no (0); yes (1).

53. Ovules per carpel: two or more (0); one (1).

54. Style length: absent or shorter than ovary (0); equal to or longer than ovary (1).

55. Stylar fusion: free (0); fused (1).

56. Stigma exposure: exposed (0); enclosed in cavity (1).

57. Stigma type: punctate (0); transversly expanded (1); linear (2).

58. Stigma surface: smooth (0); rounded papillate (1); pointed papillate (2).

59. Fruit type: indehiscent (0); septicidal or septifragal dehiscence (1); loculicidal dehiscence (2).

60. Seeds with aril: no (0); yes (1).

61. Seeds winged: no (0); yes (1).

62. Seeds with surface glands: no (0); yes (1).

63. Testa complex: no (0); yes (1).

64. Lignified exotegmen: absent (0); present(1).

65. Ratio of cotyledon to hypocotyl + radicle: less than 0.2 (0); greater than 0.2 to less than 2 (1); greater than 2 (2).

66. Cotyledons cordate at the base: no (0); yes (1).

67. Germination type: epigeal (0); hypogeal (1).

68. Seedling with accessory roots: no (0); yes (1).

69. Dioecy: absent (0); present (1).

Appendix 2.2. Morphological data matrix for the clusioid clade and the fossil taxon *Paleoclusia*. Polymorphisms: A=0&1; B= 0&2; C =0&5; D=1&2; E=2&3; F=3&4; G=4&5; H=0&1&2; I=0&2&4; J=0&4&5; K=3&4&5; L=0&3&4&5; M=2&3&4&5; N=0&2&3&4&5.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
PALEOCLUSIA	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	1	0	?	?	?	?	?	?	?	?	?	0	0
Archytaea	1	?	0	1	1	0	0	0	0	1	0	0	-	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0
Ploiarium	1	0	0	1	1	0	0	0	0	1	0	1	-	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0
Bonnetia	1	0	0	1	1	0	0	0	0	1	0	A	-	0	0	0	0	0	3	0	0	0	0	0	1	0	1	0	?	A	?	?	?	0	0
Calophyllum	1	1	0	2	1	A	0	0	1	0	0	1	1	-	1	B	0	1	0	1	0	0	0	2	0	0	1	0	A	0	0	1	0	0	1
Caraipa	1	1	0	2	A	A	0	0	0	1	0	1	0	A	1	1	0	1	1	1	0	0	0	B	0	0	1	0	A	0	0	1	1	0	0
Clusiella	1	1	0	0	1	1	0	0	1	1	1	1	0	0	1	D	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0
Haploclathra	1	?	0	2	1	0	0	0	1	1	0	1	0	A	1	1	0	1	1	1	0	0	1	2	0	0	1	0	1	0	0	1	1	0	0
Kayea	1	1	0	1	1	1	0	0	1	1	0	1	0	A	1	1	0	1	1	1	0	0	0	0	0	0	1	0	A	0	0	1	A	0	1
Kielmeyera	1	1	0	1	1	1	0	0	0	1	0	1	0	0	1	1	0	A	1	1	0	0	A	B	0	0	1	0	1	0	0	1	1	0	0
Mahurea	1	?	0	0	1	1	0	0	0	1	0	1	0	1	1	1	0	1	1	1	0	0	1	0	0	0	A	0	1	0	0	1	1	0	0
Marila grandiflora group	1	?	0	D	1	0	0	0	1	0	0	1	0	1	1	1	0	1	1	1	0	0	0	D	0	0	1	?	1	0	0	0	1	0	0
Marila tomentosa group	1	1	0	2	1	0	0	0	1	0	0	1	0	1	1	1	0	1	D	1	0	0	0	H	0	0	1	?	0	0	0	0	1	0	0
Mesua ferrea	1	1	0	2	0	-	1	0	1	?	0	1	0	0	1	1	0	1	2	1	0	0	A	0	0	0	1	0	0	2	-	1	-	0	1
Mesua thwaitesii group	1	?	0	2	0	-	1	0	1	?	0	1	0	0	1	1	0	1	2	1	0	0	1	0	0	0	?	0	0	0	0	0	1	0	0
Mammea americana group	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1	D	0	1	2	1	0	0	0	0	0	0	1	0	0	1	-	1	1	0	2
Mammea bongo	1	1	0	D	1	1	0	1	1	1	0	1	0	0	1	D	1	1	2	0	0	0	0	0	0	0	1	?	0	1	-	1	1	0	2
Mammea siamensis group	1	1	0	D	1	1	0	1	1	1	0	1	0	0	1	D	1	1	2	1	0	0	0	0	0	0	1	0	0	1	-	1	1	0	2
Mammea touriga	1	?	0	1	1	1	0	1	1	1	0	1	0	0	1	1	0	1	2	1	0	0	0	0	0	0	?	?	0	1	-	1	1	0	2
Neotatea columbiana	1	?	0	0	1	0	0	0	0	0	0	1	1	-	1	0	0	A	1	1	0	0	1	0	0	0	1	?	1	1	-	1	-	0	0
Poeciloneuron indicum	1	1	0	2	1	1	0	1	1	1	0	1	0	0	1	1	0	1	1	1	1	0	1	0	0	0	1	?	1	0	0	1	1	0	0
Poeciloneuron pauciflorum	1	1	?	?	1	1	0	0	1	1	1	1	0	0	1	1	0	1	1	1	0	0	1	0	0	0	?	?	0	1	-	1	?	0	1
Endodesmia calophylloides	1	?	0	0	0	-	0	0	1	1	1	1	0	0	1	1	0	0	3	0	0	0	1	0	0	0	1	?	1	0	0	1	1	0	0
Lebrunia bushaie	1	?	0	1	0	-	0	0	1	1	1	1	0	0	1	1	0	?	3	0	0	0	1	0	0	0	?	?	0	0	0	1	0	0	1
Clusia alata	1	0	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0
Clusia gundlachii	1	?	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	1
Clusia major	1	?	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0
Clusia panapanari	1	?	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0
Decaphalangium peruvianum	1	?	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0

Appendix 2.2 (Continued).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Clusia columbiana (Havetia)	1	?	?	0	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	?	1	0	0	1	1	0	1
Clusia p p (Quapoya spp)	1	0	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0
Clusia p p (Oedomatopus spp)	1	?	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	?	1	0	0	1	1	0	1
Clusia caudatum	1	?	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	1
Clusia p p (Havetiopsis)	1	?	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	?	1	0	0	1	1	0	1
Clusia p p (Renggeria)	1	?	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	1	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0
Chrysochlamys	1	0	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	?	1	0	0	1	1	0	0
Dystovomita	1	0	?	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	0	0	0	1	1	0	1
Tovomita	1	0	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1
Tovomita weddelliana	1	?	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	1
Tovomitopsis	1	?	?	?	1	0	0	0	1	1	0	1	0	0	1	2	?	?	?	?	?	?	0	0	0	0	?	?	1	0	0	1	1	0	2
Allanblackia	1	?	0	0	1	A	0	0	1	0	0	1	0	0	1	D	0	0	1	0	0	0	0	0	0	0	1	0	A	?	?	1	1	0	0
Septogarcinia sumbawaensis	1	?	0	0	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	0	0	1	-	?	1	0	1
Garcinia morella	1	?	?	1	1	A	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	0	1	-	?	1	0	1
Garcinia dulcis	1	0	0	0	1	A	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	A	0	0	0	1	0	0	1	-	1	1	0	0
Garcinia p p (Pentaphalangium spp)	1	?	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1
Garcinia p p (Rheedia spp)	1	?	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	A	0	0	0	0	0	1	0	0	1	-	?	1	0	1
Garcinia cymosa	1	0	0	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	3
Lorostemon bombaciflorum	1	?	0	0	1	1	0	0	1	0	A	1	0	0	1	2	0	0	1	0	0	0	1	0	0	0	1	0	1	2	-	1	-	1	0
Lorostemon coelhoi	1	?	0	0	1	1	0	?	1	0	1	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	0	1	2	-	1	-	1	0
Montrouzeria	1	?	A	1	1	1	0	0	1	A	1	1	0	0	1	D	0	0	1	0	0	0	0	0	0	0	1	?	A	2	-	1	-	0	0
Moronobea	1	?	0	1	1	1	0	1	1	0	1	1	0	0	1	2	0	0	1	0	1	0	0	0	0	0	1	?	1	2	-	1	-	A	0
Pentadesma	1	?	0	0	1	1	0	1	1	0	1	1	0	0	1	D	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1	0	0
Platonia	1	?	0	1	1	1	0	1	1	0	1	1	0	0	1	2	0	0	1	?	1	0	0	0	0	0	1	0	1	2	-	1	-	0	0
Symphonia	1	0	0	1	1	1	0	1	1	0	1	1	0	0	1	2	0	0	1	0	A	0	0	0	0	0	1	0	1	1	-	1	1	0	0
Thysanostemon pakaraimae	1	?	0	0	1	1	0	0	1	0	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	?	?	1	2	-	1	-	1	0
Cratoxylum sect Isopterygium	1	?	1	0	1	1	0	0	1	0	0	1	0	0	1	1	0	1	0	1	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0
Cratoxylum sects Cratoxylum and Tridesmos	1	?	1	0	1	1	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	B	0	0	1	0	A	0	0	A	1	0	0
Eliaea articulata	1	?	1	0	1	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0

Appendix 2.2 (Continued).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Harungana madagascariensis	1	?	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	1	1	1	0	0	1	2	0	0
Psorospermum cerasifolium	1	?	1	0	0	-	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	?	1	0	?	1	2	0	0
Psorospermum lamianum	1	?	1	0	1	1	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	?	1	0	0	1	2	0	0
Psorospermum febrifugum	1	?	1	0	0	-	0	0	A	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	1	1	0	?	1	2	0	0
Psorospermum staudtii group	1	?	1	0	0	-	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	?	1	0	0	1	2	0	0
Vismia affinis	1	?	1	0	0	-	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	?	1	0	0	1	2	0	0
Vismia cayennensis	1	?	1	0	1	A	0	0	1	0	0	1	0	0	1	1	0	0	A	1	0	0	0	1	0	0	1	1	1	0	0	1	D	0	0
Vismia laurentii	1	?	1	0	0	-	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	1	1	0	1	1	2	0	0
Vismia orientalis	1	?	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	?	1	0	1	1	2	0	0
Vismia rubescens	1	?	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	1	?	1	0	0	1	2	0	0
Hypericum Adenotrias	1	?	?	?	?	0	0	?	1	0	0	-	?	-	1	1	0	0	0	-	0	1	0	0	0	0	?	0	1	0	0	1	1	0	0
Hypericum sect Elodes	1	?	?	?	?	0	0	?	1	0	0	1	?	0	1	1	0	0	0	?	?	1	0	2	0	0	?	0	1	0	0	1	1	0	0
Hypericum sect Myriandra	1	?	?	?	?	0	0	?	1	0	0	A	?	0	1	1	0	0	0	?	?	1	0	0	0	0	?	0	1	0	0	1	1	0	A
Hypericum sects Brathys and Trignobrathys	1	?	?	?	?	0	0	?	1	0	0	A	?	0	1	1	0	0	0	?	?	1	A	0	0	0	?	0	1	0	0	1	1	0	A
Hypericum Ascyreia s l	1	?	?	?	?	0	0	?	1	0	0	A	?	0	1	D	0	0	0	?	?	1	0	0	0	0	?	0	1	0	0	1	1	0	0
Hypericum Euhypericum	1	?	?	?	?	0	0	?	1	0	0	A	?	0	1	D	0	0	0	?	?	1	A	0	0	0	?	0	1	0	0	1	1	0	A
Hypericum ellipticifolium (Lianthus)	1	?	?	?	-	-	0	0	1	0	0	1	0	0	1	D	?	?	?	?	?	?	0	0	0	0	?	?	1	0	0	1	1	0	0
Hypericum (Santomasia) steyermarkii	1	?	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	0	0	0	0	?	?	1	0	0	1	1	0	0
Hypericum p p (Thornea spp)	1	?	1	0	1	0	0	0	1	0	0	1	0	0	1	D	0	0	0	?	0	1	0	0	0	0	0	?	1	0	0	1	1	0	0
Hypericum p p (Triadenum spp)	1	1	?	0	-	-	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	?	1	0	0	1	1	0	0
Podostemoideae	0	?	?	?	?	?	?	?	?	?	?	?	?	?	A	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	1	?	0	?
Weddellinoideae	0	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	0	?	2	?	1	?	0	?
Tristichoideae	0	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	1	?	0	?

Appendix 2.2 (Continued).

	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
PALEOCLUSIA	0	0	1	0	-	0	1	0	1	0	0	0	0	0	0	5	1	0	0	1	0	1	0	?	0	0	0	?	?	?	?	?	?	1
Archytaea	0	0	1	0	A	1	1	0	0	0	0	0	0	0	0	5	1	0	1	1	0	0	1	1	0	0	0	0	1	1	0	?	?	0
Ploiarium	0	0	1	0	1	A	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0
Bonnetia	0	0	0	0	0	A	1	0	0	0	0	0	0	0	0	K	1	0	A	A	0	0	1	1	0	0	0	0	1	1	0	?	?	0
Calophyllum	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	M	0	1	1	1	0	1	0	0	0	0	0	1	0	2	0	1	0	1
Caraipa	0	0	0	0	0	?	1	0	0	0	0	A	0	0	0	3	1	A	1	1	0	1	0	1	0	A	0	1	0	2	1	?	0	0
Clusiella	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	J	1	0	0	1	0	1	0	0	0	0	1	0	1	1	0	?	?	1
Haploclathra	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	3	1	A	1	1	0	1	0	1	0	1	0	1	0	2	1	?	?	0
Kayea	0	0	0	0	0	A	1	0	0	0	0	0	0	0	0	F	0	0	1	1	0	0	0	1	0	0	0	1	0	2	?	1	0	0
Kielmeyera	0	0	0	0	0	A	1	0	0	A	0	A	0	0	0	E	1	0	1	1	0	1	0	1	0	1	0	0	0	2	1	0	0	0
Mahurea	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	F	1	0	1	1	0	1	0	1	0	1	0	0	1	1	0	?	?	0
Marila grandiflora group	0	0	A	0	0	1	1	0	0	0	0	?	1	0	0	K	1	0	?	1	0	1	0	1	0	0	0	0	1	1	0	?	?	0
Marila tomentosa group	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	L	1	0	?	1	0	1	0	1	0	0	0	0	1	1	0	?	?	0
Mesua ferrea	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	1	0	1	1	0	1	0	1	0	0	0	1	0	2	0	1	0	0
Mesua thwaitesii group	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	1	0	1	1	0	1	0	1	0	0	0	1	0	2	0	?	?	0
Mammea americana group	0	0	0	0	0	1	1	0	0	0	0	0	0	A	0	2	1	0	1	1	0	1	0	0	0	0	0	1	0	2	0	1	0	1
Mammea bongo	0	0	0	0	0	1	1	0	0	0	0	A	0	0	0	1	1	0	1	1	0	1	0	A	0	0	0	1	0	2	0	1	0	1
Mammea siamensis group	0	0	0	0	0	1	1	0	0	0	0	A	0	0	0	2	1	0	1	1	0	1	0	0	0	0	0	1	0	2	0	1	0	1
Mammea touriga	0	0	0	0	0	1	1	0	0	0	0	0	0	?	?	2	1	0	1	1	0	1	0	0	0	0	0	1	0	2	0	1	0	1
Neotatea columbiana	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	3	1	0	1	1	0	1	0	1	0	1	0	0	1	1	0	?	?	0
Poeciloneuron indicum	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	2	1	0	1	0	0	0	0	1	0	0	0	1	0	2	?	A	0	0
Poeciloneuron pauciflorum	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	2	1	0	1	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0
Endodesmia calophylloides	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	-	1	1	-	0	0	0	0	0	0	0	1	0	2	0	?	?	0
Lebrunia bushaie	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	-	1	1	-	0	0	0	0	0	0	0	1	0	2	0	?	?	0
Clusia alata	0	0	0	0	0	1	0	0	1	0	0	0	0	?	?	0	1	0	?	0	0	1	0	1	1	0	0	0	1	0	0	?	?	1
Clusia gundlachii	0	0	0	0	0	1	0	0	0	0	0	0	0	?	?	5	1	0	0	?	0	1	0	1	1	0	0	0	1	0	0	?	?	1
Clusia major	0	0	0	0	0	1	0	0	0	0	0	0	0	?	?	5	1	0	0	?	0	1	0	1	1	0	0	0	1	0	0	0	0	1
Clusia panapanari	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	5	1	0	0	0	0	1	2	1	1	0	0	0	1	0	0	?	?	1
Decaphalangium peruvianum	0	0	0	0	0	1	0	0	0	1	0	0	-	?	?	5	1	0	0	0	0	1	2	1	1	0	0	0	1	0	0	?	?	1

Appendix 2.2 (Continued).

	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
Clusia columbiana (Havetia)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	5	1	0	0	?	0	1	0	1	1	0	0	0	?	?	?	?	?	1
Clusia p p (Quapoya spp)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	5	1	0	0	?	0	1	0	1	1	0	0	0	1	0	0	?	?	1
Clusia p p (Oedomatopus spp)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	G	1	0	?	0	0	1	0	1	1	0	0	0	?	0	0	?	?	1
Clusia caudatum (Pilosperma)	0	0	0	0	0	1	0	0	?	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	0	1	0	0	?	?	1
Clusia p p (Havetiopsis)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	1
Clusia p p (Renggeria)	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	5	1	0	0	0	0	1	2	1	1	0	0	0	1	0	0	?	?	1
Chrysochlamys	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	5	1	1	0	?	0	1	0	1	1	0	0	1	0	0	0	0	0	1
Dystovomita	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	5	1	A	0	0	0	1	0	1	1	0	0	A	1	0	0	?	?	1
Tovomita	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	G	1	1	A	0	0	1	0	1	1	0	0	1	0	0	0	1	1	1
Tovomita weddelliana	0	0	0	0	0	1	0	0	0	0	0	0	0	?	?	C	1	1	0	0	0	1	0	1	1	0	0	1	0	0	0	?	?	1
Tovomitopsis	0	0	0	0	0	1	0	0	0	0	0	0	0	?	?	G	1	1	0	0	0	1	0	1	1	0	?	1	?	?	?	?	?	1
Allanblackia	0	0	1	0	0	1	1	0	1	0	0	0	0	1	0	5	1	0	0	1	0	1	0	0	0	0	0	1	1	0	?	1	0	1
Septogarcinia sumbawaensis	0	0	0	0	0	1	1	0	?	0	0	0	0	1	0	0	1	1	0	?	0	1	0	1	0	0	?	1	0	0	?	?	?	1
Garcinia morella	0	0	0	0	0	1	1	0	?	0	0	0	0	0	0	J	1	1	0	?	0	1	0	0	0	0	0	1	0	0	?	1	1	1
Garcinia dulcis	0	0	1	0	0	1	1	0	0	0	0	0	0	1	A	G	1	1	0	1	0	1	0	0	0	0	?	1	0	0	0	1	1	1
Garcinia p p (Pentaphalangium spp)	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	N	1	1	0	?	0	1	0	0	0	0	0	1	1	0	0	0	0	1
Garcinia p p (Rheedia spp)	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	4	1	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1
Garcinia cymosum	0	0	1	1	0	1	?	0	?	0	0	0	0	1	1	3	1	1	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	1
Lorostemon bombacıflorum	0	1	1	0	1	1	0	0	1	0	1	0	0	1	1	5	1	0	0	1	1	-	-	0	0	0	0	1	?	0	?	?	?	0
Lorostemon coelhoi	0	1	1	0	1	1	0	1	1	0	1	0	0	1	?	5	1	0	0	1	?	?	?	0	0	0	0	1	1	0	?	?	?	0
Montrouzeria	0	1	1	0	A	1	0	A	1	0	1	0	0	A	0	5	1	0	0	1	1	-	-	0	0	0	0	1	1	0	?	?	?	0
Moronobea	0	1	1	0	1	1	0	1	1	1	1	0	0	1	0	5	1	0	1	1	1	-	-	0	0	0	0	1	1	0	?	1	0	0
Pentadesma	0	1	1	0	1	1	0	0	1	1	1	0	0	A	0	5	1	0	1	1	1	-	-	0	0	0	0	1	0	0	?	1	1	0
Platonia insignis	0	1	1	0	1	1	0	1	1	1	1	0	0	1	0	5	1	0	1	1	1	-	-	0	0	0	0	1	0	0	?	1	0	0
Symphonia	0	0	1	0	?	1	0	0	1	0	0	0	0	A	0	5	1	0	?	1	1	-	-	0	0	0	0	1	0	0	?	1	1	0
Thysanostemon pakaraimae	0	1	1	0	1	1	0	1	1	0	1	0	0	A	1	5	1	0	0	1	1	-	-	0	0	0	0	1	0	0	?	?	?	0

Appendix 2.2 (Continued).

	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
Cratoxylum sect Isopterygium	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	1	0	1	0	0	0	1	2	0	1	0	0	1	1	0	?	?	0
Cratoxylum sects Cratoxylum and Tridesmos	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	1	0	1	0	0	A	1	2	0	1	0	0	1	1	0	?	?	0
Eliaea articulata	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	1	0	1	0	0	1	1	D	0	1	0	0	1	1	0	?	?	0
Harungana madagascariensis	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	A	1	0	0	1	0	0	0	0	0	0	1	?	0	0	0	0
Psorospermum cerasifolium	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	?	?	0
Psorospermum lamianum	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	1	1	0	0	1	0	0	0	0	1	0	1	2	0	?	?	0
Psorospermum febrifugum	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	A	1	0	0	1	0	0	0	0	1	0	A	2	0	?	?	0
Psorospermum staudtii group	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	1	1	0	0	1	0	0	0	0	1	0	1	2	0	?	?	0
Vismia affinis	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	1	0	0	0	0	1	0	1	2	0	?	?	0
Vismia cayennensis	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	1	0	0	0	0	A	0	1	1	0	0	0	0
Vismia laurentii	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	?	?	0
Vismia orientalis	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	?	?	0
Vismia rubescens	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	5	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	?	?	0
Hypericum sect Adenotrias	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	?	0	A	0	0	0	1	1	0	0	0	0	1	1	?	?	?	0
Hypericum sect Elodes	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	?	0	0	0	0	1	1	1	0	0	0	0	1	1	?	?	?	0
Hypericum sect Myriandra	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	M	?	0	A	0	0	0	1	1	0	0	0	0	1	1	?	?	?	0
Hypericum sects Brathys and Trignobrathys	0	0	A	0	0	0	1	0	0	0	0	0	0	0	0	M	?	0	A	0	0	A	1	1	0	0	0	0	1	1	?	?	?	0
Hypericum Ascyreia s l	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	5	?	0	A	A	0	A	1	A	0	0	0	0	1	1	?	?	?	0
Hypericum Euhypericum	0	0	1	0	0	0	1	0	0	0	0	0	0	A	0	K	?	0	A	0	0	A	1	A	0	0	0	0	1	1	?	?	?	0
Hypericum ellipticifolium (Lianthus)	0	0	1	0	1	0	1	0	?	0	0	0	0	?	?	3	1	0	1	0	0	0	?	1	0	0	0	?	?	?	?	?	?	0
Hypericum (Santomasia) steyermarkii	0	0	1	0	1	0	1	0	0	0	0	0	0	0	?	5	1	0	1	0	0	0	0	1	0	0	0	0	1	1	0	?	?	0

Appendix 2.2 (Continued).

	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
Hypericum p p (Thornea spp)	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	1	0	1	0	0	0	1	1	0	0	0	0	1	1	0	?	?	0
Hypericum p p (Triadenum spp)	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	3	1	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0
Podostemoideae	?	0	A	?	0	1	1	0	0	0	0	0	0	A	1	2	1	0	0	A	0	2	1	1	0	0	0	?	1	?	0	?	?	0
Weddellinoideae	?	0	0	?	0	1	1	0	0	0	0	0	0	0	1	2	1	0	0	1	0	1	1	1	0	0	0	?	?	?	0	?	?	0
Tristichoideae	?	0	0	?	0	1	1	0	0	0	0	0	0	1	1	3	1	0	0	0	0	2	A	1	0	0	0	?	1	?	0	?	?	0

Appendix 2.2 (Continued).

Appendix 2.3. Voucher information (in addition to vouchers listed in Appendix 2.4) for clusioid taxa used to score morphology in this study.

BONNETIACEAE.

Archytaea – A. angustifolia: Venezuela, Maguire et al. 37566 (GH: anat.); *A. multiflora*: British Guiana, Maguire & Fanshaw 23108 (A: anat., fr.), Tillet & Tillet 45521 (GH: anat.).

Bonnetia – B. crassa: Venezuela, Maguire & Maguire 35069 (GH: anat.); *B. cubensis*: Cuba, Shafer 8232 (A; anat.); *B. neblinae*: Venezuela, Maguire et al. 37111 (GH: anat.).

Ploiarium – P. alternifolium: Malaya, Stevens et al. 1074 (A; anat.); *P. sessile* (Scheff.) Hallier f.: Irian Jaya, van Royen 5557 (anat.)

CALOPHYLLACEAE.

Calophyllum – C. chapeleiri: Madagascar, Dorr et al. 4628A (seedling), Dorr et al. 4625 (anat.), Dorr 4626 (anat.).

Caraipa –C. insignis: Colombia, Schultes & Liogier 9520 (GH: anat.)

Clusiella - C. axillaris: Brazil, Kruckoff 8933 (A: anat.); Venezuela, Maguire et al. 37439 (GH: anat); Colombia, Schultes & Cabrera 16089 (GH: anat,).; *C. elegans*: Colombia, Soejarto et al. 2869 (GH: anat.), Killip & Cuatrecasas 39862 (GH: anat.).

Endodesmia calophylloides – Ekat, Talbot & Talbot 3058 (K: fr.)

Haploclathra – H. leiantha Ducke 656 (GH: anat.), Nelson 1244 (fr.: K); *H. paniculata* Campbell et al. 21833 (GH: anat.), Brazil, Ducke 926 (GH: fr)

Kayea – K. borneensis: Sarawak, S 18613 (A: anat., fr.); *K. ferruginea*: SFN 23900 (fr.); *K. myrtifolia*: Sarawak, Stevens et al. 186 (A: anat, fr.); *K. scalarinervosa*: Sabah, SAN 17441 (A: anat., fr.); wrayi: Malaya, Chew-Wee-Lek 915 (A: fr.).

Kielmeyera – K. coriacea: Brazil, Irwin et al. 17823 (US: fr.); Bolivia, Solomon 7730 (fr.); *K. decipiens* Saddi: Brazil, Kuhlmann 505 (US: fr); *K. grandiflora* (Wawra) Saddi: Cuatrecasa 26604 (US: fr); *K. netiifolia* Camb.: Brazil, Irwin et al. 11596 (US: fr); *K. rizziana*: Brazil, Sucre et al. 5336 (US: fr); *K. sp.*: Eiten & Eiten 9465 (US: fr.)

Lebrunia bushaie: Congo, Dubois 816 (A; anat.).

Mahurea – *M. exstipulata*: Prance et al. 4546 (GH: anat., fr.); *M. palustris*: Brazil, Amazonia, Prance et al. 20017 (GH: anat.).

Mammea americana group- *M. americana*: Matuda 3580 (A); *M. immansueta*: Mori & Kallunki 4699 (holotype, MO); *M. africana*: Small 558 (K, MO).

Mammea bongo – Dorr et al. 4454, Madagascar, (A: anat.); sp. nov. Dorr et al. 4430 (A; anat.).

Mammea siamensis group – *M. odorata* Kornassi 224 (A: fr.), BW 4641 (fr.).

Mammea touriga – O'Farrell 45 (BRI, L).

Marila grandiflora group – *M. grandiflora* Broadway s.n. iii.1928 (: anat.); *M. magnifica* Steyermark & Espinosa 123775 (VEN).

Marila tomentosa group – *M. biflora*, Ekman 4662 (A: anat.); *M. laxiflora*: Schunke 5003 (anat.); *M. macrophylla* Bentham: Panama, Johnston 685 (GH: anat.); *M. pluricostata*: Allen 6538 (anat.).

Mesua ferrea - *M. ferrea* Kostermans 25012 (A), 25669 (A), Comanor 1173 (GH anat)

Mesua thwaitesii group - *M.* sp. Fernandes 369 (A: anat.); *M. pulchella* CP 3404 (GH), anon s.n. (GH. fr).

Neotatea colombiana: Schultes & Cabrera 14734 (GH: anat.).

Poeciloneuron indicum - B.S.I. Southern Circle 62852 (A, MH).

Poeciloneuron pauciflorum - Broome 210 (K), Beddome 437 (BM).

CLUSIACEAE S.S.

Allanblackia – *A. floribunda*: Belgian Congo, LeBrun 1058 (A; anat.), Louis 9956 (A: anat.); *A. kisonghi* Vermoesen: Belgian Congo, Corbisier-Baland 1412: (A: anat.); *A. marieni* Staner: Belgian Congo, LeBrun 1377 (A; anat.); *A. staneriana*: Angola, Gossweiler 8221 (BM: fr.) – see also Delay & Mangenot (1960: fr.)

Chrysochlamys – *C. caribaea*: St Lucia, Beard 496 (A; fr.); *C. macrophylla* Pax: Peru, Kayap 987 (GH; fr.); *C. weberbaueri* Engler: Peru, Klug 3061 (GH: anat.), Berlin 914 (GH: fr., anat.), Woytkowski 6166 (GH: fr.); *C.* sp.: Schunke V 2497 (fr).

Clusia alata: Costa Rica, Lent 2092 (GH: anat.), Lent 2033 (GH: fr.)

Clusia caudatum (syn. *Pilosperma caudatum*): Colombia, Cuatrecasas 16987 (anat.), Killip & Cuatrecasas 38720 (US: anat.)

Clusia gundlachii Stahl: Puerto Rico, Wagner 93 (A: anat, fr.), Wagner 1276 (A: anat.)

Clusia major: Guadeloupe, R. A & E. S. Howard 19438 (A: anat., fr.); Monsterrat, R.A. & E. S. Howard 15096 (A: seedling); Dominica, Whitefoord 4386 (A: anat.).

Clusia panapanari: British Guiana, de la Cruz 3219 (GH: anat.), Hitchcock 17634 (GH: anat., fr.)

Clusia p.p. (*Havetiopsis*) –*Havetiopsis flavida* (Benth,) Planchon & Riana: Perus, Croat 20564 (GH: anat., fr.).

Clusia p.p. (*Oedomatopus spp.*) – *O. obovatus* Planchon & Triana: Prance et al. 4233 (anat., fr.), Venezuela, Maguire et al. 36049 (anat.); *O.* ?octandrus (Poepp. & Endl.) Planchon & Triana: Colombia, Schultes & Lopez 10068 (fr.); Venezuela, Maguire et al. 42628 (anat.).

Clusia p.p. (*Quapoya* spp.) – *Q. longipes* (Ducke) Maguire): Colombia, Schultes & Cabrera 15170 (anat,); *Q. peruviana*: Peru, Kayap 1317 (GH: anat.); *Q. scandens*: French Guiana, Wachenheim 2929 (A: anat.)

Clusia p.p. (*Renggeria*) – *R. comans* Black 54-1626 (NY: anat), 54-1625 (NY: fr).

Decaphalangium peruvianum: Peru, Vasquez et al. 3405 (A: anat.); Colombia, Schultes et al. 24109 (ECON: anat.).

Dystovomita – *D. clusiifolia* (Maguire) D'Arcy: Venezuela, Maguire & Steyermark 60030 (GH: anat.), Maguire & Steyermark 60031 (GH: anat.), Liesner & Gonzalez 9875 (fr.); Colombia, Gentry et al. 40433 (fr.); *D. pittieri* (Engler) D'Arcy: Mori & Kallunki 2612 (fr.); Costa Rica, Schubert et al. 812 (A: anat.); *D. sp.*: Colombia, Schultes et al. 14755 (GH: anat.).

Garcinia cymosa: Papua New Guinea, Kanehira 3992 (A: anat.), NGF 41384 (A: fr.).

Garcinia dulcis (Roxb.) Kurz: Papua New Guinea, Hartley 9943 (A: fr.); Indonesia, Irian Jaya, Moll 9622 (A: anat.).

Garcinia morella: Sri Lanka, Kostermans 24854 (A: anat.), CP 372 (GH: fr.).

Garcinia p.p. (*Pentaphalangium* spp.) – *P. brassii*: Papua New Guinea, Brass 8206 (A: anat., fr.); *P. latissimum*: Papua New Guinea, Shaw-Meyer s.n. 11.xi.1963 (A: anat.), Hoogland 3821 (A: anat.); *P. pachycarpon* (A. C. Smith) Kostermans: Irian Jaya, Brass & Versteegh 13550 (A: fr.); *P. solomonense* (A. C. Smith) Kostermans: Solomon Islands, BSIP 6714 (A; anat,), BSIP 14622 (A: anat.); *P. volkensii* (Lauterbach) Kostermans: Marianas, Kanehira 1173 (A;anat.).

Garcinia p.p. (*Rheedia spp.*)– *R. ruscifolia* Grisebach: Cuba, Clemente & Alain 4116 (A: anat.), Leon & Clemente 23246 (A: fr.).

Lorostemon bombaciflorum – Brazil, Ducke 944 (GH: anat.); Ducke 1200 (fr.)

Lorostemon coelhoi – Aluzio 245 (GH); Schultes & Cabrera 25898 (GH)

Montrouzeria – *M. balanasae*: New Caledonia, Balansa 3192 (A: fr.); *M. cauliflora*: New Caledonia, McPherson 1557 (: anat.); *M. gabriellae*: New Caledonia, Baumann-Bodenheim 15045 (anat.), Balansa 2364 (A: fr.); *M. sphaeroidea*: New Caledonia, Balansa s.n. (A: anat.); *M. verticillata*: New Caledonia, LeRat & LeRat 2483 (anat.).

Moronobea – *M. coccinea* Aublet: Colombia, Schultes et al. 18221 (anat.); *M. jenmannii* Engler var. *jenmanii*: British Guiana, Maguire & Fanshaw 23442 (A: anat.); *M. intermedia* Engler: Venezuela, Maguire 33547 (anat.); *M. riparia* (Spruce) Planchon & Triana: Colombia, Schultes et al. 18221 (anat.).

Pentadesma – *P. butyracea*: Liberia, Mayer 28 (US: fr.), Cooper 80 (US: fr) Yale school of Forestry 13730 (A: anat.); *P. exelliana*: Congo, Gilbert 553 (A; anat.); *P. reyndersii* Spirlet: Ruana, Renders 312 (A: anat.).

Platonia insignis Martius – Colombia, Schultes & Cabrera 19342 (anat.); Colombia, Schultes et al. 18272 (anat.), Surinam, BW 5588 (A; anat.)

Septogarcinia sumbawensis – Indonesia, Sumbawa, Kostermans 19125 (A: fr.), Kostermans 18789 (A; anat.).

Symphonia – *S.* cf. *louvelii* Jumelle & H. Perr.: Madagascar, Dorr & Barnett 4538 (A; anat.); *S. macrophylla* Vesque: Madagascar, Dorr & Barnett 4537 (anat.); Madagascar, Station Agric. de l'Alaotra 3482 (MO: fr.); *S. nectarifera*: Madagascar, Station Agric. de l'Alaotra 1901 (MO: anat.).

Thysanostemon pakaraimae: British Guiana, Maguire et al. 44026 (GH: anat.).

Tovomita – *T. calodictyos* Sandwith: British Guiana, Maguire & Fanshaw 22198 (A: anat.); *T. membranacea* (Planchon & Triana) D'Arcy: Ecuador, Napos (fr); *T.* cf. *umbellata*: Brazil, Amazonas, Krukoff 7019 (A: fr); *T. silvicola*: Hammel 16042 (GH: seedlings); *T. sp.*: Colombia, Vaupes, Schultes and Cabrera 15918 (fr); Krukoff 7242 (A; fr).

HYPERICACEAE.

Cratoxylum sects. Cratoxylum + Tridesmos – *C. formosum* (Jack) Dyer: ssp. formosum: Indonesia, Bangka, Kostrmans & Anta 453 (A: anat.); *C. sumatranum* (Jack) Blume: Sumatra, Lorzing 12338 (A: anat.).

Cratoxylum sect. Isopterygium – *C. arborescens* Blume: Sumatra, Rahmat si Toroes 4859 (A; anat.); Sabah, SAN 89495 (A: anat.); *C. glaucum* Korthals: Sarawak, S 16702 (A: anat.).

Eliea articulata: Madagascar, Humbert 5758 (A: anat), Areny & Rakotozafy 15350 (MO: fr).

Harungana madagascariensis: Australia, L. S. Smith 5321 (A: anat.); Congo, Leonard 1457 (A: anat.); Kersting 208A (fr.).

Hypericum ellipticifolium (syn. *Lianthus*)–T.T. Yü 20125 (A).

Hypericum p.p. (*Thornea spp.*)– Guatemala, Steyermark 48946 (A: anat., fr.)

Hypericum p.p. (*Triadenum spp.*) – *T. fraseri* (Spach) Gleason: U.S.A., Friesner 16304 (GH: anat.); *T. japonicum* (Blume) Makino: Japan, Murata 19838 (A: anat., fr.); *T. virginicum*: Boufford & Wood 17930 (anat.).

Hypericum (*Santomasia*) *steyermarkii* – Mexico, Matuda 2894 (MICH: fr.), Matuda S-228 (A; anat.).

Psorospermum lamianum H. Perrier: Gentry 11297 (GH)

Psorospermum cerasifolium group- Madagscar, *P.* cf. *androsaemifolium* Baker: Madagascar, Dorr & Rakotozafy 4534 (K: fr.); *P. cerasifolium* Baker: Madagascar, Perrier de la Bathie 1199 (P; fr.), Perrier de la Bathie 1162 (P; fr.), Kaudern s.n. ix.1912 (A; anat.); *P. lanceolatum* (Choisy) Hochreutiner: Madagascar, Barnett & Dorr 248 (A: anat), Dorr 3909 (fr.), de Cary 17703 (US: fr., anat.), Croat 32590 (MO: fr); *P. molluscum*: Madagascar, Scott Elliot 2249 (K: fr.); *P.* cf. *revolutum* (Choisy) Hochreutiner: Madagascar, Dorr et al 4433 (A: anat.).

Psorospermum febrifugum Spach: Cameroons, Breteler et al. 2338 (A: anat.); Angola, Teixeira & Figueira 5828 (A: fr.); Breteler 2793 (fr.); Wilson 188 (fr.); Becquaert 14 (anat., fr.).

Psorospermum staudtii group: Cameroons, Zenker 4234 (K: fr); Cameroons, FNI 35043 (K: fr.), Yafunga 39 (fr.); Congo, Louis 8975 (A; anat.); *P. senegalense*: Nigeria, Dalziel s.n. 1912 (BM: fr.)

Vismia affinis: Congo, Toussaint 199 (A: anat.), Toussaint 85 (K; fr.); Leopoldville, Wagemans 2215 (K: anat., fr.) Gossweiler 6307 (BM: fr.).

Vismia cayennesis (Jacquin) Persoon: Brazil, Austin et al. 7202 (GH: anat.).

Vismia laurentii - Corbisur-Balaud 934 (A: anat, fr).

Vismia rubescens Oliver: Congo, Louis 10247 (A: anat.); Portugese Congo, Gossweiler 9169 (A: anat.); Gentry 33544 (anat.).

Vismia orientalis – Tanzania, Swynnerton s,.n. 3.i.1922 (BM: anat.); Tanzania, Bruce 1058 (BM: fr.); Kenya, R. M. Graham 2119 (BM: anat.); Mwasumbi & Mhoro 2591 (fr.).

Appendix 2.4. Voucher information and GenBank accessions for sequences used in this study. Accessions in brackets are from a different voucher source. A dash (—) indicates that the sequence was unavailable. Herbaria acronyms follow Holmgren and Holmgren (1998 [continuously updated]). **FAMILY. Species,** *voucher* (herbarium), GenBank accessions: *matK*, *ndhF*, *rbcL*, *matR*.

BONNETIACEAE. ***Archytaea triflora*** Mart., *Kubitzki & Feuerer 97-26* (HBG), HQ331545, AY425029, AY380342, AY674475; ***Bonnetia sessilis*** Benth., *Berry s.n. 25.7.98* (MO), EF135509, HQ331849, HQ332010, EF135292; ***Ploiarium alternifolium*** Melchior, *Sugumaran 165* (US), FJ669999, FJ670063, FJ670161, FJ670352.

CALOPHYLLACEAE. ***Calophyllum inophyllum*** L., *Ruhfel 115* (A), HQ331553, HQ331856, HQ332016, HQ331709; ***Caraipa savannarum*** Kubitzki, *G. Aymard s.n.* (PORT), HQ331565, HQ331867, HQ332026, HQ331720; ***Clusiella isthmensis*** Hammel, *M. Whitten 2657* (FLAS), HQ331585, HQ331889, AY625019, HQ331738; ***Endodesmia calophylloides*** Benth., *Burgt 762* (WAG), FJ670005, FJ670069, FJ670163, FJ670356; ***Haploclathra paniculata*** Benth., *C. Grandez 16246* (FLAS), HQ331614, HQ331919, HQ332068, HQ331765; ***Kayea oblongifolia*** Ridl., *Ruhfel 116* (A), HQ331638, HQ331940, HQ332088, HQ331786; ***Kielmeyera petiolaris*** Mart., *F. Feres 75* (UEC), HQ331642, HQ331944, AY625016, HQ331790; ***Mahurea exstipulata*** Benth., *Kubitzki et al. 97- 27* (HBG), HQ331650, HQ331954, AY625018, HQ331799; ***Mammea americana*** L., *C. Notis 392* (FLAS), HQ331652, HQ331956, AY625029, HQ331801; ***Mammea siamensis*** T. Anderson, *Chase 1216* (K), FJ670006, FJ670070, AY625028, FJ670357; ***Mammea touriga*** (C.T. White & W.D. Francis) L.S. Sm., *H. van der Werff and B. Gray 17055* (MO), HQ331656, HQ331960, HQ332101, HQ331804; ***Marila tomentosa*** Poepp. & Endl., *van der Werff et al. 16215* (MO), HQ331660, HQ331964, AY625010, HQ331808; ***Mesua ferrea*** L., *M. Sugumaran et al. SM 120* (KLU), HQ331661, HQ331965, [*C. Notis 390* (FLAS), AY625024], HQ331809; ***Poeciloneuron indicum*** Bedd., *U. Ghate s.n.* (FLAS), HQ331673, HQ331977, AY625023, HQ331819.

CLUSIACEAE S.S. ***Allanblackia sp.***, *E. Ndive s.n.* (YU), HQ331542, HQ331843, HQ332004, HQ331699; ***Chrysochlamys allenii*** (Maguire) Hammel, *R. Kriebel 2289* (INB), HQ331569, HQ331871, HQ332030, HQ331723; ***Clusia* cf. *flavida*** (Benth.) Pipoly, *M. H. G. Gustafsson 454* (AAU), HQ331575, HQ331878, HQ332035, HQ331728; ***Clusia gundlachii*** Stahl, *Chase 341* (NCU), EF135520, AY425041, Z75673, AY674493; ***Clusia hammeliana*** Pipoly, *M. H. G. Gustafsson 451* (AAU), HQ331578, HQ331882, HQ332038, HQ331732; ***Clusia major*** L., *M. H. G. Gustafsson 396* (AAU), HQ331581, HQ331885, HQ332041, HQ331735; ***Dystovomita paniculata*** (Donn. Sm.) Hammel, *B. Hammel 25295* (MO), HQ331594, HQ331897, [*B. Hammel 22728* (INB), HQ332051], HQ331746; ***Garcinia cowa*** Roxb., *M. Sugumaran et al. SM 146* (KLU), HQ331596, HQ331900, HQ332054, HQ331748; ***Garcinia cymosa*** (K. Schum.)

I.M.Turner & P.F.Stevens, *P. Sweeney 1000* (MO), HQ331597, HQ331901, [*T. Motley s.n.* (AAU) AF518379], HQ331749; ***Garcinia latissima*** Miq., *Chase 2100* (K), FJ670008, FJ670072, AF518386, FJ670359; ***Garcinia macrophylla*** Mart., *Chase 1219* (K), —, FJ670073, FJ670165, FJ670360; ***Garcinia spicata*** Hook. f., *C. Notis 388* (FLAS), HQ331608, HQ331913, HQ332063, HQ331760; ***Garcinia urophylla*** Scort. ex King, *P. W. Sweeney 1081* (MO), HQ331611, HQ331916, HQ332066, HQ331763; ***Lorostemon coelhoi*** Paula, *V. Bittrich 95-170* (UEC), HQ331648, HQ331952, [*Assuncão 492* (UEC), AF518401], HQ331797; ***Montrouziera cauliflora*** Planch. & Triana, *Lowry 5601* (MO), FJ670007, FJ670071, FJ670164, FJ670358; ***Moronobea coccinea*** Aubl., *SM 24698* (NY), HQ331665, HQ331969, AF518378, HQ331813; ***Pentadesma butyracea*** Sabine, *Kitjima s.n.* (A), HQ331669, HQ331973, [*Nagata 951*, (HLA), AF518383], HQ331817; ***Platonia insignis*** Mart., *V. Bittrich s.n. 3.01.05* (INB), HQ331670, HQ331974, [*Mori 23699* (NY), AF518394], HQ331818; ***Symphonia globulifera*** L. f., *Ruhfel 21* (A), HQ331680, HQ331985, [*Mori 24792* (NY), AF518381], HQ331826; ***Tovomita calophyllophylla*** García-Villacorta & Hammel, *J.Vormisto 579* (AAU), HQ331683, HQ331988, HQ332119, HQ331828; ***Tovomita weddelliana*** Planch. & Triana, *M. H. G. Gustafsson 478* (AAU), HQ331686, HQ331991, HQ332122, HQ331831; ***Tovomitopsis saldanhae*** Engl., *V. Bittrich s.n.* (UEC), HQ331687, HQ331992, HQ332123, —.

CTENOLOPHONACEAE. ***Ctenolophon englerianus*** Mildbr., *McPherson 16911* (MO), EF135524, FJ670074, AJ402940, AY674499.

HYPERICACEAE. ***Cratoxylum arborescens*** (Vahl) Blume, *Ruhfel 121* (A), HQ331586, HQ331890, HQ332045, HQ331739; ***Cratoxylum cochinchinense*** (Lour.) Blume, *Church et al. 2699* (A), HQ331587, HQ331891, HQ332046, HQ331740; ***Eliea articulata*** Cambess., *Razakamalala 295* (MO), FJ670023, FJ670096, FJ670167, FJ670374; ***Harungana madagascariensis*** Poir., *B. Pettersson and L. A. Nilson 37* (UPS), HQ331615, HQ331920, [*Naugona 139* (NY), AF518396], HQ331766; ***Hypericum aegypticum*** L., *M. Gustafsson MG 1148* (AAU), HQ331617, HQ331922, HQ332069, HQ331767; ***Hypericum elodes*** L., *Halliday s.n., 6/7 1964* (AAU), HQ331622, —, HQ332073, HQ331772; ***Hypericum irazuense*** Kuntze ex N. Robson, *Ruhfel 8* (A), —, —, HQ332078, HQ331776; ***Hypericum kalmianum*** L., *C.C. Davis s.n.* (A), HQ331627, HQ331930, HQ332079, —; ***Hypericum perforatum*** L., *Ruhfel s.n.* (A), HQ331630, HQ331933, HQ332081, —; ***Psorospermum febrifugum*** Spach, *M. Hedren et al. 394* (UPS), HQ331677, HQ331980, HQ332113, HQ331822; ***Santomasia steyermarkii*** (Standl.) N. Robson, *E. Matuda S-228* (A), —, HQ331982, —, —; ***Thornea calcicola*** (Standl. & Steyerm.) Breedlove & E.M. McClint., *D.E. Breedlove 37070* (MO), HQ331682, [*J.A. Steyermark 48946* (A), HQ331987], —, —; ***Triadenum fraseri*** (Spach) Gleason, *C.C. Davis s.n.* (A), HQ331688, HQ331993, HQ332124, [*C.C. Davis s.n.* (A), HQ331832]; ***Vismia bilbergiana*** Beurl., *B. Hammel 25285* (MO), HQ331693, HQ331997, [*STRI:BCI 734543* (STRI), GQ981917], HQ331836; ***Vismia guineensis*** (L.) Choisy, *M. Merello et al. 1149* (UPS), HQ331695, HQ331999, —, HQ331838; ***Vismia***

rubescens Oliv., *R. Niangadouma et al. 374* (MO), —, HQ332001, HQ332127, HQ331840;

OCHNACEAE. ***Ochna multiflora*** DC., Chase 229 (NCU), EF135572, AY425072, Z75273, EF135302.

PANDACEAE. ***Panda oleosa*** Pierre, *Schmidt et al. 2048* (MO), FJ670032, FJ670111, AY663644, FJ670383.

PODOSTEMACEAE. ***Podostemum ceratophyllum*** Michx., *Ruhfel s.n.* (A), HQ331671, HQ331975, HQ332108, [*Horn s.n.* (DUKE), EF135304]; ***Tristicha trifaria*** (Bory ex Willd.) Spreng., *C.T. Philbrick 6090* (WCSU), HQ331691, HQ331995, [*BR-01*, AB113746], HQ331834; ***Weddellina squamulosa*** Tul., *C.T. Philbrick 5827* (WCSU), HQ331697, HQ332002, [not listed, AB113758], HQ331841.

Appendix 2.5. Ancestral state reconstruction figures A2.1-A2.18.

Figure A2.1. Maximum likelihood ancestral state reconstruction of carpel number (character 51) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.2. Maximum likelihood ancestral state reconstruction of leaf insertion (character 9) in the clusioid clade. Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.3. Maximum likelihood ancestral state reconstruction of exudate in the plant body (character 15) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

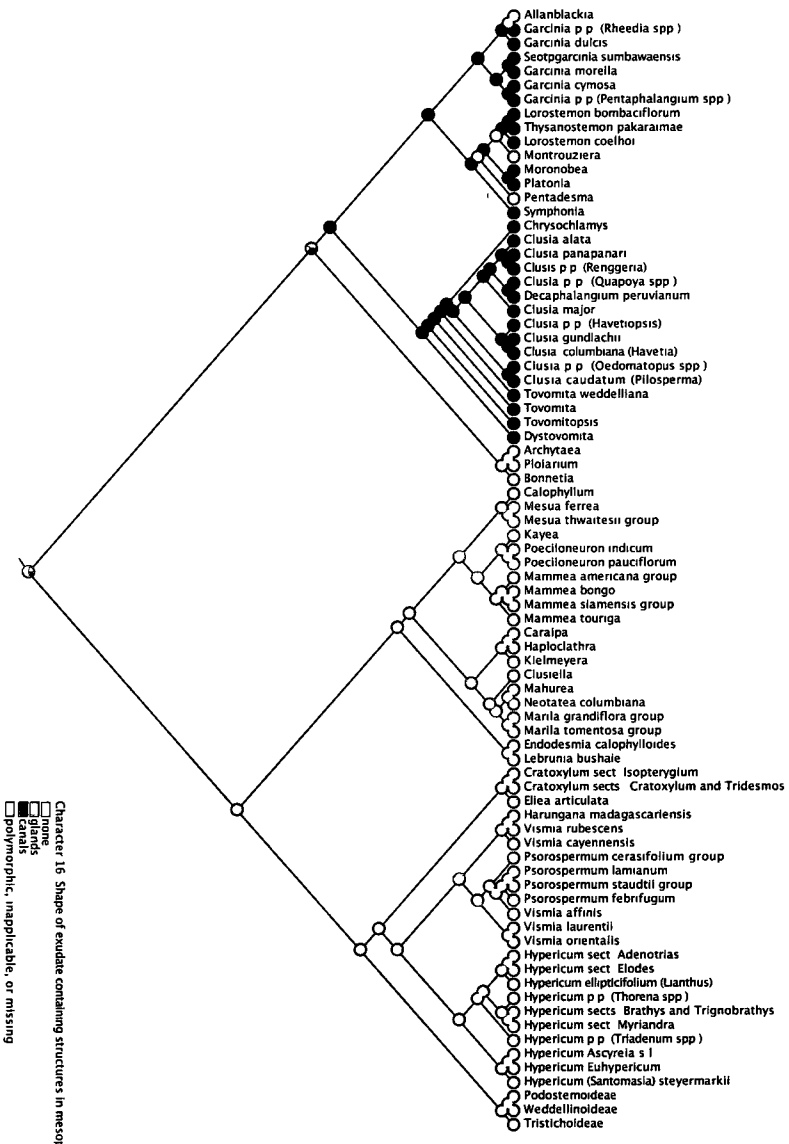

Figure A2.4. Maximum likelihood ancestral state reconstruction of the shape of exudate containing structures in the mesophyll (character 16) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.5. Maximum likelihood ancestral state reconstruction of sepal number (character 35) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.6. Maximum likelihood ancestral state reconstruction of stamen arrangement (character 38) in the clusioid clade. Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.7. Maximum likelihood ancestral state reconstruction of fasciclodia presence in staminate or perfect flowers (character 40) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.8. Maximum likelihood ancestral state reconstruction of dioecy (character 69) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.9. Maximum likelihood ancestral state reconstruction of anther orientation (character 44) in the clusioid clade. Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.10. Maximum likelihood ancestral state reconstruction of stylar fusion (character 55) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.11. Maximum likelihood ancestral state reconstruction of style length (character 54) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.12. Maximum likelihood ancestral state reconstruction of filament attachment (character 41) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.13. Maximum likelihood ancestral state reconstruction of an indumentum of unbranched unicellular hairs (character 23) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

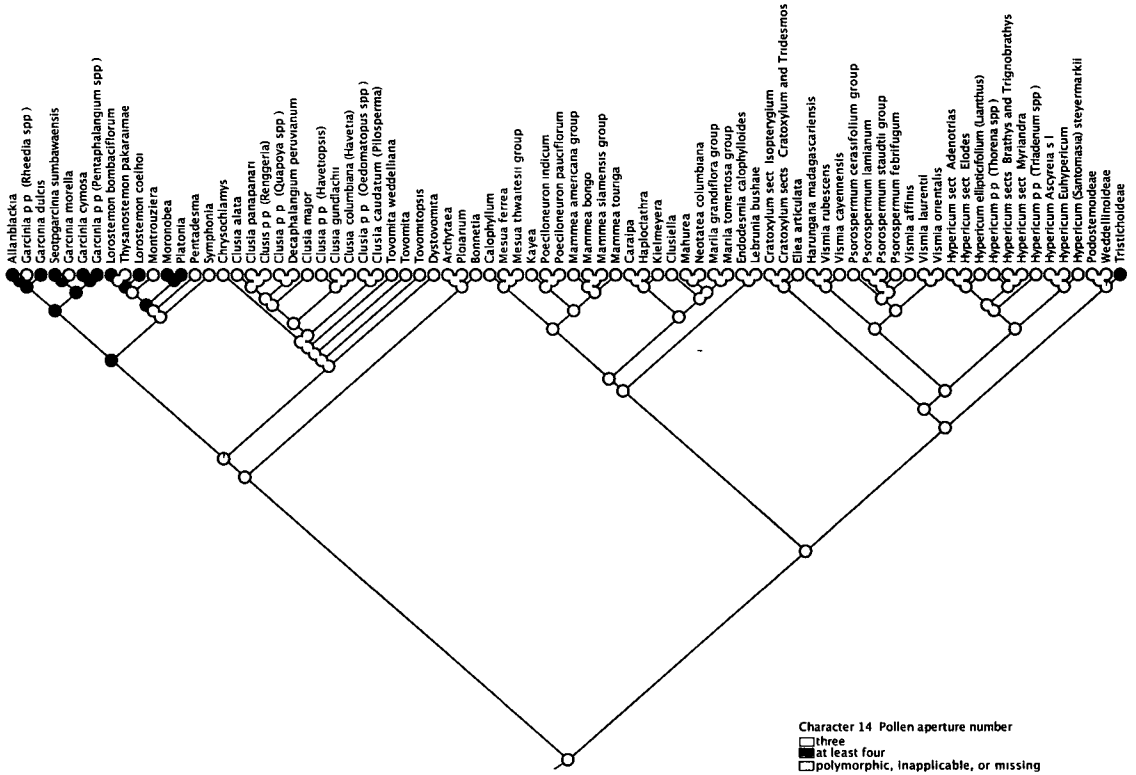

Figure A2.14. Maximum likelihood ancestral state reconstruction of pollen aperture number (character 14) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

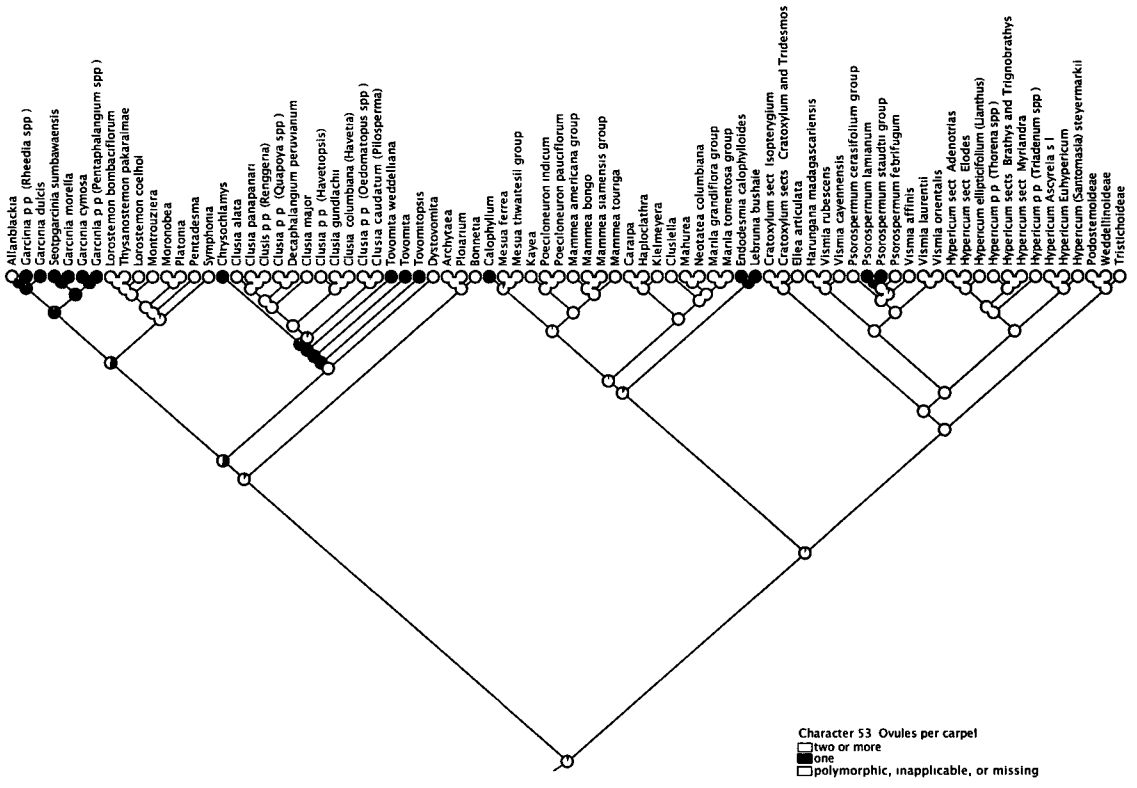

Figure A2.15. Maximum likelihood ancestral state reconstruction of ovules per carpel (character 53) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.16. Maximum likelihood ancestral state reconstruction of aril presence (character 60) in the clusioid clade. Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.17. Maximum likelihood ancestral state reconstruction of filament thickness relative to the anthers (character 42) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

Figure A2.18. Maximum likelihood ancestral state reconstruction of stigma surface (character 58) in the clusioid clade Proportion of pie graph indicates the relative degree of support for alternative ancestral character states

CHAPTER 3:

Dispersal largely explains the Gondwanan distribution of the ancient tropical clusioid plant clade

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ABSTRACT

The clusioid clade (Malpighiales) has a widespread pantropical distribution, and is present on all former Gondwanan landmasses (Africa, Australia, India, Madagascar, and South America) except Antarctica. Furthermore, this clade has an ancient fossil record dating back to the Turonian (~90 Ma). Several biogeographers have previously hypothesized that their distribution is the result of ancient Gondwanan vicariance. Our estimates of molecular divergence times and ancestral ranges for the clusioids, however, revealed only a single cladogenic event that is potentially consistent with ancient Gondwanan vicariance involving the separation of Africa and South America. Instead, we detected that the clade's distribution is most likely the result of extensive dispersal during the Cenozoic, mostly occurring after the middle Eocene. Our analyses indicate that the distribution across former Gondwanan landmasses involves at least 20 dispersal events between these areas, and in some cases also involves Laurasian landmasses in the north (e.g., North America and Eurasia). Many of these dispersal events, however, do not appear to be randomly distributed in space and time. Instead, we detect several repeated patterns of dispersal between similar areas involving distantly related clades. For example, dispersal from South America into North America and dispersal between the various areas of present-day, tropical Southeast Asia appears to have occurred after the Eocene-Oligocene boundary (~34 Ma), whereas dispersal into Madagascar occurred throughout the last 70 million years. These results support growing evidence that suggests many traditionally recognized angiosperm clades are far too young for their distributions to have been influenced strictly by Gondwanan vicariance. Instead, it

appears that dispersal is the best explanation for many angiosperm clades with Gondwanan distributions such as those observed in the clusioid clade.

Keywords: dispersal, vicariance, Gondwana, Guttiferae

INTRODUCTION

Tropical forests contain tremendous angiosperm diversity (Richards, 1996; Whitmore, 1998; Morley, 2000) and are included in 15 of the 25 biodiversity hotspots of the world (Myers et al., 2000). The origination time of this diversity, and how it has been maintained, are a central focus of ecology and evolutionary biology (Raven and Axelrod, 1974; Richardson et al., 2001; Ricklefs, 2004; Wiens and Donoghue, 2004; Davis et al., 2005; Fine and Ree, 2006; Jaramillo et al., 2006; Ricklefs, 2006; Mittelbach et al., 2007). Principally important in these ideas is determining the place and time of origin of large, ecologically important, tropical clades of organisms. Phylogenetic studies examining the biogeographic history of clades have grown at an exponential pace, but detailed studies of groups distributed in the tropics, where much of early angiosperm diversification occurred, are still rare. To better understand the assembly of the tropical forest biome, and determine the roles of ancient vicariance versus more recent dispersal, we need to elucidate the timing and origin of major plant clades that inhabit these regions (Pennington and Dick, 2004).

The tropics are particularly well represented on the former fragments of Gondwana, including the present day continents of Africa, Australia, India, Madagascar, and South America. Numerous tropical plant clades share a Gondwanan distribution pattern, and the separation of these southern landmasses has been used to explain the distribution of many such groups (Raven and Axelrod, 1974). However, separation of Gondwana began in the Jurassic (~180 Ma; McLoughlin, 2001), well before many angiosperm lineages are likely to have been present, particularly eudicot angiosperms (Magallón et al., 1999; Sanderson and Doyle, 2001). Some portions of Gondwana,

however, retained connections until much more recently, thus allowing for the possibility that some of these more recent separations influenced the diversification of angiosperms since the Upper Cretaceous. For example, western Gondwana (including present day South America and Africa) separated between 100-80 Ma, Madagascar and India separated between 95-84 Ma, and the final connections between South America, Antarctica and Australia were broken between 35-30 Ma (McLoughlin, 2001; Sanmartín, 2002; Upchurch, 2008).

An emerging paradigm regarding many pantropical distributions is that relatively few angiosperm clades exhibit ages that are clearly consistent with Gondwanan time frames (Chanderbali et al., 2001; Renner et al., 2001; Davis et al., 2002; Davis et al., 2004; Zerega et al., 2005; Couvreur et al., 2010) and that their current distribution is more likely the result of long-distance, transoceanic dispersal or dispersal through the Northern hemisphere. The largely southern temperate clades Proteaceae and Nothofagaceae have been implicated in Gondwanan scenarios (Manos, 1993; Weston and Crisp, 1994) and are classic examples of Gondwanan vicariance (eg., Lomolino et al., 2010). However, recent molecular divergence time estimates of even these groups suggest that strict Gondwanan vicariance scenarios are likely too simplistic. Instead, initial Gondwanan vicariance, in combination with recent dispersal, appears to have played a key role in facilitating their distributions (Knapp et al., 2005; Barker et al., 2007). A similar combination of Gondwanan vicariance and recent dispersal appears to be true in some tropical groups with Gondwanan distributions, including Hernadiaceae, Monimiaceae, and Myrtaceae (Sytsma et al., 2004; Michalak et al., 2010; Renner et al., 2010). The paucity of examples in which Gondwanan vicariance explains the

distributions of tropical angiosperm clades may be due in part to the concentration on formal taxonomic groups, which in many instances are far too young to be consistent with Gondwanan vicariance (e.g., clades at the rank of family; Wikström et al., 2001; Bell et al., 2010). Furthermore, extinction combined with prevalent recent dispersal may be hindering our ability to clearly detect ancient biogeographical patterns in angiosperm distributions (Pennington and Dick, 2004; Upchurch, 2008; Clayton et al., 2009). Thus, the need to expand biogeographical studies that address Gondwanan vicariance to include both older and larger angiosperm clades, especially those without formal taxonomic designations, is warranted.

The clusioid clade (Malpighiales; Wurdack and Davis, 2009; Ruhfel et al., 2011) provides a test case of the potential impact of Gondwanan vicariance on tropical angiosperms. Features of the clusioids that make them especially amenable for this purpose include: i) their well-sampled and strongly supported phylogeny (Ruhfel et al., 2011), ii) their pantropical distribution, and iii) their ancient Cretaceous fossil record (~90 Ma; Crepet and Nixon, 1998). This clade includes five families (Bonnetiaceae, Calophyllaceae, Clusiaceae s.s., Hypericaceae, and Podostemaceae) that are strongly supported as monophyletic (Wurdack and Davis, 2009; Ruhfel et al., 2011). Recent phylogenetic analyses of the clusioids (Ruhfel et al., 2011; Ruhfel et al., unpublished) have greatly resolved their phylogeny and further clarified the placement of the fossil taxon *Paleoclusia* (Crepet and Nixon, 1998), which is one of the oldest rosid macrofossils (Crepet et al., 2004; Schönenberger and von Balthazar, 2006). These analyses provide a much more informed use of *Paleoclusia* as an age constraint for molecular divergence time estimation. In addition to *Paleoclusia*, fossil pollen from the

clusioid clade is known from the Eocene (*Pachydermites*, ~45 Ma; Germeraad et al., 1968; Salard-Cheboldaeff, 1979). These fossils directly indicate that the clusioid clade is ancient, a finding that is further supported by molecular divergence time estimates of Malpighiales: stem group clusioids have been estimated to be of Aptian–Albian age (~106-115 Ma) and crown group clusioids to be of Albian–Turonian age (~92-104 Ma; Davis et al., 2005). Although no previous biogeographical study has been conducted on the entire clusioid clade, two studies have indicated that at least some intercontinental disjunctions are more consistent with recent dispersal than ancient Gondwanan vicariance (Dick et al., 2003; Kita and Kato, 2004).

Each of the clusioid families is distributed primarily in the tropics and is represented on two or more former Gondwanan landmasses (Fig. 3.1). These numerous instances of transcontinental disjunctions provide independent opportunities to test the influence of Gondwanan vicariance versus more recent dispersal (Ruhfel et al., 2011). Either these disjunctions arose repeatedly though continental breakup of these southern landmasses or were achieved more recently via dispersal when these land areas were more separate. Several researchers have previously commented on the possibility that the clusioid clade and its constituent subclades are ancient. For example, Raven and Axelrod (1974) hypothesized that various clusioid clades date to times when Africa and South America were in close proximity to one another. Others (Robson, 1977, Hypericum [Hypericaceae]; Kato, 2006, Podostemaceae) have also proposed similar biogeographical hypotheses that invoke Gondwanan vicariance for members of this clade. The goal of our study is to test the hypothesis that the modern distribution of the clusioid clade is due to ancient Gondwanan vicariance. To accomplish

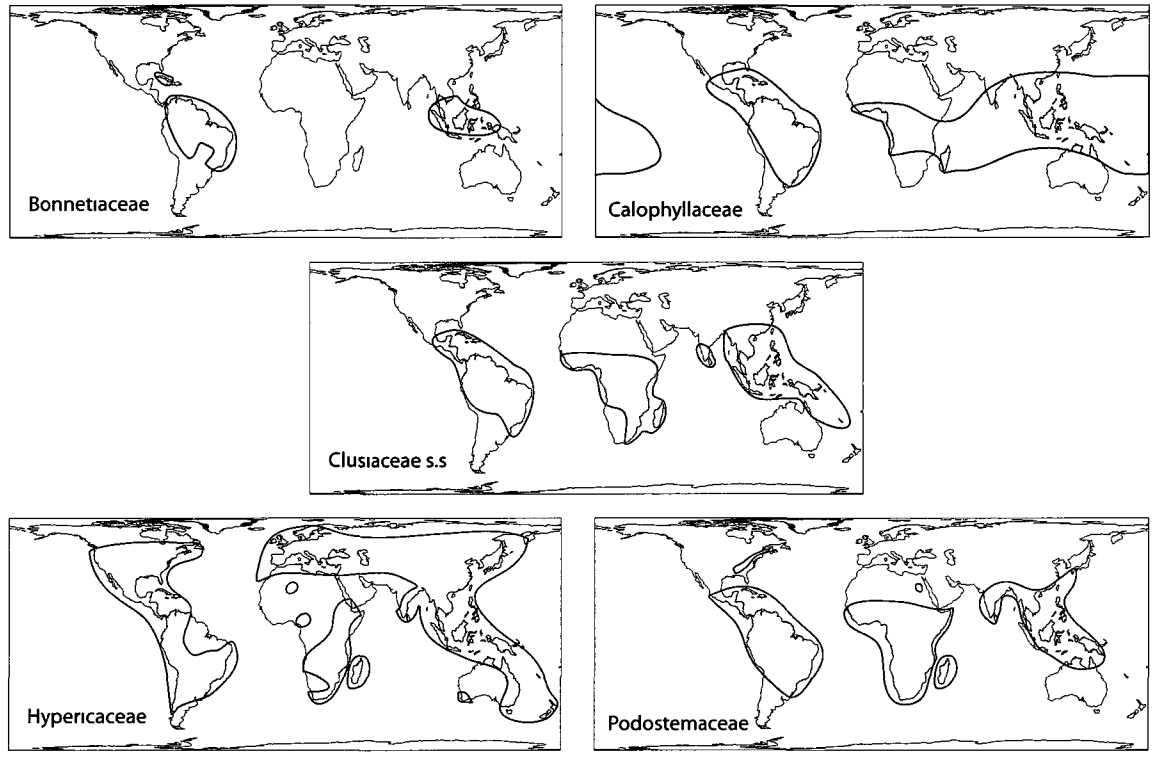

Figure 3.1. Current distributions of families in the clusioid clade (Stevens 2001 onwards and references therein).

this we will estimate divergence times using Bayesian methods that allow for lineage-specific rate heterogeneity. Next, we will reconstruct ancestral ranges of all major clusioid clades using methods that account for uncertainty in phylogenetic relationships and divergence-time estimates. And finally, we will identify patterns of dispersal under conditions when vicariance seems unlikely.

METHODS

Taxon sampling and molecular methods–We used the expanded taxon sampling scheme from Ruhfel et al. (2011). This included 194 clusioid species sampled for three plastid genes (*matK*, *ndhF*, and *rbcL*) plus the mitochondrial gene *matR*. This sampling includes all major morphological and biogeographical representatives of all five clusioid families. Additionally, we added new data for seven species of Podostemaceae (*Castelnavia multipartida* Tul. &Wedd., *Castelnavia princeps* Tul. &Wedd., *Cipoia ramosa* C.P. Bove, C.T. Philbrick, & Novelo *Hydrodiscus koyamae* [M. Kato & Fukuoka] Koi & M. Kato, *Lophogyne lacunosa* [Gardner] C.P. Bove & C.T. Philbrick), *Macarenia clavigera* P. Royen, and *Saxicolella amicorum* J.B. Hall) and one species of Clusiaceae s.s. (*Tovomitopsis paniculata* [Spreng.] Planch. & Triana). These taxa include five Podostemaceae genera that were not included in the Ruhfel et al. (2011) sampling (*Cipoia*, *Hydrodiscus*, *Lophogyne*, *Macarenia*, and *Saxicolella*). *Cipoia, Macarenia,* and *Tovomitopsis paniculata* (the type species of the genus), have never been included in a molecular phylogenetic analysis. *matK* data from *Saxicollela* are from the study by Kelley et al. (2010). This is the first time that this genus has been included in a broad phylogenetic analysis of the clusioid clade.

In addition, we also added data for previously unsampled gene regions (Ruhfel et al., 2011). In some instances, we also replaced previously sampled, but lesser quality, gene sequences. Molecular methods, sequence assembly, and alignment strategy followed those in Ruhfel et al. (2011). Data matrices and trees are available from the first author. We added a total of six, six, seven, and thirteen sequences for *matK*, *ndhF*, *rbcL*, and *matR* respectively. Voucher information for all sequences is provided in Appendix 3.1; new sequences for this study have GenBank accession numbers beginning with JF (JF828242-JF828273). Based on recent analyses by Xi et al. (2010) we chose the following taxa to serve as outgroups: *Bruguiera gymnorhiza* (Rhizophoraceae; representing Rhizophoraceae + Erythroxylaceae), *Ctenolophon englerianus* (Ctenolophonaceae), *Irvingia malayana* (Irvingiaceae), *Panda oleosa* (Pandaceae), and *Ochna multiflora* (Ochnaceae s.l.).

Phylogenetic analyses and divergence time estimation–A Bayesian Markov chain Monte Carlo (MCMC) approach to simultaneously estimate the phylogenic history and divergence times of the clusioid clade was conducted using BEAST v.1.6.1 (Drummond and Rambaut, 2007). Data were partitioned by gene region following Ruhfel et al. (2011) and a GTR+ Γ model with four rate categories was applied to each partition with base frequencies estimated from the data. We implemented a relaxed molecular clock (uncorrelated lognormal; Drummond et al., 2006) and a Yule tree prior. A maximum likelihood starting tree was created using RAxML v.7.2.6 (Stamatakis, 2006; distributed by A. Stamatakis at http://wwwkramer.in.tum.de/exelixis/software.html) following the search strategy of Ruhfel et al. (2011) with branch lengths approximately adjusted for time using PATHd8 v.1.0 (Britton et al., 2007); branch lengths and topology

satisfied all topological and fossil prior constraints (see below). Fossil age-constraint priors were enforced as probability distributions (Ho and Phillips, 2009; see below). We ran 5 independent MCMC chains for 50 million generations each sampling every 1000 steps in order to obtain an estimated sample size (ESS) greater than 200 for all parameters. We assessed convergence and stationarity of estimated parameter values using Tracer v.1.5 (Rambaut and Drummond, 2009). When the independent chains converged, the samples of each run were combined after discarding the burin (~25 % of each run), using LogCombiner v.1.6.1. TreeAnnotator v.1.6.1 was then used to generate a maximum clade credibility (MCC) tree and estimate the mean node age, 95% highest posterior density (HPD) of divergence time estimates, and posterior probability for all nodes in the topology. Divergence time estimation in BEAST provides two main advantages compared to other approaches (Sanderson, 2003; Lartillot et al., 2009) that implement relaxed molecular clock methods. First, fossil calibrations in BEAST can be treated as probability distributions, rather than simply minimum or maximum dates. Second, BEAST does not require a fixed topology to estimate divergence times, thus allowing the incorporation of phylogenetic uncertainty in the estimation of divergence times.

Topological and fossil constraints–Topological constraints were enforced in our BEAST analyses to accommodate fossil constraints and incorporate recent phylogenetic discoveries. Relationships among our outgroups were constrained to well-supported relationships (>70% BP or 0.95 PP) in Xi et al. (2010): 1) *Irvingia malayana* (Irvingiaceae) + *Panda oleosa* (Pandaceae), 2) *Bruguiera gymnorhiza* (Rhizophoraceae) + *Ctenolophon englerianus* (Ctenolophonaceae), and 3) *Ochna multiflora* (Ochnaceae

s.l.) + the clusioid clade. We also enforced several constraints within the Garcineae clade (Clusiaceae s.s.). These constraints were enforced based on the well supported findings by Sweeney (2008; see his Figs. 3 and 4) who used two nuclear genes to resolve the group. To ensure that our data were not in conflict with constraints in our outgroups and Garcinieae, we performed an alternative topology test using the approximately unbiased test (AU; Shimodaira, 2002) as implemented in the R software package, scaleboot ver. 0.3-2 (Shimodaira, 2008; distributed by CRAN at http://www.r-project.org). The constrained maximum likelihood topology could not be rejected by our data ($p= 0.12$).

The root node was set to a uniform distribution between 89.3 Ma and 125 Ma. The former age corresponds to the minimum age of the oldest known fossil within Malpighiales (Crepet and Nixon, 1998). The later date corresponds to the earliest evidence of tricolpate pollen (Magallón et al., 1999; Sanderson and Doyle, 2001), a synapomorphy of the eudicot clade to which the Malpighiales belong (APG III, 2009). In addition to the root node fossil constraint, we included three additional fossils, two of which are clusioids, to estimate divergence times (Table 3.1). These three fossil age constraints were modeled as lognormal distributions with separate means and standard deviations (Table 3.1). While there are many factors to consider when assigning the mean and standard deviation values to these lognormal prior distributions (Ho and Phillips, 2009) we have taken what we believe to be conservative approach to determining these values. The minimum age of each fossil constraint was assigned based on the youngest boundary of the geological stage in which the fossil was found [geological time scale following Gradstein et al. (2004)]. For example, the *Paleoclusia* fossil is Turonian (89.3-93.5 Ma) and therefore the minimum age for this prior was set as the youngest age of that

Table 3.1. Fossils used as age constraints in divergence time estimations. Constraints were applied to the most recent common ancestor (MRCA) listed. Mean and standard deviation (SD) for the lognormal prior on each fossil are also given.

Fossil (Clade)	Minimum age (Ma)	MRCA	References	Mean (SD)
Ctenolophonidites costatus	65.5	*Ctenolophon* and *Bruguiera*	(Edet and Nyong, 1994; Schrank, 1994)	2.5 (0.4)
Paleoclusia chevalieri	89.3	OC. Ochna and clusioid clade BC: Bonnetiaceae and Clusiacae s.s	(Crepet and Nixon, 1998, Ruhfel et al in prep)	2.0 (0.5)
Pachydermites diederixi	40.4	*Pentadesma* and *Symphonia*	(Germeraad et al., 1968; Salard-Cheboldaeff, 1979)	4.5 (0.3)

stage, 89.3 Ma. The means of the lognormal prior distributions were chosen so that the median value was approximately at the midpoint of the stage. The standard deviation was chosen so that the 97.5% quantile fell roughly at the lower boundary of the layer. Thus, the tail of each prior distribution extends well beyond the age of the fossil, allowing for the possibility that the origination of the group is much older. Due to the uncertain phylogenetic position of the *Paleoclusia* fossil we conducted two independent analyses using alternate placements of *Paleoclusia* as an age constraint as suggested by Ruhfel et al. (unpublished). In the first analysis the *Paleoclusia* constraint was placed at the most recent common ancestor (MRCA) of Bonnetiaceae + Clusiaceae (BC placement). In the second analysis the constraint was placed deeper in the phylogeny at the MRCA of Ochnaceae s.l. + clusioid clade (OC placement).

Ancestral Range Reconstructions–Ancestral range reconstructions were conducted in a likelihood framework using the dispersal-extinction-cladogenesis model (Ree et al., 2005) as implemented in the C++ program LAGRANGE v.0.1BETA2 (Ree and Smith, 2008; available at http://code.google.com/p/lagrange). Rather than the "splits" that are reconstructed in traditional LAGRANGE analyses we reconstructed "states", a new option available in the C++ version of the program. States were chosen because summarizing split results over a set of topologies with differing phylogenetic relationships is not easily interpreted (S. Smith, personal communication). We conducted these analyses on 1000 trees randomly selected from the posterior distribution of dated BEAST trees. This approach accounts for phylogenetic and divergence time uncertainty and has been applied in several recent studies (Smith, 2009; Bendiksby et al., 2010; Smith and Donoghue, 2010). Our input LAGRANGE topologies were pruned of several

terminals to leave a single placeholder for each genus that could be meaningfully scored for our biogeographic areas. Where molecular phylogenetic studies have been conducted, single terminals representing monophyletic clades within genera (e.g., *Garcinia*) were also retained. For example if we have seven species of a genus in our full BEAST topologies but all are found in South America, six of those species were pruned from the tree and the remaining terminal was coded as occurring in South America. Tree pruning preserved branch lengths and was conducted using the R (http://www.r-project.org/) package APE v.2.6-2 (Paradis et al., 2004; available at http://cran.r-project.org/web/packages/ape). Our fully dated BEAST trees (207 total taxa) were pruned of 120 taxa leaving a total of 87 ingroup taxa and used for ancestral range reconstructions. Results of our ancestral range reconstructions using the distribution of 1000 trees were then summarized onto a target tree by recording the frequency of the ancestral range with the greatest proportional likelihood at each node. Target trees for the BC and OC analyses were obtained by pruning the full MCC trees as above. To ensure that our target trees were topologically identical, and thus our biogeographic analyses directly comparable, we resolved three uncertain nodes (< 50 PP) based on their highest PP or using phylogenetic evidence from previous studies where these relationships were supported. First, the clade containing *Mammea*, *Poeciloneuron*, and *Kayea* is supported by combined morphological and molecular phylogenetic analysis as being sister to the *Calophyllum* + *Mesua* clade (Ruhfel et al., unpublished). Second, the sister group relationship of *Kayea* + *Poeciloneuron* (Calophyllaceae) is supported by morphology and other molecular data sets (Notis, 2004; Ruhfel et al., unpublished). Third, *Saxicollela* (Podostemaceae) is considered to be an early diverging lineage of the African

Podostemoideae clade (Thiv et al., 2009; Kelly et al., 2010); this relationship also received the highest PP in each analysis, however, this relationship was not in the OC MCC tree.

Eight biogeographic areas were circumscribed in our LAGRANGE analyses (Fig. 3.2): 1) North America, Central America, and the Caribbean, 2) South America, 3) Eurasia; 4) Africa, 5) Madagascar, plus the Comoros, Seychelles, and Mascarenes 6) India and Sri Lanka, 7) Southeast Asia (those regions west of Wallace's Line, but not part of continental Eurasia), and 8) Australia (those regions east of Wallace's line including New Caledonia and the Pacific Islands). Each terminal was coded based on its present distribution, or for some terminals, based on the likely ancestral area of the group according to the best current phylogenetic and biogeographical information available. Ranges were obtained from the literature and explanations for our area coding can be found in Appendix 3.2.

We applied two models in our LAGRANGE analyses, an unconstrained model and a model that incorporates information on i) biologically feasible ancestral ranges and ii) dispersal probabilities scaled according to area connections through four stratified windows of time, reflecting changing land configurations during the period of interest. In the unconstrained model, all combinations of our eight biogeographical areas were allowed and no constraints were imposed on the ability of a taxon to disperse from one area to another. This model has two main disadvantages. First, the inclusion of all possible ranges presents the possibility that biologically unrealistic ancestral ranges may be inferred (e.g., North America + Australia). Second, dispersal events that are extremely unlikely are considered equally probable. An advantage of conducting biogeographic

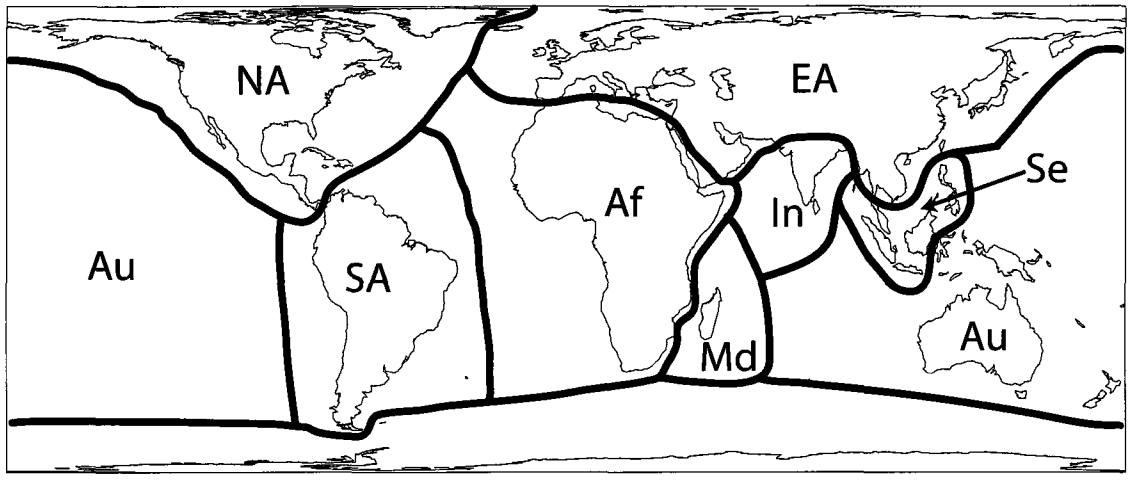

Figure 3.2. The eight biogeographic areas used in our ancestral range reconstructions. NA= North America, Central America, and the Caribbean; SA= South America; EA= Eurasia; Af = Africa; Md = Madagascar, plus the Comoros, Seychelles, and Mascarenes; In = India and Sri Lanka; Se = Southeast Asia (those regions west of Wallace's Line, but not part of continental Eurasia); and Au = Australia (those regions east of Wallace's line including New Caledonia and the Pacific Islands).

analyses in LAGRANGE compared to using parsimony based approaches such as DIVA (Ronquist, 1997) is the ability to incorporate information regarding the changing relationships of areas though time. This is especially relevant in our case because the clusioid clade is at least 90 Ma, and thus spans a period of great change in climate and continental configurations. In our second model, we imposed several assumptions that reflect our knowledge of the current distributions of clusioid taxa and of land configurations through time. First, we limited the maximum range size to three areas. Most (~90%) of our terminal taxa have range sizes of three areas or fewer and larger ranges are unlikely to be maintained over long periods of time without being further reduced via cladogenesis. However, including some terminal taxa with larger ranges (>3) was unavoidable. This is because LAGRANGE requires any ranges present in terminal taxa to be included in the transition matrix: nine taxa in our data set have distributions of four areas or greater (e.g., *Mammea*, present in six areas; Appendix 3.2). We further reduced possible ancestral ranges by excluding those that seemed unlikely. A full list of the ranges included in our analyses is available in Appendix 3.3 (Table A3.1). In addition to restricting possible ranges, we also included information on dispersal probabilities across several discrete windows of time. Dispersal probabilities and time slices used in our analyses were derived from the LAGRANGE model parameters proposed by Buerki et al. (2011). Their model considered four time slices (120-80 Ma, 80-60 Ma, 60-30 Ma, and 30-0 Ma) and three dispersal rate probabilities: 1) 1.0 for dispersal between areas that were physically connected, 2) 0.5 for areas connected through dispersal by abiotic factors such as equatorial ocean currents, and 3) 0.01 for areas that were not connected during a given period of time. Slight modifications to the Buerki et al. model were necessary to

also include India, which was not considered as a separate landmass in their analyses. Thus, we added dispersal probabilities between India and the other landmasses for each of the four windows of time (Appendix 3.3; Table A3.2). Because of recent evidence suggesting that India maintained biological connections to Africa during the late Cretaceous and Paleogene as it drifted northwards toward Eurasia (Briggs, 2003; Ali and Aitchison, 2008), we incorporated this information into our dispersal probability matrices.

RESULTS

Phylogenetic analyses–Our BEAST analyses of the clusioid clade resulted in a robust phylogeny for the group that was very similar to that presented by Ruhfel et al. (2011). No strongly supported conflicts were present among the topologies from the BI analyses using the BC or OC *Paleoclusia* constraints (Appendix 3.4; Figs. A3.1 and A3.2, respectively). The newly added taxa from Podostemaceae and Clusiaceae s.s. were mostly strongly placed. The neotropical taxa *Castelnavia multipartida*, *C. princeps*, and *Lophogyne lacunosa* (Podostemaceae) were strongly placed (100 PP) within the strictly neotropical Podostemoideae clade in positions that agree with Tippery et al. (2011). *Cipoia* was strongly supported as a member of the primarily Old World Podostemoideae clade (100 PP). Within this clade it was well supported (> 98 PP) in a clade containing mostly African and Malagasy taxa. *Macarenia,* a monotypic genus from Columbia, was placed sister to *Rhyncholacis* with strong support (100 PP). The African genus *Saxicollela* was strongly placed (100 PP) in the primarily Old World clade of Podostemoideae. However, its position within that clade was not well supported. The

type species of *Tovomitopsis* (Clusiaceae s.s.), *T. paniculata,* was strongly placed (100 PP) as sister to its congener *T. saldanhae*. Finally, the addition of *matR*, *ndhF*, and *rbcL* data for *Diamantina* (Podostemaceae) did not resolve the placement of this taxon. In Ruhfel et al. (2011), *Diamantina* was weakly placed (56 ML BP) as sister to the remaining Podostemoideae. This placement agrees with previous authors hypotheses (Philbrick et al., 2004; Rutishauser et al., 2005; Koi et al., 2006), but has very little support here (< 5 PP).

Divergence time estimation–The complete MCC tree using each placement of *Paleoclusia* is presented in Figs. A3.1 and A3.2 (Appendix 3.4). The pruned topologies used in our biogeographic reconstructions are presented in Figs. 3.3 and 3.4. Divergence times for the major clusioid subclades using each placement of the *Paleoclusia* fossil are shown in Table 3.2. The alternate placements of the fossil appear to have a greater effect on the early diverging nodes: these nodes are obviously younger with the OC placement and older with the BC placement. Node ages closer to the tips of the tree are more similar (Fig. 3.5). Using the OC placement, we estimate that the stem clusioid clade originated in the Upper Cretaceous, during the Turonian or Cenomanian (min =89.8, mean=91.7, max=94.4) and that the crown clusioids began to diverge in the Campanian–Coniacian (min =78.0, mean=83.4, max=88.7). Using the BC *Paleoclusia* placement, the stem clusioids originated in the Lower Cretaceous, during the Albian or Aptian (min =104.2, mean=115.3, max=124.3) and the crown clusioids began to diverge in the Turonian–Aptian (min =92.3, mean=102.9, max=113.7).

Results of our dating analyses are largely concordant with Davis et al. (2005). However, three items should be considered when comparing their dates to ours. First,

Fig. 3.3. Pruned phylogeny of the clusioid clade based on the analysis of a combined four-gene data set (BC placement of *Paleoclusia*; see text for details). The phylogeny and divergence times were simultaneously estimated using BEAST. Divergence time estimates were obtained by using three fossil constraints and assigning a uniform distribution to the root node between 89.9 and 125 Ma based on the youngest age possible for the *Paleoclusia* fossil and the oldest occurrence of tricolpate pollen grains representing the eudicot clade, respectively. Fossil names and arrows indicate the placement of fossil constraints. Posterior probabilities converted to percentages are given above the branches; only nodes receiving > 50% supported are annotated. Error bars at each node represent the 95% highest posterior distributions of divergence times. Scale bar represents the major Cretaceous and Cenozoic intervals. Numbers next to terminals in Garcinieae represent clades present in Sweeney (2008). Bon. = Bonnetiaceae, Cr. = Cratoxyleae, End. = Endodesmieae, Hy. = Hypericeae, L. = Lower, P. = Pleistocene, Pl. = Pliocene, Trist. = Tristichoideae, Vis. = Vismeae, W. = Weddellinoideae.

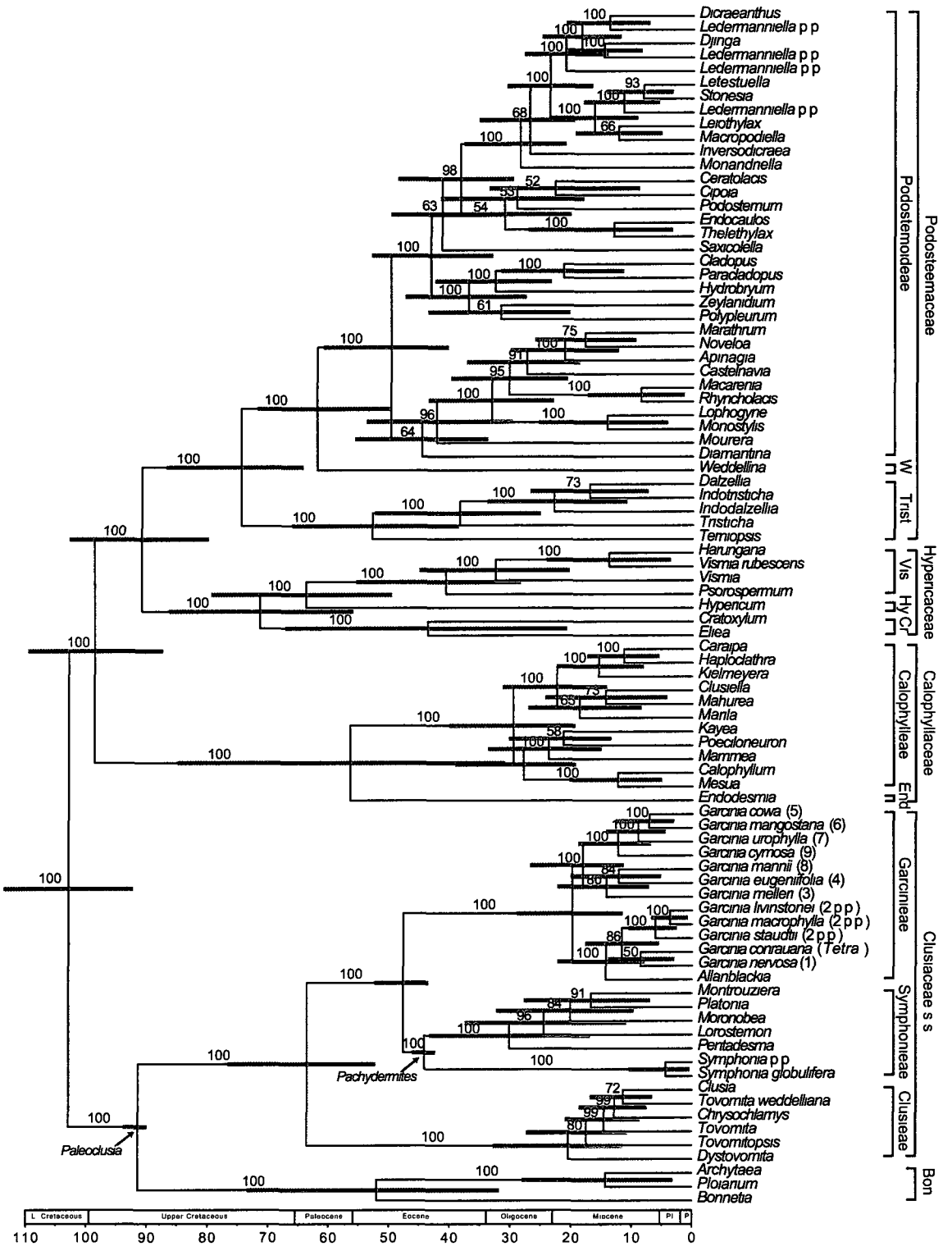

Figure 3.3 (Continued).

Fig. 3.4. Pruned phylogeny of the clusioid clade based on the analysis of a combined four-gene data set (OC placement of *Paleoclusia*; see text for details). The phylogeny and divergence times were simultaneously estimated using BEAST. Divergence time estimates were obtained by using three fossil constraints and assigning a uniform distribution to the root node between 89.9 and 125 Ma based on the youngest age possible for the *Paleoclusia* fossil and the oldest occurrence of tricolpate pollen grains representing the eudicot clade, respectively. Fossil name and arrow indicates the placement of a fossil constraint. Posterior probabilities converted to percentages are given above the branches; only nodes receiving > 50% supported are annotated. Error bars at each node represent the 95% highest posterior distributions of divergence times. Scale bar represents the major Cretaceous and Cenozoic intervals. Numbers next to terminals in Garcinieae represent clades present in Sweeney (2008). Bon. = Bonnetiaceae, Cr. = Cratoxyleae, End. = Endodesmieae, Hy. = Hypericeae, P.= Pleistocene, Pl. = Pliocene, Trist. = Tristichoideae, Vis. = Vismeae, W.= Weddellinoideae.

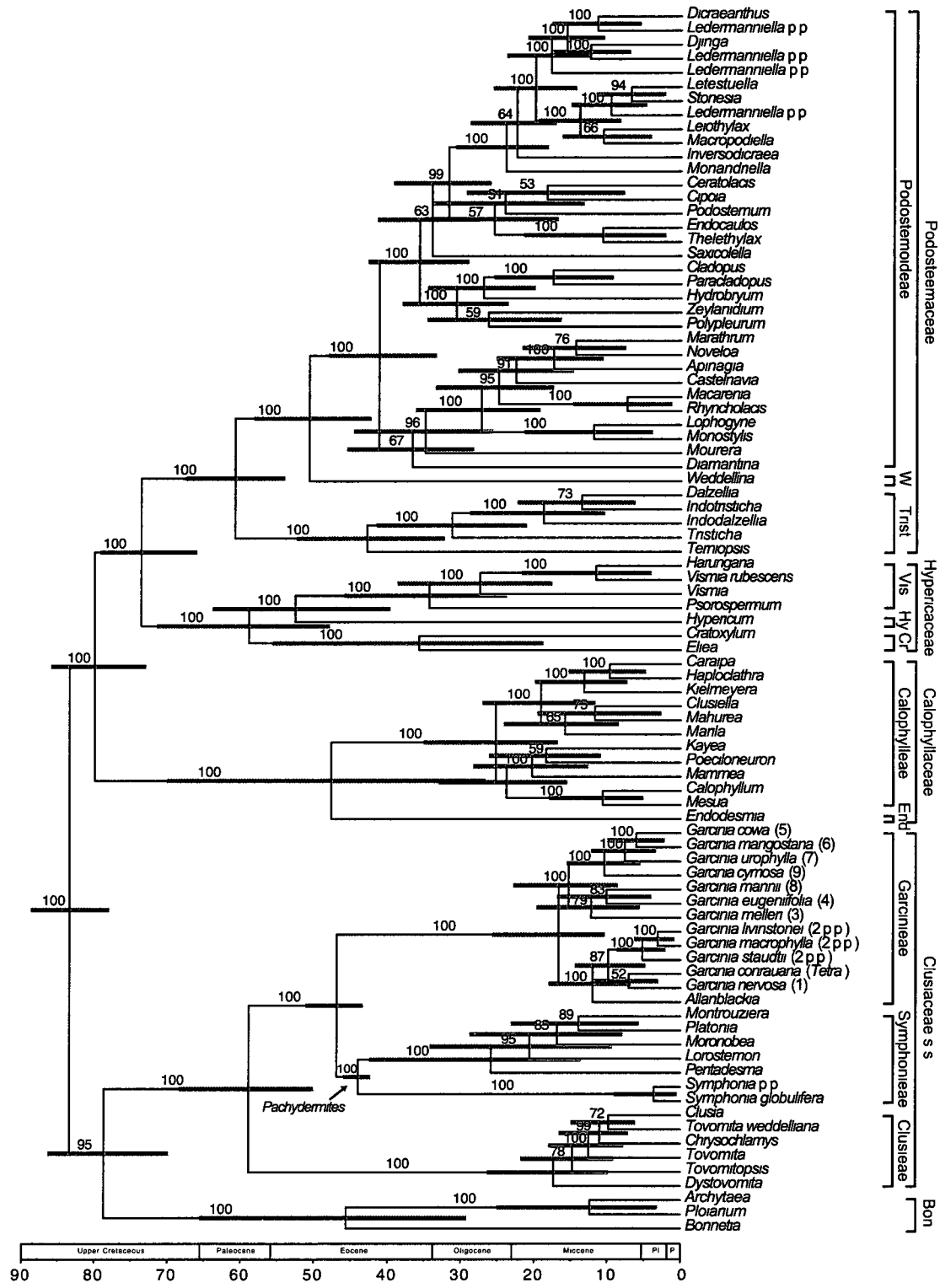

Fig. 3.4 (Continued).

Table 3.2. Crown and stem ages of the clusioid clade and its major subclades inferred using different placements of the *Paleoclusia* fossil. OC = *Paleoclusia* placed at the node between Ochnaceae s.l. and the clusioid clade. BC = *Paloeclusia* placed at the node between Bonnetiaceae and Clusiaceae s.s. Means are the average age for the clade taken from the distribution of 1000 BEAST trees. Minimum (min) and maximum (max) dates are the lower and upper values of the 95 % highest posterior density intervals of the posterior probability of distribution of node ages. No dates are listed for crown groups with only one taxon.

	Stem OC			Crown OC			Stem BC			Crown BC		
Clade	min	mean	max	min	mean	max	min	mean	max	min	mean	max
clusioids	89 8	91 7	94 4	78 0	83 4	88 7	104 2	115 3	124.3	92 3	102 9	113 7
Calo + Hyp + Podo	78 0	83 4	88 7	72 9	80 0	86 0	92 3	102 9	113 7	87 4	98 7	109 7
Bon + Clus.	78 0	83 4	88.7	69 8	78 7	86.3	92 3	102 9	113.7	89 8	91 5	93.8
Podo + Hyp	72 9	80 0	86.0	66 0	73 6	79 3	87 4	98 7	109.7	79 9	90 9	102 8
Bonnetiaceae	69 8	78 7	86.3	29 1	45 6	65 6	89.8	91 5	93 8	31 7	52 0	73.5
Calophyllaceae	72 9	80 0	86 0	26 7	47.7	70 1	87 4	98 7	109 7	30 9	56 4	85 0
Calophylleae	26 7	47 7	70 1	16 8	25 2	35 1	30 9	56 4	85 0	19 4	29 5	40 1
Endodesmieae	26 7	47 7	70 1	-	-	-	30 9	56 4	85 0	-	-	-
Clusiaceae s s	69 8	78 7	86 3	50 1	58 9	68 4	89 8	91 5	93 8	52 2	63 6	76 7
Clusieae	50 0	58 9	68 4	9 8	17 3	26 4	52 2	63 6	76 7	11 4	20 5	32 8
Garcinieae	43 3	46 9	51 1	10 3	16 7	25 7	43 4	47 6	52 3	11 5	19 8	29 0
Symphonieae	43 3	46 9	51 1	42 3	44 0	46 0	43.4	47 6	52 3	42 3	44.1	46 1
Hypericaceae	66 0	73 6	79 3	47 9	58 9	71 5	79.9	90 9	102 8	56 0	71 5	86 4
Hypericeae	39 7	52 6	63 9	20 5	30 8	40 9	49 6	63 8	79 5	26 1	37.3	52 2
Cratoxyleae	47 9	58 9	71 5	18 8	35 7	55 8	56 0	71 5	86 4	20 8	43 7	67 4
Vismieae	39 7	52 6	63 9	23.8	34 4	46 0	49 6	63 8	79 5	28 3	40 7	55 5
Podostemaceae	66 0	73 6	79 3	54 0	60 8	67 6	79 9	90 9	102 8	64 3	74 5	86.9
Podostemoideae	42 3	50 7	58.2	33 4	41 2	48.1	49 9	62 0	71 9	40 3	49 7	61 0
Tristichoideae	54 0	60 8	67 6	32 2	42 8	52 4	64 3	74 5	86 9	38 6	52 8	66 2
Weddellinoideae	42 3	50 7	58 2	-	-	-	49 9	62 0	71 9	-	-	-

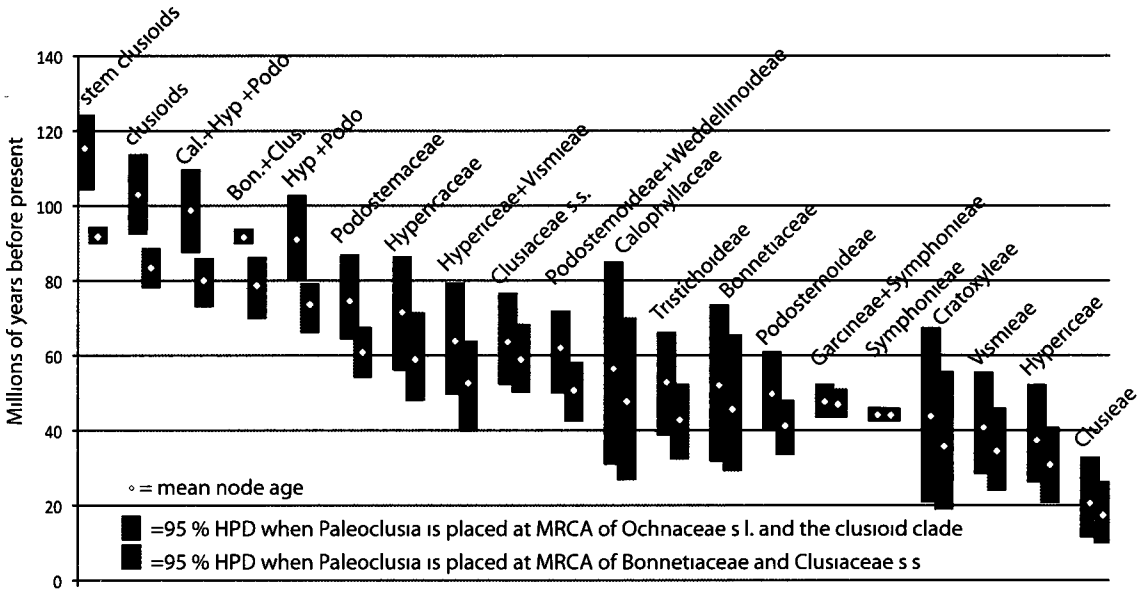

Fig. 3.5. Estimated ages of the clusioid clade and its major subclades using the two different placements of the *Paleoclusia* fossil (see text for details). Nodes deeper in the clade are less similar in age. Bon. = Bonnetiaceae, Cal. = Calophyllaceae, HPD = highest posterior density, Hyp. = Hypericaceae, MRCA= most recent common ancestor, Podo. = Podostemaceae.

their placement of *Paleoclusia* as an age constraint was different than either the BC or OC placements used in this study. Davis et al. (2005) placed the *Paleoclusia* constraint at the crown node of the clusioid clade. Second, the topology of the clusioid clade in Davis et al. was not the same as presented here: Clusiaceae s.s. were instead weakly placed as sister to the remaining clusioid families, rather than being well supported as sister to Bonnetiaceae. Third, only stem group dates are available for clusioid subclades because only four clusioid taxa were included in their sampling (no Calophyllaceae taxa were included).

Dates for two nodes in our phylogeny are younger than previously published divergence time estimations. Dick et al. (2003) estimated the divergence between the Malagasy species of *Symphonia* (Clusiaceae s.s.) and *Symphonia globulifera,* the only species of the genus occurring outside of Madagascar, to be ~28.5 Ma. Our estimates for this node are considerably younger (OC: min=0.5, mean=3.6, max=9.1; BC: min=0.4, mean=4.4, max=10.5). Similarly, Kita and Kato (2004) estimated the divergence time between *Tristicha* (Podostemaeceae) and its sister group to have occurred between 52 and 75 Ma. Our results for this node are also younger than their estimates (OC: min=21.1, mean=31.3, max=41.6; BC: min=25.2, mean=38.4, max=52.4).

Biogeographic reconstructions–Biogeographic reconstructions based on our unconstrained model were generally similar to the results of our more complex model-based reconstructions. However, several nodes in the unconstrained analyses were reconstructed as having widespread ancestors, in some cases including up to 7 areas. We consider such widespread ancestors to be unlikely and therefore will not discuss the results of the unconstrained analyses here. Instead, we will focus our discussion on the

results of the second model, which includes information on biologically feasible ranges as well as land connections and dispersal probabilities through time (Figs. 3.6 and 3.7). Importantly, however, in the unconstrained model the crown node and the first two divergence events within the clusioid clade were most frequently reconstructed as having ranges identical to those reconstructed using our second model based approach.

The inferred biogeographical history of the clusioid clade is generally similar when using the alternate placements of *Paleoclusia* and differs only in four main areas (compare Figs 3.6 and 3.7): 1) the crown Calophyllaceae node, 2) within Vismieae at the node of the most recent common ancestor of *Harungana madagascariensis* and *Vismia,* 3) the crown Podostemaceae node, and 4) five adjacent nodes at the base of primarily Old World clade of Podostemoideae. The crown node of Calophyllaceae is reconstructed as widespread in Africa + India + Eurasia (freq=32%) with the OC placement and reconstructed as Africa+India+Australia (freq=59%) with the BC placement. Within Vismieae, the difference between the two fossil placements changes the branch along which a range expansion into the neotropics is inferred. With the OC placement this event is placed deeper in the Vismieae clade, along the branch subtending the MRCA of *Harungana* and *Vismia*. With the BC placement dispersal occurs along the branch leading to the neotropical *Vismia* clade. The crown node of Podostemaceae is reconstructed as South America + Africa with the OC placement (freq= 50%) and strictly African (freq=73%) with the BC placement. Finally, several differences occur in five early diversification events in the primarily Old Word clade of Podostemoideae. The crown node of this clade is reconstructed as Eurasia + Africa (freq=36%) in the OC placement and South America + Africa (freq=48%) in the BC placement. The descendant

Fig. 3.6. Maximum likelihood ancestral area reconstruction (AAR) for the clusioid clade (BC placement of *Paleoclusia*; optimal topology from Fig. 3.3) estimated with Lagrange C++. AAR used our second model that incorporates information on biologically feasible ancestral ranges and dispersal probabilities through four stratified windows of time (model modified from Buerki et al., 2011; see text for details). Eight areas were included in the analysis: North America (NA), South America (SA), Eurasia (EA), Africa (Af), Madagascar (Md), India (In), Southeast Asia (Se), and Australia (Au); see text for full circumscriptions of areas and Appendix 3.2 for details regarding areas scored for each terminal. Letters above branches represent the most frequently reconstructed optimal ancestral range for that node summarized from 1000 randomly chosen trees from the posterior distribution of dated phylogenies; numbers to the right of nodes give the frequency of that reconstruction. Ranges preceded by "*" are reconstructed with 100% frequency. Colored triangles represent dispersal events. Scale bar represents the major Cretaceous and Cenozoic intervals. Filled boxes to the left of taxon names represent our area scoring for that taxon. Numbers next to terminals in Garcinieae represent clades present in Sweeney (2008). Bon. = Bonnetiaceae, Cr. = Cratoxyleae, End. = Endodesmieae, Hy. = Hypericeae, P. = Pleistocene, Pl. = Pliocene, Trist. = Tristichoideae, Vis. = Vismeae, W. = Weddellinoideae.

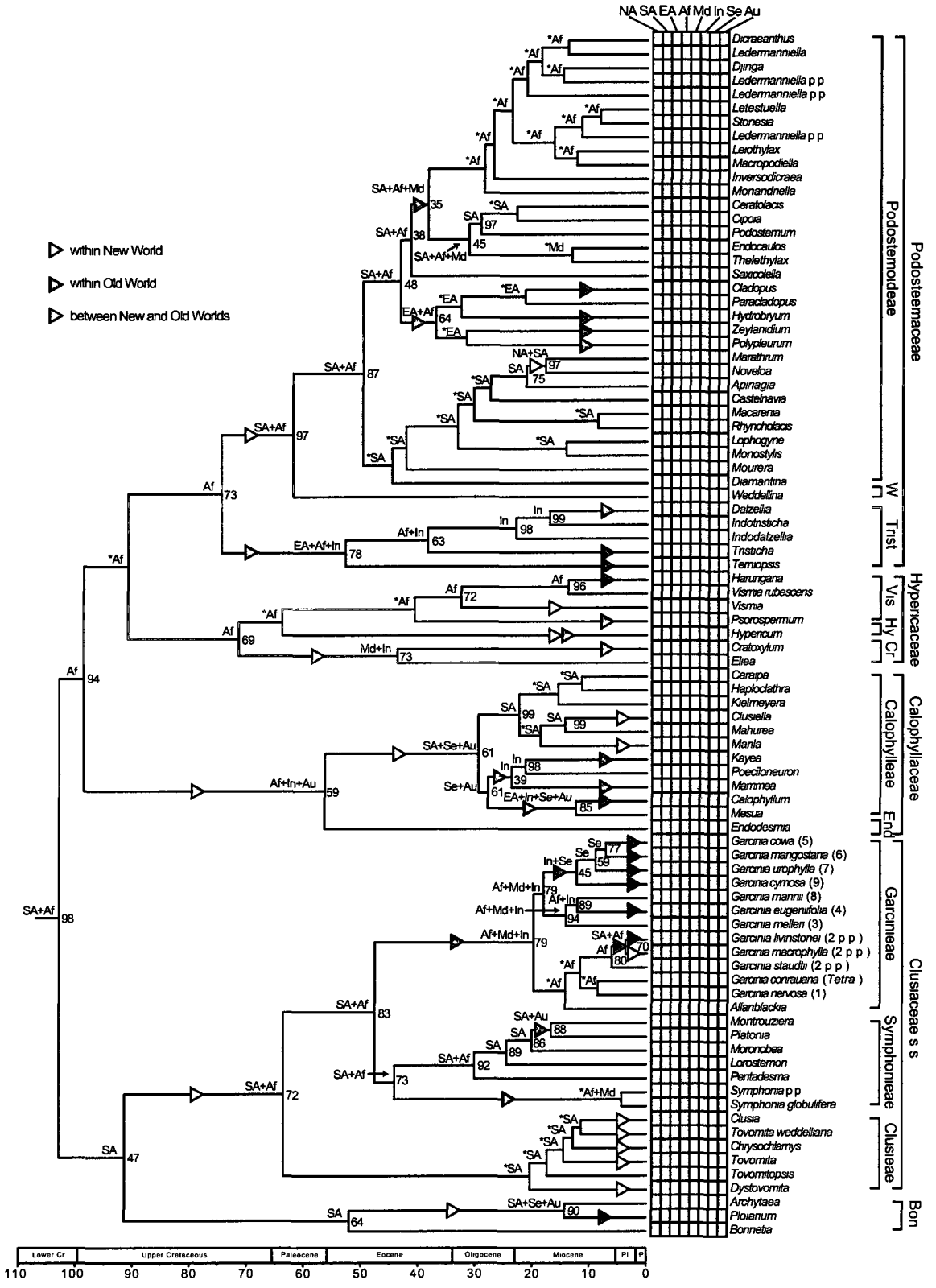

Fig. 3.6 (Continued).

Fig. 3.7. Maximum likelihood ancestral area reconstruction (AAR) for the clusioid clade (OC placement of *Paleoclusia*; optimal topology from Fig. 3.4) estimated with Lagrange C++. AAR used our model that incorporates information on biologically feasible ancestral ranges and dispersal probabilities through four stratified windows of time (model modified from Buerki et al., 2011; see text for details). Eight areas were included in the analysis: North America (NA), South America (SA), Eurasia (EA), Africa (Af), Madagascar (Md), India (In), Southeast Asia (Se), and Australia (Au); see text for full circumscriptions of areas and Appendix 3.2 for details regarding areas scored for each terminal. Letters above branches represent the most frequently reconstructed optimal ancestral range for that node summarized from 1000 randomly chosen trees from the posterior distribution of dated phylogenies; numbers to the right of nodes give the frequency of that reconstruction. Ranges preceded by "*" are reconstructed with 100% frequency. Colored triangles represent dispersal events. Scale bar represents the major Cretaceous and Cenozoic intervals. Filled boxes to the left of taxon names represent our area scoring for that taxon. Numbers next to terminals in Garcinieae represent clades present in Sweeney (2008). Bon. = Bonnetiaceae, Cr. = Cratoxyleae, End. = Endodesmieae, Hy. = Hypericeae, P. = Pleistocene, Pl. = Pliocene, Trist. = Tristichoideae, Vis. = Vismeae, W. = Weddellinoideae.

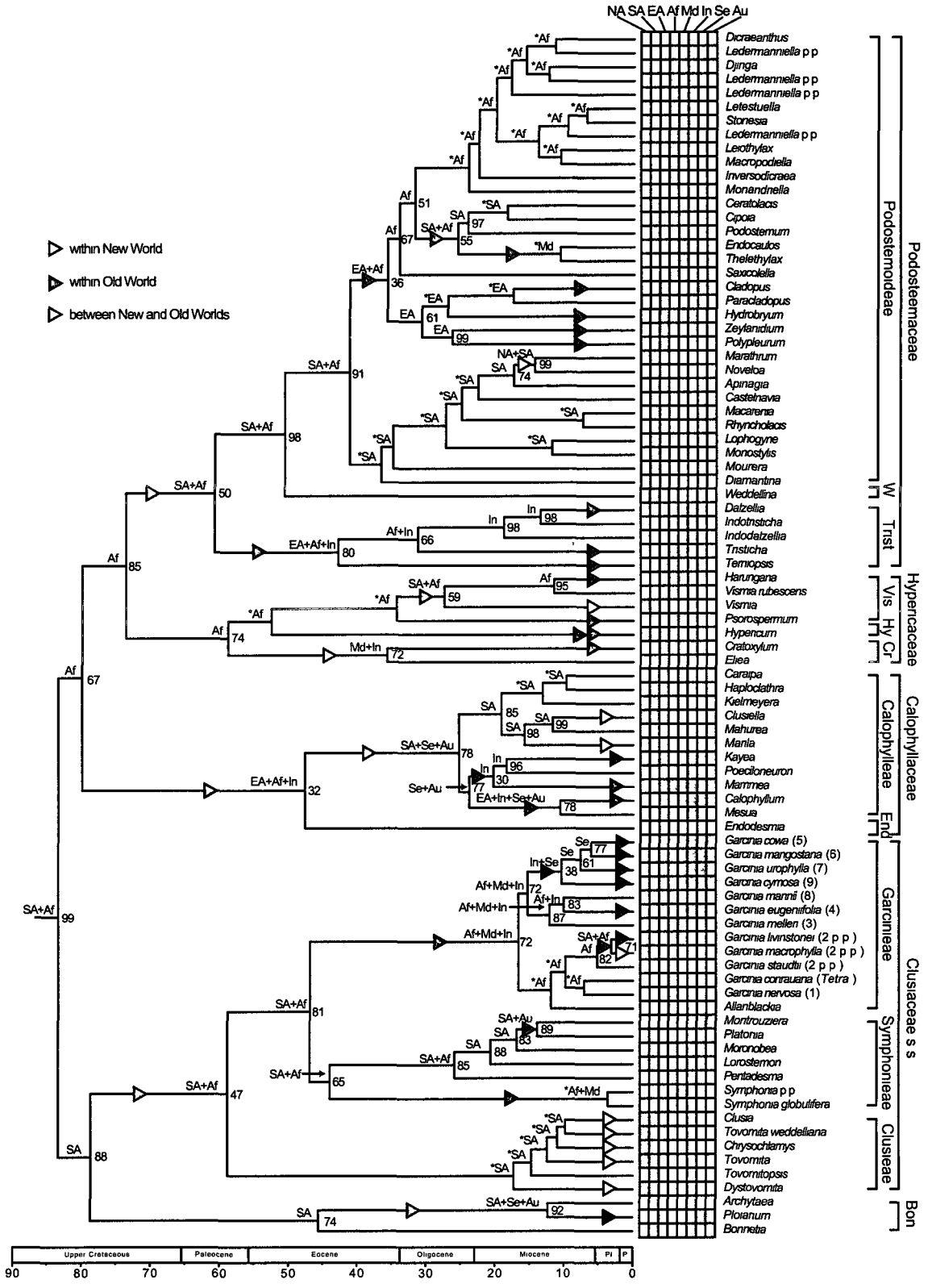

Fig. 3.7. (Continued).

nodes in each scenario also differ in their biogeographic reconstructions (compare Figs. 3.6 and 3.7).

Range reconstructions that are potentially consistent with vicariant events appear to have occurred 13 (BC placement; Fig: 3.6) or 16 times (OC placement: Fig 3.7) in the clusioid clade. Among those, area relationships of twelve putative vicariant events are consistent with the break-up of portions of Gondwana (Table 3.3). However, only the vicariance event at the crown clusioid node occurs within a widow of time when the areas involved, South America and Africa, would have been contiguous or in very close proximity (>80 Ma). Most vicariance events associated with areas of Gondwana (Table 3.3) would need to be > 20 million years older to be consistent with Gondwanan vicariance.

Forty-eight and 50 dispersal events are inferred using the BC and OC placements of *Paleoclusia*, respectively. This indicates that a dispersal event takes place along one in every four branches (~28%). The majority of these range expansion events occur well after the break-up of Gondwana, even when considering the 95% HPDs. In the BC analyses 38 of 48 (~79%) inferred dispersal events occur after the beginning of the Eocene (55.8 Ma) and 24 of those (~51%) since the beginning of the Oligocene (33.9 Ma). In the OC analyses 41 out of 50 (82%) occurred since the beginning of the Eocene and 28 of those (56%) since the beginning of the Oligocene. Furthermore, our analyses indicate that the distribution across former Gondwanan landmasses involves at least 20 dispersal events between these areas.

The crown node of the clusioid clade is inferred to have a range of Africa + South America (OC freq=99%; BC freq=98%). This node appears to have experienced a

Table 3.3. Nodes with area relationships consistent with vicariance events associated with the separation of Gondwanan landmasses The most recent common ancestors (MRCA) of the node of interest are listed Parent node ranges listed split into two daughter node ranges, eg , SA+Af splits into SA and Af or SA+Se+Au splits into SA and Se+Au See Figs 3 6 and 3 7 for details a '-' indicates not present or inapplicable, t = a terminal

			Biogeographic scenario				Frequency		Support (PP)		BC placement parent node age			OC placement parent node age		
node	MRCA	clade	fossil placement	parent node range	daughter node 1 range	daughter node 2 range	BC	OC	BC	OC	min	mean	max	min	mean	max
1	*Dicrantheus* and *Bonnetia*	crown clusioids	both	SA+AF	Af	SA	98/94/47	99/67/88	100/100/100	100/100/95	92 3	102 9	113 7	78 0	83 4	88 7
2	*Pentadesma* and *Lorostemon*	Symphonieae	both	SA+Af	SA	Af	92/89/t	85/88/t	100	100/95/t	16 9	30 1	43 3	13 6	25 9	42 5
3	*Garcinia macrophylla* and *G livinstonei*	Garcinieae	both	SA+Af	Af+Md	NA+SA	70/t/t	71/t/t	100	100/t/t	0 7	3 7	6 8	0 8	3 2	6 3
4	*Harungana* and *Vismia macrophylla*	Vismeae	OC	SA+Af	Af	NA+SA	-	59/95/t	100/100/t	100/100/t	20 3	32 5	45 0	17 6	27 4	38 7
5	*Diamantina* and *Dicrantheus*	Podostemoideae	OC	SA+Af	EA+Af	SA	87/-/100	91/36/100	100/100/64	100/100/67	40 3	49 7	61 0	33 4	41 2	48 1
6a	*Endocaulos* and *Ceratolacis*	Podostemoideae	BC	SA+AF+Md	SA	Md	45/97/100	-/97/100	54/53/100	57/51/100	20 2	31 2	43 5	16 8	25 5	35 2
6b	*Endocaulos* and *Ceratolacis*	Podostemoideae	OC	SA+Af	SA	Md	-/97/100	55/97/100	54/53/100	57/51/100	20 2	31 2	43 5	16 8	25 5	35 2
7	*Archytaea* and *Ploiarium*	Bonnetiaceae	both	SA+Se+Au	SA	EA+Se+Au	90/t/t	92/t/t	100	100/t/t	3 1	14 3	28 0	3 1	12 3	25 0
8	*Montrouziera* and *Platonia*	Symphonieae	both	SA+Au	Au	SA	88/t/t	89/t/t	91/t/t	89/t/t	6 9	16 7	27 8	5 7	13 9	23 1

Table 3.3 (Continued)

			Biogeographic scenario				Frequency		Support (PP)		BC placement parent node age			OC placement parent node age		
node	MRCA	clade	fossil placement	parent node range	daughter node 1 range	daughter node 2 range	BC	OC	BC	OC	min	mean	max	min	mean	max
9	*Mesua* and *Caraipa*	Calophylleae	both	SA+Se+Au	SA	Se+Au	61/99/61	78/85/77	100/100/-	100/100/-	19 4	29 5	40 1	16 8	25 3	35 1
10	*Symphonia globulifera* and *S fasciculata*	Symphonieae	both	Af+Md	Md	Af	100/t/t	100/t/t	100	100/t/t	0 4	4 4	10 5	0 5	3 6	9 1
11	*Endodesmia* and *Caraipa*	Calophyllaceae	BC	Af+In+Au	SA+Se+Au	Af	59/61/t	-/78/t	100/100/t	100/100/t	30 9	56 4	85 0	26 7	47 7	70 1
12	*Cratoxylum* and *Eliea*	Cratoxyleae	both	Md+In	EA+In+Se	Md	73/t/t	72/t/t	100/t/t	100/t/t	20 8	43 7	67 3	18 8	35 7	55 8

vicariance event with the Bonnetiaceae + Clusiaceae s.s. clade inheriting a South American range, and the Calophyllaceae + Hypericaceae + Podostemaceae clade inheriting an African range. Thus, stem group Bonnetiaceae and Clusiaceae s.s. are inferred as having a South American origin while stem groups of the remaining three families are inferred to have arisen in Africa. Crown group ancestral ranges for each family are as follows: Bonnetiaceae, South America (OC freq=74%; BC freq=64%); Clusiaceae s.s., South America + Africa (OC freq=47%; BC freq=72%); in Calophyllaceae, reconstructions differ between the placements of *Paleoclusia*, (Eurasia + Africa + India, OC freq=32%; Africa + India + Australia, BC freq=59%); Hypericaceae, Africa (OC freq=74%; BC freq=69%); and in Podostemaceae, reconstructions also differ (South America + Africa, OC freq=50%; Africa, BC freq=73%).

DISCUSSION

Wegener (1915) introduced the theory of continental drift in the early 20th century and nearly 50 years later this idea began to be widely accepted by the scientific community. In a seminal paper, Raven and Axelrod (1974), incorporated this new understanding of the Earth's geological history in an attempt to understand the distributions of angiosperms. With this publication they essentially provided a null model for the biogeographical studies of organisms. For instance, if a clade of plants is distributed on Africa and South America, it is most parsimonious to invoke that they originated before the separation of these two landmasses and achieved their distribution through vicariance rather than dispersal. With the advent of divergence time estimation from molecular data (Zuckerkandl and Pauling, 1965) and its continued development

(reviewed in Rutschmann, 2006) it is possible to test these biogeographic hypotheses (Crisp et al., 2011). As mentioned in the Introduction, it appears that most angiosperm clades are far too young for their distributions to have been influenced strictly by Gondwanan vicariance. Instead, it seems that dispersal, sometimes combined with initial Gondwanan vicariance, is a more likely explanation for many Gondwanan distributions in angiosperms such as those observed in the clusioid clade.

Ancient Gondwanan vicariance in the clusioid clade–We identified one new example of putative Gondwanan vicariance in the ancient, pantropical clusioid clade. In all analyses the crown node of the clusioid clade is well supported as having undergone a vicariance event associated with the splitting of Africa and South America. After divergence at the crown node, the Calophyllaceae + Hypericaceae + Podostemaceae ancestral lineage inherited an African range and the Bonnetiaceae + Clusiaceae s.s. lineage inherited a South American range (Figs. 3.6 and 3.7). Using either placement of *Paleoclusia* as an age constraint, the 95% HPD for the crown node of the clusioid clade overlaps with the time when Africa and South America were still connected or were in close proximity to one another (>80 Ma). Using the OC placement (Fig. 3.4; Table 3.2) the crown clusioid node is 83.4 Ma (min= 78.0, max=88.7), while with the BC placement (Fig. 3.3; Table 3.2), the estimated age for this node is older, 102.9 Ma (min=92.3, max=113.7). All other cladogenic events involving Gondwanan land masses are far too young (Table 3.3) to be attributable to strict vicariance: in most cases age estimates would need to be tens of millions of years older to be consistent with Gondwanan vicariance. This suggests that as biogeographic studies are expanded to examine even

more inclusive (and thus older) clades, such as Malpighiales, it is possible that vicariance events associated with the break-up of Gondwana will be much more common.

Determining the relationship of *Paleoclusia* to other clusioids is especially important for understanding the biogeographic history of the clade. The current understanding of the relationship of *Paleoclusia* to other clusioids is somewhat uncertain. At the time of deposition the *Paleoclusia* fossil locality in New Jersey, USA, was in Southern Laurasia in a subtropical to tropical environment (Crepet and Nixon, 1998 and references therein). However, most extant members of the clusioid clade are now found only in similar environments in more southern regions that are mostly on former Gondwanan fragments (Fig. 3.1). None of the early diverging lineages in our ancestral range reconstructions include North America, at least in part because *Paleoclusia* was not included in our ancestral area reconstructions. If the more nested BC placement is correct, the occurrence of *Paleoclusia* in southern Laurasia may be indicative of a broader historical clusioid distribution including South America, Africa, and regions of Laurasia (e.g., North America). If *Paleoclusia* is a member of the stem clusioid lineage (OC placement), much more would need to be known about the ancestral distribution of the clusioid sister group, Ochnaceae s.l., to better interpret the presence of the clusioid fossil in Laurasia.

Other vicariance events of interest– We also detect several putative vicariance events that are likely associated with the changing availability of dispersal routes for tropical angiosperms during the late Cretaceous and Paleogene. The first occurs at the crown Cratoxyleae node (Figs. 3.6 and 3.7). The crown node of Cratoxyleae is inferred as having an ancestral range of Madagascar + India. *Eliea* inherited a range of Madagascar

and *Cratoxylum* inherited an Indian range with subsequent expansion into Eurasia and Southeast Asia. Separation between Madagascar and India occurred ~84-95 Ma (McLoughlin, 2001) and using either the OC or BC placements the crown Cratoxyleae node occurs well after this split (BC 95% HPD 20.8-67.3 Ma; OC: 95% HPD 18.8-55.8 Ma). This apparent vicariance event is thus not likely to be associated with strict vicariance between these two landmasses. However, a more recent connection between Madagascar and India could have been maintained via the Seychelles block and other islands spanning these two landmasses (Ali and Aitchison, 2008). This cladogenic event thus may have occurred when these intervening areas became submerged during the Paleocene and Eocene and gene flow ceased as India moved away from Madagascar on its northward passage to Eurasia (Ali and Aitchison, 2008). Our area scoring of *Cratoxylum* may effect our reconstruction of ancestral ranges in Cratoxyleae. This genus is primarily distributed in areas defined here as Eurasia and Southeast Asia (Gogelein, 1967) and reaches India only at the northwestern extent of its range. Further work on the biogeographic history of this genus is needed to determine its ancestral range. If Indian populations are nested within the clade, it may be inappropriate to score *Cratoxylum* as ancestrally being present in India as we have done here.

Three vicariance events occur at roughly comparable times between similar areas and are suggestive of a common pattern (Figs. 3.6 and 3.7). In two cases, vicariance involves South America and Australia + Southeast Asia, and in the third, South America and Australia (New Caledonia). The nodes at which these putative vicariance events occur are: 1) crown Calophylleae, 2) the MRCA of *Motrouziera* + *Platonia* (Symphonieae), and 3) the MRCA of *Archytaea* + *Ploiarium* (Bonnetiaceae). The 95%

HPDs of these three nodes overlap considerably in the Oligocene and Miocene, suggesting the possibility that they occurred within a similar window of time. Phylogenetic relationships in the first and third examples are strongly supported here except for the node of the MRCA of *Mesua* and *Kayea* (<50 PP; Calophylleae). This node, however, is also supported by combined analysis of morphological and molecular data (Ruhfel et al., unpublished). The sister group relationship of *Montrouziera* and *Platonia,* however, is not well supported (< 95 PP). Regardless, the New Caledonian clade, *Montrouziera,* is embedded in a clade with strictly South American taxa, and is also morphologically very similar to the South American taxa (Stevens, 2007a; Ruhfel et al., unpublished). Therefore, regardless of support values in this part of the phylogeny its seems likely that the vicariance scenario inferred here is stable. Range reconstructions for these scenarios are also well supported; all reconstructed ranges receive a frequency of > 61%.

The timing of these disjunctions, however, is unlikely due to Gondwanan vicariance. Although South America and Australia were connected through Antarctica until ~30-35 Ma, the last time that this route was likely available to tropical taxa was during the Paleocene-Eocene Thermal Maximum (PETM, ~56 Ma; Morley, 2003; Pennington and Dick, 2004). The 95% HPD for all three nodes of interest are outside of this timeframe. It may be possible that these distributions were attained via connections involving a tropical belt of vegetation in the Northern hemisphere (Wolfe, 1975; Tiffney, 1985; Lavin and Luckow, 1993; Davis et al., 2002). Cooling events in the Oligocene (Zachos et al., 2001) may have resulted in a retreat into southern areas now occupied by

these taxa. This scenario would imply that tropical connections involving the Northern hemisphere were maintained, perhaps more sporadically, after the PETM.

A third instance of vicariance caused by the changing positions of fragments of Gondwana is dependent on the placement of *Paleoclusia* as an age constraint. This event occurs at the crown node of Calophyllaceae, but only with the more nested placement of *Paleoclusia* (BC; Fig. 3.6). This example involves an ancestral range of Africa + India + Australia (freq=59%) where one daughter lineage, *Endodesmia* (Endodesmieae, including *Lebrunia* [African, not sampled]), inherits an African range, while the other inherits a range of South America + South East Asia + Australia. Using the OC placement, the ancestral range reconstruction of crown Calophyllaceae differs: the range is reconstructed as Eurasia + Africa + India, with a lower frequency (freq= 32%); the daughter lineages are reconstructed as having the same ranges as in the BC placement. We interpret the vicariance event reconstructed using the BC placement at the crown Calophyllaceae node as the ancestral range being split into Africa + India and Australia based on our understanding of changing area relationships through time. Thus, the range along the branch leading to Endodesmieae would have been India + Africa, with extinction occurring in India along that branch. The Australian lineage (Calophylleae) would have subsequently dispersed into South America and South East Asia along the branch leading to crown Calophylleae (dispersal events are considered separately below). In terms of age reconstructions, this vicariance scenario is possible because the branch leading to crown Calophyllaceae occurs during a time when dispersal between Africa, India, Madagascar, and Australia was perhaps possible via the Kerguelen Plateau (KP) which connected these land masses until ~80 Ma (McLoughlin, 2001; Sanmartín and

Ronquist, 2004). After the plateau was submerged and India began to move northward, this could have broken the connection between Africa and Australia resulting in the inferred vicariant event. Some authors however, dispute the availability of migration by way of the KP (Ali and Aitchison, 2009).

Gondwanan distributions in the clusioid clade are largely the result of post-Eocene dispersal– Rates of dispersal are astonishingly high in the clusioid clade. Forty-eight or 50 dispersal events are inferred depending on the placement of *Paleoclusia*. This translates into a dispersal event along > 25% of the branches. Moreover, most of these dispersals (>75%) are recent and have occurred since the beginning of the Eocene (~56 Ma) and the majority (>50%) since the beginning of the Oligocene (~34 Ma). These results support the growing body of literature (Chanderbali et al., 2001; Renner et al., 2001; Davis et al., 2002; Davis et al., 2004; Zerega et al., 2005; Couvreur et al., 2010) that invokes dispersal, rather than vicariance, as a major explanatory factor for the distribution of many pantropical taxa.

Changing land configurations and accompanying climatic changes since the origin of the clusioid clade likely presented new opportunities for movement between areas during some windows of time but not others. Along these lines we detect patterns in plant disjunctions involving similar areas in distantly related clades suggesting common dispersal pathways to achieving these distributions. Highly relevant to this discussion is our knowledge of the dispersal biology of these plants. Dispersal occurs by various means within the clusioid clade. For instance, wind and water dispersed seeds are common in Calophyllaceae, Hypericaceae, and Podostemaceae (Cook and Rutishauser, 2007; Stevens, 2007a, b) and dispersal by birds and mammals occurs in Calophyllaceae,

Clusiaceae s.s., Hypericaceae, and possibly Podostemaceae (Cook and Rutishauser, 2007; Stevens, 2007a, b). Oceanic fruit dispersal is likely in some widespread taxa (e.g., *Calophyllum* and *Mammea*; Stevens, 2007a), but in *Symphonia* vegetative propagules are deemed more likely for marine dispersal as its seeds are not likely to be salt water tolerant (Dick et al., 2003). Vegetative propagules may also be dispersed by water in clonal species of Podostemaceae (Philbrick and Novelo, 2004) but it is unlikely that these freshwater plants could survive extened periods of salt water exposure. In general, it may be that taxa with limited dispersal capabilities are more likely to disperse over water only over small distances and that vegetative propagules, or floating mats of vegetation, are responsible for long-distance oceanic dispersal events as has been suggested in *Symphonia* (Dick et al., 2003). However, it should be kept in mind that the probability of very unlikely long-distance dispersal events increases over long periods of time making even the most unlikely events possible (Simpson, 1952).

We divide our discussion of dispersal patterns in the clusioid clade into three broad categories: i) dispersal between the Old and New Worlds, ii) dispersal within the New World, and iii) dispersal within the Old World. For simplicity, we will focus on the mean ages inferred in our divergence time estimates for dispersal events.

Dispersal between the Old and New Worlds–Eight or nine dispersal events between the New and Old Worlds were inferred based on the alternate placements of *Paleoclusia*. In all but one instance, these events appear to be limited to dispersal between Africa and the New World or Australia (or Australia + Southeast Asia) and South America. Dispersal from South America to Africa is inferred only once, but dispersal from Africa to the New World occurred at least four times. The South America

to Africa dispersal event occurred during the Upper Cretaceous or Paleocene (~90-60 Ma) along the branch leading to crown Clusiaceae s.s. (Figs. 3.6 and 3.7). If this event occurred early along the branch it might have occurred via direct overland dispersal or by an island-hopping event because South America and Africa were still in very close proximity at this time (Morley, 2003). However, if dispersal occurred more recently during the Paleocene this would more likely be attributed to either a long distance dispersal event over the Atlantic Ocean or an overland boreotropical route through the Northern Hemisphere. A dispersal event in the opposite direction (Africa to South America) occurs during a similar, though slightly younger, window of time in Podostemaceae. The branch in Podostemaceae along which this event occurs however, depends on the placement of *Paleoclusia*. In this case mean age estimates place this dispersal event outside the period of time when South America and Africa were in close proximity (>80 Ma). It is perhaps more likely that overland dispersal via the Northern Hemisphere occurred during this window of time. Dispersal between Africa and the New World is present in three other areas of the topology. These events occur along the branch leading to *Hypericum* and within Vismieae and Garcineae. *Hypericum* appears to have originated in Africa and dispersed outward from there. This could have occurred as early as the Paleocene (~64 Ma). The branch along which dispersal from Africa into the New World occurs in Vismieae differs with the placement of *Paleoclusia*. However, in both cases it is inferred to have occurred no earlier than the late Eocene. The earlier windows of time during which dispersal may have occurred in Vismieae are potentially consistent with migration via Laurasia. Finally, there seems to have been a more recent dispersal event from Africa into South America in the *Garcinia* clade (Figs. 3.6 and 3.7, *Garcinia*

macrophylla terminal). This occurred since the latest Miocene and was likely during a time when migration via Laurasia may not have been available for the movement of tropical taxa.

Dispersal from South America into Australia or Australia + Southeast Asia seems to have occurred two times, once each in Bonnetiaceae and Symphonieae (Clusiaceae s.s.). The branch along which dispersal occurred in Bonnetiaceae is quite long, spanning over 30 million years, and thus dispersal could have occurred anytime between the early Eocene and the mid Miocene. If dispersal occurred early along this branch it could have been via Antarctica or the North Atlantic Land Bridge as these routes may have been available to tropical elements during the PETM (Morley, 2003; Pennington and Dick, 2004). If this occurred more recently, this must either have occurred through the northern hemisphere or via long distance dispersal across the southern oceans, perhaps facilitated by the Antarctic Circumpolar Current (Sanmartin et al., 2007). Dispersal from South America to New Caledonia in Symphonieae is too recent for dispersal via direct land connections between Antarctica and Australia (28-32 Ma; McLoughlin, 2001)—paleoclimates were not likely conducive for this migration during this time. This leaves open the possibility of a migration route through Laurasia or as a result of long distance dispersal across the Pacific. The latter has been suggested by Heads (2010).

In two of these events between the Old and New Worlds, (along the branch leading to crown Calophylleae and along the branch leading to *Ceratolacis*, *Cipoia*, *Endocaulos*, and *Thelethylax* [Podostemaceae]), the scenarios between the OC and BC analyses differ considerably. Due to this uncertainty we will not discuss these further.

Dispersal within the New World–Dispersal events are unidirectional from South America into North America for tropical clusioid taxa. This pattern is broadly consistent with numerous tropical plant groups (Burnham and Graham, 1999). The Isthmus of Panama was completely formed approximately three million years ago and has long been understood to be an important event for the interchange of biota between North and South America (Burnham and Graham, 1999; Cody et al., 2010). Dispersal prior to the closing of this land bridge has been observed for many taxa (Burnham and Graham, 1999), but appears to be more prevalent for plants than animals (Cody et al., 2010). We infer eight or nine independent events reflecting this south-to-north dispersal pattern, most commonly in Calophyllaceae, Clusiaceae s.s., and Podostemaceae. These dispersal events are all inferred to have occurred recently since the Miocene. In two instances we were able to infer more specific dates of these dispersal events. In these cases, one event appears to have occurred after the closing of the land bridge and the other is older and most likely occurred prior to its formation. In Garcineae (*G. macrophylla* clade) dispersal into North America seems to have occurred <3.7 Ma, which is consistent with dispersal across the Isthmus of Panama. In Podostemoideae (along the branch to *Marathrum* and *Noveloa*), dispersal was inferred during the Miocene (> 7 Ma), prior the formation of the Isthmus. Finally, this south-to-north pattern is further evident in seven additional instances involving *Clusiella* and *Marila* (Calophyllaceae) and in all genera of Clusieae except *Tovomitopsis*. In these cases the timeline for these events is likely not much older than the Miocene (~23 Ma), but could be much younger. Species level phylogenies are needed to determine a more precise timing of the dispersal events in these clades.

Similar south-to-north dispersal events may have also happened in other clusioid subclades, but are not clearly captured in our analyses. This may be the case in Vismieae, along the branch leading to the clade of neotropical *Vismia*. However, reconstructions differ between the BC and OC analyses and only a species level phylogeny of the group will help to clarify this issue. This pattern is also suggested by the nested placement of North American or Caribbean taxa in South American clades in molecular studies of *Bonnetia* (Bonnetiaceae; Ruhfel et al., 2011), and *Podostemum* and *Tristicha* (Podostemaceae; Moline et al., 2006). Molecular studies of *Symphonia globulifera* (Clusiaceae s.s.) additionally suggest evidence for dispersal between South America and North America before the formation of the Isthmus of Panama (Dick et al., 2003; Dick and Heuertz, 2008). These events in *Bonnetia*, *Podostemum*, *Symphonia,* and *Tristicha*, however, are not evident in our range reconstructions because the ancestral ranges of these taxa are known and are thus not scored as present in North America here (see Appendix 3.2 for details). The temperate and tropical montane genus *Hypericum* is likely an exception to this south-to-north pattern. Preliminary molecular results suggest that *Hypericum* entered South America from North America (Nürk et al., 2010). This must have occurred after the origination of crown group Hypericeae, which according to our estimates happened some time between 20 and 52 Ma (Table 3.2).

Dispersal within the Old World–The biogeographical history of the clusioid clade in the Old World is complex and involves numerous dispersal events among adjacent areas during very different windows of time. This is likely the result of an equally complex geological history in the Old World, particularly in the region of tropical Southeast Asia (Hall, 1998), which includes areas scored here as Australia, Eurasia,

India, and Southeast Asia. Inferred dispersal events between these regions of the Old World do occur between the Upper Cretaceous and Eocene (~100-34 Ma), but these are few and mostly involve movements out of Africa into Eurasia, India, and Madagascar in the early diverging lineages of the Calophyllaceae + Hypericaceae + Podostemaceae clade. Most dispersal events, however, occur after the Eocene-Oligocene boundary (33.9 Ma) and primarily involve movements out of India, Southeast Asia, and Eurasia (likely tropical continental South East Asia) into the areas immediately adjacent to each of these regions. During this period continental Southeast Asia and Sundaland experienced wide fluctuations in sea level and rain forest cover (Hall, 1998; Morley, 2007) that may have affected the dispersal pathways between these areas. Particularly, rainforest cover was severely reduced between 35 and 20 Ma, yet before and after this event, extended periods of suitable rainforest climate existed (Morley, 2007). Movements from India into adjacent areas of Eurasia, Southeast Asia and Australia occur in *Cratoxylum* (Hypericacae), *Dalzelia* (Podostemaceae), *Kayea* and *Mammea* (Calophyllaceae), and Garcinieae (Clusiaceae s.s.). Most recent literature describes the collision of India with Eurasia as having occurred between 50 and 55 million years ago, but recent evidence suggests that India collided with Asia much more recently, around ~35 Ma (Ali and Aitchison, 2008; and references therein). Our inferred dispersal events out of India are most consistent with overland dispersal routes becoming available during this later time. In contrast to these other clades, the dispersal out of India into Eurasia and Southeast Asia in *Cratoxylum*, is earlier and could have occurred prior to the Eocene-Oligocene boundary. At this time India may not yet have collided with continental Asia, and India and Southeast Asia were at similar latitudes. It is possible that *Cratoxylum* dispersed into

Southeast Asia at this time via over-water dispersal. This pattern is evident in the pollen fossil record where many pollen types found in India begin to appear in Southeast Asia at this time (Morley, 2003). Furthermore, the *Dalzelia* and *Cratoxylum* examples are consistent with scenarios of origination in Africa and reaching Eurasia by rafting on the Indian plate. A similar pattern is found in Crypteroniaceae (Rutschmann et al., 2004) and in several other plant and animal clades (reviewed in Datta-Roy and Karanth, 2009). Movement out of Southeast Asia and Eurasia into nearby areas during this time were also likely influenced by the changing land configurations and climate mentioned above. Clades with an ancestral range in Southeast Asia or Southeast Asia +Australia that disperse into nearby areas within the region include *Ploiarium* (Bonnetiaceae), a subclade of Garcinieae, and the primarily Old World clade of Calophylleae. Origination in Eurasia, most likely what is now continental tropical Southeast Asia, with subsequent movement into nearby areas occurs in the Podostemoideae subclade containing *Cladopus*, *Paracladopus*, *Hydrobryum*, *Zeylanidium*, and *Polypluerum*. Dispersal in this clade seems to have been predominantly from Eurasia into India, with three repeated events, and once from Eurasia into Southeast Asia. Similar "into-India" dispersal during this same time period has been invoked for amphibians (Van Bocxlaer et al., 2009).

The final repeated pattern of dispersal detected in our results is movement into, but not out of, Madagascar. This pattern occurs at least nine times: once in *Symphonia* (Clusiaceae s.s.), at least three times each in Garinieae (Clusiaceae s.s.) and Hypericaceae, and once each in Podostemoideae and Tristichoideae (Podostemaceae). These events appear to originate mostly in Africa, but dispersal into Madagascar from Southeast Asia (*Garcinieae*) or India (*Mammea*) is also inferred. *Calophyllum* also

dispersed into Madagascar, but the area of origin is unclear; the ancestor of the genus is inferred as being widespread in the Old World. Dispersal into Madagascar does not seem to be clustered in time. For example in Cratoxyleae, dispersal into Madagascar could have occurred as early as the Upper Cretaceous (71.5 Ma; BC placement). In contrast, dispersal into Madagascar in Garcinieae appears to have occurred at least twice in the last 10 million years. These results are consistent with a recent literature review (Yoder and Nowak, 2006 and references therein) which found that most Malagasy clades dispersed from Africa to Madagascar sometime during the Cenozoic. These dispersal events do not seem unrealistic for many plant groups because Madagascar is relatively close to Africa (~400 km). Furthermore, there is evidence of a land bridge between Africa and Madagascar that was present in the mid-Eocene and early Miocene that may have facilitated dispersal between these areas (McCall, 1997), but the availability of these land connections has been contested (Yoder and Nowak, 2006).

Conclusions and future directions– We present here one likely case of Gondwanan vicariance at that origin of the clusioid clade that occurred when South America and Africa were in close proximity to one another. However, our results overwhelmingly suggest that dispersal, not vicariance, is largely responsible for the pantropical distribution of the clusioids. These results further suggest that dispersal has likely played a major ongoing role in the assembly, maintenance, and distribution of tropical diversity since the Upper Cretaceous (Pennington and Dick, 2004). In general, dispersal in the clusioid clade between the Old and New Worlds and within the Old World has occurred throughout this time. Dispersal events within the New World, mostly

from South America to North America, and between areas of tropical Southeast Asia seem to occur mostly after the Oligocene-Miocene boundary.

Future work on the biogeography of the clusioid clade should concentrate on two main topics: fossils and phylogenies. The placement of *Paleoclusia* has an obvious effect on both divergence time estimates for the clusioid clade as well as biogeographic reconstructions. Given the material available for *Paleoclusia* it is unlikely that a more precise placement for this fossil will be obtained. However, if vegetative material of *Paleoclusia* is discovered, this may greatly improve our knowledge of its phylogenetic placement: vegetative characters appear to be important for placing clusioid taxa scored only for morphology (see Ruhfel et al., unpublished). Furthermore, reliable fossils are presently lacking for several clusioid subclades, including Calophyllaceae, Hypericaceae, and Podostemaceae. This is problematic because it is known that nodes furthest from fossil calibration points are more difficult to estimate (Linder et al., 2005). Particularly useful would be fossils that could be placed within or at least near to Podostemaceae. This group of plants seems to have an exceptionally elevated rate of molecular evolution (Davis et al., 2007) that may confound age estimates (Smith and Donoghue, 2008).

In addition, more detailed and well-supported phylogenies are needed for several genera, tribes and subfamilies in the clusioid clade. Increased phylogenetic resolution within these clades would be particularly helpful in elucidating the biogeographic history of the clusioids. These include especially: *Calophyllum*, Clusieae, Garcinieae, *Hypericum*, *Mammea*, Podostemoideae, and Vismieae. Well-sampled and resolved phylogenies for these clades will avoid the complications of scoring widespread terminal

taxa, and allow a more informed view of the timing and location of biogeographic events in the clusioid clade.

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CHAPTER 3 APPENDICES

Appendix 3.1. Voucher information and GenBank accession numbers for sequences used in this study. New data have GenBank numbers beginning with JF (JF828242-JF828273), and accessions in brackets are from a different voucher source. A dash (—) indicates that the sequence was unavailable. Herbaria acronyms follow Thiers (continuously updated). **FAMILY. Species,** *voucher* (herbarium), GenBank accessions: *matK*, *ndhF*, *rbcL*, *matR*.

BONNETIACEAE. ***Archytaea triflora*** Mart., *Kubitzki & Feuerer 97-26* (HBG), HQ331545, AY425029, AY380342, AY674475; ***Bonnetia ahogadoi*** (Steyer.) A.L. Weitzman & P.F. Stevens, *Weitzman et al. 409* (K), HQ331546, AY425035, HQ332007, —; ***Bonnetia cubensis*** (Britton) R.A. Howard, *J. Gutierrez et al. HAJB 81795* (WIS), HQ331547, HQ331846, HQ332008, HQ331702; ***Bonnetia paniculata*** Spruce ex Benth., *P. Berry 7789* (MICH), HQ331548, HQ331847, HQ332009, HQ331703; ***Bonnetia roraimae*** Oliv., *Weitzman et al. 402* (K), —, HQ331848, AJ402930, —; ***Bonnetia sessilis*** Benth., *Berry s.n. 25.7.98* (MO), EF135509, HQ331849, HQ332010, EF135292; ***Bonnetia steyermarkii*** Kobuski, *Weitzman et al. 403* (K), —, HQ331850, HQ332011, HQ331704; ***Bonnetia stricta*** (Nees) Nees & Mart., *Amorim 3958* (CEPEC), HQ331549, HQ331851, HQ332012, HQ331705; ***Bonnetia tepuiensis*** Kobuski & Steyerm., *P. Berry 7788* (MICH), —, HQ331852, HQ332013, —; ***Ploiarium alternifolium*** Melchior, *Sugumaran 165* (US), FJ669999, FJ670063, FJ670161, FJ670352.

CALOPHYLLACEAE. ***Calophyllum brasiliense*** Cambess., *C. Notis 387* (FLAS), HQ331550, HQ331853, —, HQ331706; ***Calophyllum castaneum*** P.F. Stevens, *Ruhfel 111* (A), HQ331551, HQ331854, HQ332014, HQ331707; ***Calophyllum goniocarpum*** P.F. Stevens, *F. Damon 318* (MO), HQ331552, HQ331855, HQ332015, HQ331708; ***Calophyllum inophyllum*** L., *Ruhfel 115* (A), HQ331553, HQ331856, HQ332016, HQ331709; ***Calophyllum lanigerum*** Miq., *Ruhfel 104* (A), HQ331554, HQ331857, HQ332017, HQ331710; ***Calophyllum longifolium*** Willd., *Aguilar 11657* (NY), HQ331555, HQ331858, HQ332018, HQ331711; ***Calophyllum soulattri*** Burm. f., *Chase 1217* (K), HQ331556, AY425037, [*F. Damon 320* (MO), AY625021], AY674484; ***Calophyllum sp. 1***, *Ruhfel 108* (A), HQ331557, HQ331859, HQ332019, HQ331712; ***Calophyllum sp. 2***, *Ruhfel 113* (A), HQ331558, HQ331860, HQ332020, HQ331713; ***Calophyllum sp. 3***, *Ruhfel 114* (A), HQ331559, HQ331861, HQ332021, HQ331714; ***Calophyllum teysmannii*** Miq., *Ruhfel 112* (A), HQ331560, HQ331862, HQ332022, HQ331715; ***Calophyllum verticillatum*** P.F. Stevens, J. *Rabenantoandro et al. 733* (MO), HQ331561, HQ331863, HQ332023, HQ331716; ***Calophyllum vexans*** P.F. Stevens, *F. Damon 321* (MO), HQ331562, HQ331864, HQ332024, HQ331717; ***Caraipa densifolia*** Mart., *C. Grandez 16239* (FLAS), HQ331563, HQ331865, AY625012, HQ331718; ***Caraipa grandifolia*** Mart., *C. Grandez 16244* (FLAS), HQ331564, HQ331866, HQ332025, HQ331719; ***Caraipa savannarum*** Kubitzki, *G. Aymard s.n.* (PORT),

HQ331565, HQ331867, HQ332026, HQ331720; ***Caraipa tereticaulis*** Tul., *Vormisto 578* (AAU), HQ331566, HQ331868, HQ332027, HQ331721; ***Clusiella isthmensis*** Hammel, *M. Whitten 2657* (FLAS), HQ331585, HQ331889, AY625019, HQ331738; ***Endodesmia calophylloides*** Benth., *Burgt 762* (WAG), FJ670005, FJ670069, FJ670163, FJ670356; ***Haploclathra cordata*** R.Vásquez, *C. Grandez 16237* (FLAS), HQ331613, HQ331918, AY625017, HQ331764; ***Haploclathra paniculata*** Benth., *C. Grandez 16246* (FLAS), HQ331614, HQ331919, HQ332068, HQ331765; ***Kayea elmeri*** Merr., *Ruhfel 110* (A), HQ331636, —, HQ332086, HQ331784; ***Kayea hexapetala*** Pierre, *Ruhfel 119* (A), HQ331637, HQ331939, HQ332087, HQ331785; ***Kayea oblongifolia*** Ridl., *Ruhfel 116* (A), HQ331638, HQ331940, HQ332088, HQ331786; ***Kayea sp.***, *E. Wood and G. A. Teck 5500* (A), HQ331639, HQ331941, HQ332089, HQ331787; ***Kayea stylosa*** Thw., *Kostermans 11106* (HUH), HQ331640, HQ331942, AY625025, HQ331788; ***Kielmeyera lathrophyton*** Saddi, *F. Feres s.n.* (UEC), HQ331641, HQ331943, AY625015, HQ331789; ***Kielmeyera petiolaris*** Mart., *F. Feres 75* (UEC), HQ331642, HQ331944, AY625016, HQ331790; ***Mahurea exstipulata*** Benth., *Kubitzki et al. 97-27* (HBG), HQ331650, HQ331954, AY625018, HQ331799; ***Mammea africana*** Sabine, *D. Kenfack 2055* (MO), HQ331651, HQ331955, HQ332098, HQ331800; ***Mammea americana*** L., *C. Notis 392* (FLAS), HQ331652, HQ331956, AY625029, HQ331801; ***Mammea sessiliflora*** Planch. & Triana, *McPherson 18377* (MO), HQ331653, HQ331957, AY625027, HQ331802; ***Mammea siamensis*** T. Anderson, *Chase 1216* (K), FJ670006, FJ670070, AY625028, FJ670357; ***Mammea sp. 1***, *P. Sweeney 1305* (MO), HQ331654, HQ331958, HQ332099, HQ331803; ***Mammea sp. 2***, *T.G. Laman et al. TL 727* (A), HQ331655, HQ331959, HQ332100, —; ***Mammea touriga*** (C.T. White & W.D. Francis) L.S. Sm., *H. van der Werff and B. Gray 17055* (MO), HQ331656, HQ331960, HQ332101, HQ331804; ***Mammea zeereae*** P.F. Stevens, *P. Sweeney 1273* (MO), HQ331657, HQ331961, HQ332102, HQ331805; ***Marila laxiflora*** Rusby, *van der Werff et al. 16246* (MO), HQ331659, HQ331963, —, HQ331807; ***Marila tomentosa*** Poepp. & Endl., *van der Werff et al. 16215* (MO), HQ331660, HQ331964, AY625010, HQ331808; ***Mesua ferrea*** L., *M. Sugumaran et al. SM 120* (KLU), HQ331661, HQ331965, [*C. Notis 390* (FLAS), AY625024], HQ331809; ***Poeciloneuron indicum*** Bedd., *U. Ghate s.n.* (FLAS), HQ331673, HQ331977, AY625023, HQ331819.

CLUSIACEAE S.S.. ***Allanblackia sp.***, *E. Ndive s.n.* (YU), HQ331542, HQ331843, HQ332004, HQ331699; ***Chrysochlamys allenii*** (Maguire) Hammel, *R. Kriebel 2289* (INB), HQ331569, HQ331871, HQ332030, HQ331723; ***Chrysochlamys eclipes*** L.O. Williams, *BCI 158121* (STRI), HQ331570, HQ331872, HQ332031, HQ331724; ***Chrysochlamys grandifolia*** (L.O. Williams) Hammel, *R. Aguilar ra12291* (NY), —, HQ331873, HQ332032, HQ331725; ***Chrysochlamys silvicola*** (Hammel) Hammel, *B. Hammel 25293* (MO), HQ331571, HQ331874, —, HQ331726; ***Chrysochlamys skutchii*** Hammel, *R. Aguilar ra12292* (NY), HQ331572, HQ331875, —, —; ***Clusia* cf. *flavida*** (Benth.) Pipoly, *M. H. G. Gustafsson 454* (AAU), HQ331575, HQ331878, HQ332035, HQ331728; ***Clusia clusioides*** (Griseb.) D'Arcy, *M. H. G. Gustafsson 272* (NY), —, HQ331879, AF518388, HQ331729; ***Clusia fructiangusta*** Cuatrec., *M. H. G. Gustafsson 485*

(AAU), HQ331576, HQ331880, HQ332036, HQ331730; ***Clusia gracilis*** Standl., *Ruhfel 23* (A), HQ331577, HQ331881, HQ332037, HQ331731; ***Clusia gundlachii*** Stahl, *Chase 341* (NCU), EF135520, AY425041, Z75673, AY674493; ***Clusia hammeliana*** Pipoly, *M. H. G. Gustafsson 451* (AAU), HQ331578, HQ331882, HQ332038, HQ331732; ***Clusia lanceolata*** Cambess., *C. Notis 389* (FLAS), HQ331579, HQ331883, HQ332039, HQ331733; ***Clusia loretensis*** Engl., *M. H. G. Gustafsson 500* (AAU), HQ331580, HQ331884, HQ332040, HQ331734; ***Clusia major*** L., *M. H. G. Gustafsson 396* (AAU), HQ331581, HQ331885, HQ332041, HQ331735; ***Clusia pallida*** Engl., *M. H. G. Gustafsson 464* (AAU), HQ331582, HQ331886, HQ332042, HQ331736; ***Clusia rosea*** Jacq., *Kent s.n.* (A), HQ331583, HQ331887, HQ332043, JF828263; ***Clusia viscida*** Engl., *M. H. G. Gustafsson 444* (AAU), HQ331584, HQ331888, HQ332044, HQ331737; ***Dystovomita* cf. *brasiliensis*** D'Arcy, *Sothers 452* (UEC), —, —, AF518387, [Procópio, L.C. 07 PFRD 3794 (INPA), JF828261]; ***Dystovomita paniculata*** (Donn. Sm.) Hammel, *B. Hammel 25295* (MO), HQ331594, HQ331897, [*B. Hammel 22728* (INB), HQ332051], HQ331746; ***Garcinia* aff. *afzelii*** Engl., *P. W. Sweeney 1411* (MO), HQ331595, HQ331898, HQ332052, HQ331747; ***Garcinia conrauana*** Engl., *S. Moses 961* (MO), —, HQ331899, HQ332053, —; ***Garcinia cowa*** Roxb., *M. Sugumaran et al. SM 146* (KLU), HQ331596, HQ331900, HQ332054, HQ331748; ***Garcinia cymosa*** (K. Schum.) I.M.Turner & P.F.Stevens, *P. Sweeney 1000* (MO), HQ331597, HQ331901, [*T. Motley s.n.* (AAU) AF518379], HQ331749; ***Garcinia eugeniifolia*** Wall. ex T. Anderson, *P. W. Sweeney 985* (MO), HQ331598, HQ331902, HQ332055, HQ331750; ***Garcinia hessii*** (Britton) Alain, *Axelrod 4537* (UPR), EF135543, —, AJ402952, DQ110341; ***Garcinia hombroniana*** Pierre, *M. Sugumaran et al. SM 124* (KLU), HQ331599, HQ331903, HQ332056, HQ331751; ***Garcinia intermedia*** (Pittier) Hammel, *M.J Balick 3570* (GH), HQ331600, HQ331904, —, HQ331752; ***Garcinia latissima*** Miq., *Chase 2100* (K), FJ670008, FJ670072, AF518386, FJ670359; ***Garcinia livingstonei*** T. Anderson, *P. Sweeney 1007* (MO), —, HQ331905, —, HQ331753; ***Garcinia macrophylla*** Mart., *Chase 1219* (K), —, FJ670073, FJ670165, FJ670360; ***Garcinia mangostana*** L., *Kent s.n.* (A), HQ331601, HQ331906, HQ332057, —; ***Garcinia mannii*** Oliver, *G. Walters et al. 604* (MO), HQ331602, HQ331907, —, HQ331754; ***Garcinia melleri*** Baker, *J. Rabenantoandro and G. McPherson 689* (MO), HQ331603, HQ331908, HQ332058, HQ331755; ***Garcinia nervosa*** Miq., *Ruhfel 106* (A), HQ331604, HQ331909, HQ332059, HQ331756; ***Garcinia penangiana*** Pierre, *Ruhfel 118* (A), HQ331605, HQ331910, HQ332060, HQ331757; ***Garcinia rostrata*** Hassk. ex Hook. f., *P. W. Sweeney 1071* (MO), HQ331606, HQ331911, HQ332061, HQ331758; ***Garcinia scortechinii*** King, *P. W. Sweeney 994* (MO), HQ331607, HQ331912, HQ332062, HQ331759; ***Garcinia spicata*** Hook. f., *C. Notis 388* (FLAS), HQ331608, HQ331913, HQ332063, HQ331760; ***Garcinia staudtii*** Engl., *P. Sweeney et al. 1445* (MO), HQ331609, HQ331914, HQ332064, HQ331761; ***Garcinia tsaratananensis*** (H. Perrier) P. Sweeney & Z.S. Rogers, *P. Sweeney 1232* (MO), HQ331610, HQ331915, HQ332065, HQ331762; ***Garcinia urophylla*** Scort. ex King, *P. W. Sweeney 1081* (MO), HQ331611, HQ331916, HQ332066, HQ331763; ***Lorostemon coelhoi*** Paula, *V. Bittrich 95-170* (UEC),

HQ331648, HQ331952, [*Assuncão 492* (UEC), AF518401], HQ331797; ***Montrouziera cauliflora*** Planch. & Triana, *Lowry 5601* (MO), FJ670007, FJ670071, FJ670164, FJ670358; ***Montrouziera sphaeroidea*** Planch. ex Planch. & Triana, *K. Cameron 981* (NY), HQ331664, HQ331968, [*Cameron 981* (NY), AF518390], HQ331812; ***Moronobea coccinea*** Aubl., *SM 24698* (NY), HQ331665, HQ331969, AF518378, HQ331813; ***Pentadesma butyracea*** Sabine, *Kitjima s.n.* (A), HQ331669, HQ331973, [*Nagata 951*, (HLA), AF518383], HQ331817; ***Platonia insignis*** Mart., *V. Bittrich s.n. 3.01.05* (INB), HQ331670, HQ331974, [*Mori 23699* (NY), AF518394], HQ331818; ***Symphonia fasciculata*** (Noronha ex Thouars) Vesque, *J.S. Miller et al 8836* (MO), HQ331679, HQ331984, HQ332117, HQ331825; ***Symphonia globulifera*** L. f., *Ruhfel 21* (A), HQ331680, HQ331985, [*Mori 24792* (NY), AF518381], HQ331826; ***Tovomita calophyllophylla*** García-Villacorta & Hammel, *J.Vormisto 579* (AAU), HQ331683, HQ331988, HQ332119, HQ331828; ***Tovomita longifolia*** (Rich.) Hochr., *R. Aguilar ra12290* (NY), HQ331684, HQ331989, HQ332120, HQ331829; ***Tovomita sp.***, *J. Vormisto 562* (AAU), HQ331685, HQ331990, HQ332121, HQ331830; ***Tovomita weddelliana*** Planch. & Triana, *M. H. G. Gustafsson 478* (AAU), HQ331686, HQ331991, HQ332122, HQ331831; ***Tovomitopsis paniculata*** (Spreng.) Planch. & Triana, *Amaral & Bittrich 2003/02* (UEC), —,—, JF828248, JF828262; ***Tovomitopsis saldanhae*** Engl., *V. Bittrich s.n.* (UEC), HQ331687, HQ331992, HQ332123, —.

CTENOLOPHONACEAE. ***Ctenolophon englerianus*** Mildbr., *McPherson 16911* (MO), EF135524, FJ670074, AJ402940, AY674499.

HYPERICACEAE. ***Cratoxylum arborescens*** (Vahl) Blume, *Ruhfel 121* (A), HQ331586, HQ331890, HQ332045, HQ331739; ***Cratoxylum cochinchinense*** (Lour.) Blume, *Church et al. 2699* (A), HQ331587, HQ331891, HQ332046, HQ331740; ***Cratoxylum formosum*** (Jack) Dyer, *Ruhfel 107* (A), HQ331588, HQ331892, HQ332047, HQ331741; ***Cratoxylum sumatranum*** (Jack) Blume, *Chase 1218* (K), FJ670022, FJ670095, AF518395, FJ670373; ***Cratoxylum glaucum*** Korth., *Ruhfel 102* (A), HQ331589, HQ331893, HQ332048, HQ331742; ***Eliea articulata*** Cambess., *Razakamalala 295* (MO), FJ670023, FJ670096, FJ670167, FJ670374; ***Hypericum aegypticum*** L., *M. Gustafsson MG 1148* (AAU), HQ331617, HQ331922, HQ332069, HQ331767; ***Hypericum androsaemum*** L., *J. Christiansen s.n.* (AAU), HQ331618, HQ331923, HQ332070, HQ331768; ***Hypericum annulatum*** Moris, *J. Christiansen s.n.* (AAU), HQ331619, HQ331924, HQ332071, HQ331769; ***Hypericum canariense*** L., *J. Christiansen s.n.* (AAU), HQ331620, HQ331925, HQ332072, HQ331770; ***Hypericum ellipticum*** Hook., *C.C. Davis s.n.* (A), HQ331621, HQ331926, —, HQ331771; ***Hypericum elodes*** L., *Halliday s.n., 6/7 1964* (AAU), HQ331622, —, HQ332073, HQ331772; ***Hypericum empetrifolium*** Willd., *Chase 837* (K), HQ331623, AY425060, HQ332074, AY674525; ***Hypericum garrettii*** Craib, *J. Christiansen s.n.* (AAU), HQ331624, HQ331927, HQ332075, HQ331773; ***Hypericum grandifolium*** Choisy, *M. Gustafsson MG1147* (AAU), HQ331625, HQ331928, HQ332076, HQ331774; ***Hypericum hircinum*** L., *J. Christiansen s.n.* (AAU), HQ331626, HQ331929, HQ332077, HQ331775; ***Hypericum irazuense*** Kuntze ex N. Robson, *Ruhfel 8* (A), —, —, HQ332078, HQ331776; ***Hypericum***

kalmianum L., *C.C. Davis s.n.* (A), HQ331627, HQ331930, HQ332079, JF828264; ***Hypericum linarifolium*** Vahl, *J. Christiansen s.n.* (AAU), HQ331628, HQ331931, HQ332080, HQ331777; ***Hypericum mutilum*** L., *C.C. Davis s.n.* (A), HQ331629, HQ331932, —, HQ331778; ***Hypericum perforatum*** L., *Ruhfel s.n.* (A), HQ331630, HQ331933, HQ332081, JF828265; ***Hypericum tetrapterum*** Fr., *J. Christiansen s.n.* (AAU), HQ331631, HQ331934, HQ332082, HQ331779; ***Santomasia steyermarkii*** (Standl.) N. Robson, *E. Matuda S-228* (A), —, HQ331982, —, —; ***Thornea calcicola*** (Standl. & Steyerm.) Breedlove & E.M. McClint., *D.E. Breedlove 37070* (MO), HQ331682, [*J.A. Steyermark 48946* (A), HQ331987], —, —; ***Triadenum japonicum*** (Blume) Makino, *S. Kobayashi 2713* (A), HQ331689, HQ331994, HQ332125, HQ331833; ***Triadenum fraseri*** (Spach) Gleason, *C.C. Davis s.n.* (A), HQ331688, HQ331993, HQ332124, [*C.C. Davis s.n.* (A), HQ331832]; ***Triadenum walteri*** (J.F. Gmel.) Gleason, *Brant 4792* (MO), HQ331690, FJ670097, FJ670168, FJ670375; ***Harungana madagascariensis*** Poir., *B. Pettersson and L. A. Nilson 37* (UPS), HQ331615, HQ331920, [*Naugona 139* (NY), AF518396], HQ331766; ***Psorospermum* aff. *androsaemifolium*** Baker, *R. Randrianaivo et al. 145* (UPS), HQ331675, —, HQ332111, —; ***Psorospermum corymbiferum*** Hochr., *J.E. Lawesson and Goudiaby 7578* (AAU), HQ331676, HQ331979, HQ332112, HQ331821; ***Psorospermum febrifugum*** Spach, *M. Hedren et al. 394* (UPS), HQ331677, HQ331980, HQ332113, HQ331822; ***Psorospermum revolutum*** (Choisy) Hochr., *M. Thulin, P. Kornhall, and M. Popp 10312* (UPS), HQ331678, —, HQ332114, HQ331823; ***Vismia sp.***, *Miller et al. 9313* (MO), EF135601, FJ670098, FJ670169, AY674571; ***Vismia baccifera*** (L.) Triana & Planch., *Ruhfel 20* (A), HQ331692, HQ331996, [*Gustafsson 302* (NY), AF518382], HQ331835; ***Vismia bilbergiana*** Beurl., *B. Hammel 25285* (MO), HQ331693, HQ331997, [*STRI:BCI 734543* (STRI), GQ981917], HQ331836; ***Vismia guianensis*** (Aubl.) Choisy, *Amorim 7659* (CEPC), HQ331694, HQ331998, HQ332126, JF828267; ***Vismia guineensis*** (L.) Choisy, *M. Merello et al. 1149* (UPS), HQ331695, HQ331999, —, HQ331838; ***Vismia macrophylla*** Kunth, *Amorim 3972* (CEPC), HQ331696, HQ332000, —, HQ331839; ***Vismia rubescens*** Oliv., *R. Niangadouma et al. 374* (MO), —, HQ332001, HQ332127, HQ331840.

IRVINGIACEAE. ***Irvingia malayana*** Oliv., *Simpson 2638* (K), EF135553, AY425061, AF123278, EF135300.

OCHNACEAE. ***Ochna multiflora*** DC., *Chase 229* (NCU), EF135572, AY425072, Z75273, EF135302.

PANDACEAE. ***Panda oleosa*** Pierre, *Schmidt et al. 2048* (MO) FJ670032, FJ670111, AY663644, FJ670383.

PODOSTEMACEAE. ***Apinagia longifolia*** (Tul.) P. Royen, *C.T. Philbrick 6023* (WCSU), HQ331543, HQ331844, HQ332005, HQ331700; ***Apinagia riedelii*** Tul., *C.T. Philbrick 5960* (WCSU), HQ331544, HQ331845, HQ332006, HQ331701; ***Castelnavia monandra*** Tul. & Wedd., *C.T. Philbrick 5982* (WCSU), HQ331567, HQ331869, HQ332028, HQ331722; ***Castelnavia multipartida*** Tul. & Wedd., *Bove et al. 2241* (WCSU), JF828268, JF828249, JF828242, JF828255; ***Castelnavia princeps*** Tul. & Wedd., *Bove et al. 2211* (WCSU), JF828269, JF828250, JF828243, JF828256; ***Ceratolacis pedunculatum*** C. Philbrick, *Novelo*

& *Irgang, C.T. Philbrick 5761* (MO), HQ331568, HQ331870, HQ332029, —; ***Cipoia ramosa*** C.P. Bove, C.T. Philbrick & Novelo, *Bove et al. 2251* (WCSU), JF828270, JF828251, JF828244, JF828257; ***Cladopus japonicus*** Imamura, *S. Koi and N. Katayama JP-404* (TNS), HQ331573, HQ331876, HQ332033, HQ331727; ***Cladopus queenslandicus*** (Domin) C.D.K. Cook & Rutish., *J.J. Bruhl and I.R. Telford 2542* (MO), HQ331574, HQ331877, HQ332034, —; ***Dalzellia zeylanica*** Wight, *M. Kato and N. Katayama SL-101* (TNS), HQ331590, HQ331894, [SL-04 (TNS), AB113760], HQ331743; ***Diamantina lombardii*** Novelo, *C. Philbrick & Irgang, Bove et al. 2201* (WCSU), JF828271, JF828252, JF828245, JF828258; ***Dicraeanthus zehnderi*** H.E. Hess, *Ghogue GHO-1650* (Z/ZT), HQ331592, HQ331895, HQ332049, HQ331744; ***Djinga felicis*** C. Cusset, *Ghogue et al. GAR-09* (Z/ZT), HQ331593, HQ331896, HQ332050, HQ331745; ***Endocaulos mangorense*** (H. Perrier) C. Cusset, *Kato et al. MD-02* (TI), AB038191, —, —, —; ***Griffithela hookeriana*** (Tul.) Warm., *C.T. Philbrick 4683* (WCSU), HQ331612, HQ331917, HQ332067, —; ***Hanseniella heterophylla*** C. Cusset, *Kato et al. TL-311* (TI), AB104562, —, —, —; ***Hydrobryum japonicum*** Imamura, *S. Koi and N. Katayama JP-401* (TNS), HQ331616, HQ331921, —, —; ***Hydrodiscus koyamae*** (M. Kato & Fukuoka) Koi & M. Kato, *M. Kato et al. L-06* (TNS), AB537381,—, —, —; ***Indodalzellia gracilis*** (C.J. Mathew, Jäger-Zürn, & Nileena) Koi & M. Kato, *KI-115* (TNS), AB450015, —, —, —; ***Indotristicha ramosissima*** (Wight) Royen, *M. Kato et al. KI-210* (TNS), HQ331632, HQ331935, [*KI-26* (TNS), AB124844], HQ331780; ***Inversodicraea* cf. *annithomae*** (C. Cusset) R.Rutish. and Thiv, *Ghogue et al. GAHR-23* (Z/ZT), HQ331633, HQ331936, HQ332083, HQ331781; ***Inversodicraea* cf. *bosii*** (C. Cusset) R.Rutish. & Thiv, *Ghogue et al GAR-01* (Z/ZT), HQ331634, HQ331937, HQ332084, HQ331782; ***Inversodicraea cristata*** Engler, *Ghogue GHO-1664* (Z/ZT), HQ331635, HQ331938, HQ332085, HQ331783; ***Ledermanniella bifurcata*** (Engler) C. Cusset, *Ghogue GHO-1597* (Z/ZT), HQ331643, HQ331945, HQ332090, HQ331791; ***Ledermanniella bowlingii*** (J.B. Hall) C. Cusset, *Ameka and Rutishauser AR-021010* (Z/ZT), HQ331644, HQ331946, HQ332091, HQ331792; ***Ledermanniella letouzeyi*** C. Cusset, *Ghogue et al. GAR-12* (Z/ZT), HQ331645, HQ331947, HQ332092, HQ331793; ***Ledermanniella linearifolia*** Engler, *Ghogue et al. GAHR-41* (Z/ZT), —, HQ331948, HQ332093, HQ331794; ***Ledermanniella pusilla*** (Warming) C. Cusset, *Ghogue et al. GAHR-17* (Z/ZT), HQ331646, HQ331949, HQ332094, HQ331795; ***Leiothylax quangensis*** (Engler) Warming, *Ghogue GHO-1667* (Z/ZT), FM877842, HQ331950, HQ332095, —; ***Letestuella tisserantii*** G. Taylor, *Ghogue GHO-1660* (Z/ZT), HQ331647, HQ331951, HQ332096, HQ331796; ***Lophogyne lacunosa*** (Gardner) C.P. Bove & C.T. Philbrick, *Bove et al. 2258* (WCSU), JF828272, JF828253, JF828246, JF828259; ***Macarenia clavigera*** P. Royen, *Lasso EFI-14* (WCSU), JF828273, JF828254, JF828247, JF828260. ***Macropodiella heteromorpha*** (Baillon) C. Cusset, *Ghogue et al. GAHR-24* (Z/ZT), HQ331649, HQ331953, HQ332097, HQ331798; ***Marathrum foeniculaceum*** Bonpl., *C.T. Philbrick 5958* (WCSU), HQ331658, HQ331962, HQ332103, HQ331806; ***Marathrum plumosum*** (Novelo & C.T.Philbrick) *C.T.Philbrick & C.P.Bove, MX-05* (TI), AB048378, —, [Les et al., U68090], —; ***Monandriella linearifolia***

Engler, *Ghogue GHO-1663* (Z/ZT), HQ331662, HQ331966, HQ332104, HQ331810; ***Monostylis capillacea*** Tul., *C.T. Philbrick 6076* (WCSU), HQ331663, HQ331967, HQ332105, HQ331811; ***Mourera* cf. *aspera*** (Bong.) Tul., *C.T. Philbrick 6093* (WCSU), HQ331666, HQ331970, [Les et al, U68086], HQ331814; ***Mourera fluviatilis*** Aubl., *GU-24* (TI), AB038200, —, [not listed, AB113759], —; ***Noveloa coulteriana*** (Tul.) C.T.Philbrick, *C.T. Philbrick 6270* (WCSU), HQ331667, HQ331971, HQ332106, HQ331815; ***Paracladopus chanthaburiensis*** Koi & M. Kato, *S. Koi et al. TKF-24* (TNS), HQ331668, HQ331972, HQ332107, HQ331816; ***Podostemum ceratophyllum*** Michx., *Ruhfel s.n.* (A), HQ331671, HQ331975, HQ332108, JF828266; ***Podostemum scaturiginum*** (Mart.) C. Philbrick & Novelo, *C.T. Philbrick et al. 5602* (MO), HQ331672, HQ331976, HQ332109, —; ***Polypleurum stylosum*** (Wight) J.B. Hall, *M. Kato and N. Katayama SL-103* (TNS), HQ331674, HQ331978, HQ332110, HQ331820; ***Rhyncholacis sp.***, *Amaral s.n.* (INPA), EF135564, HQ331981, HQ332115, AY674537; ***Saxicolella amicorum*** J.B. Hall, *Ameka & deGraft-Johnson 112*, FN357252,—, —, —; ***Stonesia ghoguei*** E. Pfeifer and Rutishauser, *Ghogue GHO-1665* (Z/ZT), FM877841, HQ331983, HQ332116, HQ331824; ***Terniopsis brevis*** M. Kato, *S. Koi et al. TKF-25* (TNS), HQ331681, HQ331986, HQ332118, HQ331827; ***Terniopsis malayana*** (J.Dransf. & Whitmore) M.Kato, *TL-106, 107* (TNS), AB048827, —, AB083098, —; ***Terniopsis sessilis*** Hsiu C. Chao, *CH-03* (TI), AB048377, —, AB083100, —; ***Thawatchaia trilobata*** M.Kato, Koi & Y.Kita, *Kato et al. TL-419* (TI), AB104563, —, —, —; ***Thelethylax minutiflora*** (Tul.) C. Cusset, *Kato et al. MD-01* (TI), AB038196, —, —, —; ***Tristicha trifaria*** (Bory ex Willd.) Spreng., *C.T. Philbrick 6090* (WCSU), HQ331691, HQ331995, [*BR-01*, AB113746], HQ331834; ***Weddellina squamulosa*** Tul., *C.T. Philbrick 5827* (WCSU), HQ331697, HQ332002, [not listed, AB113758], HQ331841; ***Zeylanidium lichenoides*** Engl., *Kato et al. KI-35* (TI), AB048828, —, —, —; ***Zeylanidium subulatum*** (Gardner) C. Cusset, *M. Kato and N. Katayama SL-102* (TNS), HQ331698, HQ332003, HQ332128, HQ331842.

RHIZOPHORACEAE. ***Bruguiera gymnorhiza*** Lam., *Chase 12838* (K), EF135511, AY425036, [AF127693], AY674483.

APPENDIX 3.2. Area coding and references for taxa and their ranges used in LAGRANGE ancestral area reconstruction analyses. Eight biogeographic areas were circumscribed: 1) North America, Central America, and the Caribbean (NA); 2) South America (SA); 3) Eurasia (EA); 4) Africa (Af); 5) Madagascar, plus the Comoros, Seychelles, and Mascarenes (Md); 6) India and Sri Lanka (In); 7) Southeast Asia (Se; those regions west of Wallace's Line not part of continental Eurasia); and 8) Australia (Au) those regions east of Wallace's line including New Caledonia and the Pacific Islands.

Terminal in tree	Representative species	Region(s) scored	References
Bonnetiaceae	**Bonnetiaceae**		
Archytaea	*Archytaea triflora*	SA	(Weitzman et al., 2007)
Bonnetia	*Bonnetia roraimae*	SA; see note 1	(Weitzman et al., 2007; Ruhfel et al., 2011)
Ploiarium	*Ploiarium alternifolium*	EA, Se, and Au	(Weitzman et al., 2007)
Calophyllaceae	**Calophyllaceae**		
Calophyllum	*Calophyllum inophyllum*	EA, Md, In, Se, Au; see note 2	(Stevens, 1980b, 2007b)
Caraipa	*Caraipa densifolia*	SA	(Stevens, 2007b)
Clusiella	*Clusiella isthmensis*	NA, SA	(Stevens, 2007b)
Endodesmia	*Endodesmia calophylloides*	Af; see note 3	(Stevens, 2007b; Ruhfel et al. unpublished manuscript)
Haploclathra	*Haploclathra cordata*	SA	(Stevens, 2007b)
Kayea	*Kayea stylosa*	EA, In, Se, Au	(Stevens, 2007b)
Kielmeyera	*Kielmeyera lathrophyton*	SA	(Stevens, 2007b)
Mahurea	*Mahurea exstipulata*	SA	(Stevens, 2007b)
Mammea	*Mammea siamensis*	EA, Af, Md, In, Se, Au, see note 4	(Stevens, 2007b, Ruhfel et al., 2011)
Marila	*Marila laxiflora*	NA, SA	(Stevens, 2007b)
Mesua	*Mesua ferrea*	EA, In, Se	(Stevens, 2007b)
Poeciloneuron	*Poeciloneuron indicum*	In	(Stevens, 2007b)
Clusiaceae s.s.	**Clusiaceae s.s.**		
Allanblackia	*Allanblackia* sp.	Af	(Stevens, 2007b, Sweeney, 2008)
Chrysochlamys	*Chrysochlamys eclipes*	NA, SA	(Stevens, 2007b)
Clusia	*Clusia clusioides*	NA, SA	(Stevens, 2007b)
Dystovomita	*Dystovomita paniculata*	NA, SA	(Stevens, 2007b)
Garcinia conrauana (*Tetra*)	*Garcinia conrauana*	Af	(Sweeney, 2008)
Garcinia cowa (5)	*Garcinia cowa*	EA, Md, In, Se, Au	(Sweeney, 2008)
Garcinia cymosa (9)	*Garcinia cymosa*	EA, In, Se, Au	(Sweeney, 2008)
Garcinia eugenifolia (4)	*Garcinia eugenifolia*	EA, In, Se, Au	(Sweeney, 2008)
Garcinia livinstonei (2 p.p)	*Garcinia livinstonei*	Af, Md	(Sweeney, 2008)
Garcinia macrophylla (2 p.p.)	*Garcinia macrophylla*	NA, SA	(Sweeney, 2008)
Garcinia mangostana (6)	*Garcinia mangostana*	EA, In, Se, Au	(Sweeney, 2008)
Garcinia mannii (8)	*Garcinia mannii*	Af	(Sweeney, 2008)
Garcinia melleri (3)	*Garcinia melleri*	Md	(Sweeney, 2008)

Garcinia nervosa (1)	*Garcinia nervosa*	Af	(Sweeney, 2008)
Garcinia staudtii (2 p.p.)	*Garcinia staudtii*	Af	(Sweeney, 2008)
Garcinia urophylla (7)	*Garcinia urophylla*	EA, In, Se, Au	(Sweeney, 2008)
Lorostemon	*Lorostemon coelhoi*	SA	(Stevens, 2007b)
Montrouziera	*Montrouziera cauliflora*	Au	(Stevens, 2007b)
Moronobea	*Moronobea coccinea*	SA	(Stevens, 2007b)
Pentadesma	*Pentadesma butyracea*	Af	(Stevens, 2007b)
Platonia	*Platonia insignis*	SA	(Stevens, 2007b)
Symphonia p.p.	*Symphonia fasiculata*	Md	(Abdul-Salim, 2002; Dick et al., 2003; Dick and Heuertz, 2008)
Symphonia globulifera	*Symphonia globulifera*	Af; see note 5	(Abdul-Salim, 2002; Dick et al., 2003; Dick and Heuertz, 2008)
Tovomita	*Tovomita longifolia*	NA, SA; see note 6	(Stevens, 2007b; Ruhfel et al., 2011; Ruhfel et al., unpublished)
Tovomita weddeliana	*Tovomita weddeliana*	NA, SA; see note 6	(Stevens, 2007b; Ruhfel et al., 2011; Ruhfel et al., unpublished)
Tovomitopsis	*Tovomitopsis paniculata*	SA	(Stevens, 2007b)
Hypericaceae	**Hypericaceae**		
Cratoxylum	*Cratoxylum arborescens*	EA, In, Se	(Gogelein, 1967; Stevens, 2007a)
Eliea	*Eliea articulata*	Md	(Stevens, 2007a)
Harungana	*Harungana madagascariensis*	Af, Md	(Bamps, 1966; Ruhfel et al., 2011)
Hypericum	*Hypericum perforatum*	NA, SA, EA, Af; see note 7	(Robson, 1977, Stevens, 2007b, Nürk and Blattner, 2010; Nürk et al., 2010; Ruhfel et al., 2011)
Psorospermum	*Psorospermum revolutum*	Af, Md, see notes 8, 9, and 10	(Bamps, 1966; Ruhfel et al., 2011; Ruhfel et al. unpublished manuscript)
Vismia	*Vismia macrophylla*	NA, SA; see note 9	(Bamps, 1966, Ruhfel et al., 2011, Ruhfel et al. unpublished manuscript)
Vismia rubescens	*Vismia rubescens*	Af, see notes 9 and 10	(Bamps, 1966; Ruhfel et al., 2011; Ruhfel et al. unpublished manuscript)
Podostemaceae	**Podostemaceae**		
Apinagia	*Apinagia longifolia*	SA	(Cook and

			Rutishauser, 2007, Tippery et al., in press)
Castelnavia	*Castelnavia princeps511br*	SA	(Cook and Rutishauser, 2007; Philbrick et al., 2009; Tippery et al., in press)
Ceratolacis	*Ceratolacis pedunculatum*	SA	(Cook and Rutishauser, 2007)
Cipoia	*Cipoia ramosa529br*	SA	(Cook and Rutishauser, 2007)
Cladopus	*Cladopus japonicus*	EA, SE; see note 11	(Cook and Rutishauser, 2007; Koi et al., 2008)
Dalzellia	*Dalzellia zeylanica*	EA, In, SE	(Kato, 2006a, Cook and Rutishauser, 2007; Koi et al., 2009)
Diamantina	*Diamantina lombardii526br*	SA	(Cook and Rutishauser, 2007)
Dicraeanthus	*Dicraeanthus zehnderi*	Af	(Cook and Rutishauser, 2007)
Djinga	*Djinga felicis*	Af	(Cook and Rutishauser, 2007)
Endocaulos	*Endocaulos mangorense*	Md	(Cook and Rutishauser, 2007)
Hydrobryum	*Hydrobryum japonicum*	EA, In, see note 12	(Cook and Rutishauser, 2007; Koi and Kato, 2010; Ruhfel et al., 2011)
Indodalzellia	*Indodalzellia gracilis*	In	(Koi et al., 2009)
Indotristicha	*Indotristicha ramosissima*	In	(Cook and Rutishauser, 2007; Koi et al., 2009)
Inversodicraea	*Inversodicraea_cristata*	Af	(Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Ledermanniella p p	*Ledermanniella_bifurcata*	Af	(Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Ledermanniella p.p.	*Ledermanniella_bowlingii*	Af	(Cook and Rutishauser, 2007, Ruhfel et al , 2011)
Ledermanniella p.p	*Ledermanniella_pusilla*	Af	(Cook and Rutishauser, 2007, Ruhfel et al , 2011)
Ledermanniella p.p.	*Ledermanniella_letouzeyi*	Af	(Cook and Rutishauser, 2007; Ruhfel et al.,

			2011)
Leiothylax p.p.	*Leiothylax_quangensis*	Af	(Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Letestuella	*Letestuella_tisserantii*	Af	(Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Lophogyne	*Lophogyne lacunosa*	SA	(Cook and Rutishauser, 2007)
Macarenia	*Macarenia clavigera*	SA	(Cook and Rutishauser, 2007)
Macropodiella	*Macropodiella_heteromorpha*	Af	(Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Marathrum	*Marathrum foeniculaceum*	NA, SA	(Cook and Rutishauser, 2007; Tippery et al , in press)
Monandriella	*Monandriella_linearifolia*	Af	(Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Monostylis	*Monostylis capillacea*	SA	(Cook and Rutishauser, 2007, Tippery et al., in press)
Mourera	*Mourera fluviatilis*	SA	(Cook and Rutishauser, 2007)
Noveloa	*Noveloa coulteriana*	NA	(Cook and Rutishauser, 2007, Tippery et al., in press)
Paracladopus	*Paracladopus chanthaburiensis*	EA	(Kato, 2006b; Koi et al., 2008)
Podostemum	*Podostemum ceratophyllum*	SA, see note 13	(Philbrick and Novelo, 2004; Cook and Rutishauser, 2007) (Moline et al., 2006)
Polypleurum	*Polypleurum stylosum*	EA, In, see note 14	(Kato, 2006b; Cook and Rutishauser, 2007; Ruhfel et al., 2011)
Rhyncholacis	*Rhyncholacis sp*	SA	(Cook and Rutishauser, 2007)
Saxicolella	*Saxicolella amicorum*	Af	(Cook and Rutishauser, 2007; Kelly et al., 2010)
Stonesia	*Stonesia ghoguei*	Af	(Cook and Rutishauser, 2007)

Ternıopsıs	*Ternıopsis malayana*	EA, Se; see note 15	(Kato, 2006b; Koı et al., 2009)
Thelethylax	*Thelethylax mınutıflora*	Md	(Cook and Rutıshauser, 2007)
Trısticha	*Trısticha trifarıa*	Af, Md; see note 16	(Kıta and Kato, 2004; Kato, 2006a)
Weddellina	*Weddellına squamulosa*	SA	(Cook and Rutishauser, 2007)
Zeylanidium	*Zeylanıdium lıchenoıdes*	EA, In; see note 17	(Cook and Rutıshauser, 2007)

Notes:

1) *Bonnetia cubensis* occurs in Cuba. This species is embedded within a clade of strictly South America *Bonnetia* species (Ruhfel et al., 2011).

2) Stevens (1980a) suggests that the neotropical species of *Calophyllum* may be derived from a single ancestor in the Old World. Additionally, neotropical species of *Calophyllum* sampled in Ruhfel et al. (2011) are embedded within an Old World clade.

3) *Lebrunia* (Endodesmieae), is found in western tropical Africa and is sister to *Endodesmia* (Ruhfel et al., unpublished).

4) *Mammea americana* is sister to *M. africana* (Ruhfel et al., 2011). The only other neotropical species, *M. immansueta*, is very similar to both. D'Arcy (1980) thought *M. immansueta* was more closely related to *M. africana*. P. Stevens (unpublished manuscript), however, has conducted a complete monograph of this genus and lists several morphological features that suggest a closer relationship of *M. immansueta* to *M. americana*. We follow Stevens here and therefore, do not score *Mammea* as being neotropical, as the neotropical species are embedded within an Old World clade (Ruhfel et al., 2011).

5) *Symphonia globulifera* was shown to have reached the neotropics by long-distance dispersal (Dick et al., 2003), so here is scored as African in origin. Fossil pollen data also support this view (Germeraad et al., 1968; Salard-Cheboldaeff, 1979).

6) *Tovomita* is non-monophyletic (Ruhfel et al., 2011; Ruhfel et al., unpublished). *T. weddelliana* is more closely related to *Cluisa and Chrysochlamys*. As such it is included separately here.

7) *Hypericum* as scoreded here includes *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* sensu Ruhfel et al. (2011). The most diverse areas of the genus have been scored here. This scoring includes the putative origins of the genus in either Africa as proposed by Robson (Robson, 1977) and the Mediterranean region as proposed by Nürk and Blattner (2010) and Nürk et al. (2010).

8) In Ruhfel et al. (2011) and Ruhfel et al. (unpublished) a clade containing all sampled *Psorospermum* species and some African *Vismia* species was recovered. The terminal *Psorospermum* represents these species here.

9) Neotropical members of *Vismia* form a monophyletic group in Ruhfel et al. (2011). The terminal *Vismia* represents these species here.

10) *Vismia rubescens* is sister to *Harungana* (Ruhfel et al., 2011; Ruhfel et al., unpublished).

11) *Cladopus* also occurs in Australia and New Guinea but these species are embedded in a clade of Eurasian and Southeast Asian taxa (Cook and Rutishauser, 2007; Koi et al., 2008).

12) *Hydrobryum* here represents a clade containing *Diplobryum*, *Hanseniella*, *Hydrobryum*, *Hydrodiscus*, *Thawatchaia* (Koi and Kato, 2010; Ruhfel et al., 2011)

13) *Podostemum ceratophyllum* occurs in North America, but this species is embedded within a strictly South American clade (Philbrick and Novelo, 2004; Moline et al., 2006; Cook and Rutishauser, 2007).

14) *Polypluerum* scoring here includes *Zeylanidium subulatum*. These taxa were sister in Ruhfel et al. (2011). This same study showed that *Zeylanidium* is non-monophyletic. See note 17.

15) One species of *Terniopsis* is found in northern Australia (Kato, 2006a), though this species is deeply embedded within a clade of Eurasian and Southeast Asian taxa (Koi et al., 2009).

16) *Tristicha* is distributed in Africa, Madagascar and the neotropics (Kato, 2006a). Kita and Kato (2004), however, show that neotropical *Tristicha* populations are derived from within an African clade. As such we score this genus as present in Africa and Madagascar.

17) *Zeylanidium* here includes *Griffithella* which was sister to *Z. lichenoides* in (Ruhfel et al., 2011). See also note 14.

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APPENDIX 3.3. Lagrange model information.

Table A3.1. Ranges included in the second Lagrange model. Absence in an area is indicated with a '0', presence is indicated with a '1'. Areas as listed in the following order: NA, SA, EA, Af, Md, In, Se, Au. NA= North America, SA=South America, EA= Eurasia, Af=Africa, Md= Madagascar, In=India, Se= Southeast Asia, and Au= Australia. Full circumscriptions of each area are given in the Methods.

00000000	10000000	11000000	11100000	11110000	11000001	10100000	10110000
10100100	10100010	01000000	01010000	01011000	01010001	01001001	01000101
01000011	01000001	00100000	00110000	00111000	00111111	00110100	00110010
00101100	00101111	00100100	00100110	00100111	00100010	00100011	00010000
00011000	00011100	00011001	00010100	00010101	00010001	00001000	00001100
00001110	00001101	00001011	00001001	00000100	00000110	00000111	00000101
00000010	00000011	00000001					

Table A3.2. Dispersal rate matrices for each of the four windows of time used in the second LAGRANGE model. NA= North America, SA=South America, EA= Eurasia, Af=Africa, Md= Madagascar, In=India, Se= Southeast Asia, and Au= Australia.

First window of time: 0-30 Ma.

	NA	SA	EA	Af	Md	In	Se	Au
NA	1	1	1	0.01	0.01	0.01	0.01	0.01
SA	1	1	0.01	0.5	0.01	0.01	0.01	0.5
EA	1	0.01	1	1	0.01	1	1	0.01
Af	0.01	0.5	1	1	1	1	0.01	0.5
Md	0.01	0.01	0.01	1	1	1	0.01	0.5
In	0.01	0.01	1	1	1	1	1	0.01
Se	0.01	0.01	1	0.01	0.01	1	1	1
Au	0.01	0.5	0.01	0.5	0.5	0.01	1	1

Second window of time:30-60

	NA	SA	EA	Af	Md	In	Se	Au
NA	1	0.01	1	0.01	0.01	0.01	0.01	0.01
SA	0.01	1	0.01	0.01	0.01	0.01	0.01	1
EA	1	0.01	1	1	0.01	1	1	0.01
Af	0.01	0.01	1	1	1	1	0.01	0.01
Md	0.01	0.01	0.01	1	1	1	0.01	0.01
In	0.01	0.01	1	1	1	1	1	0.01
Se	0.01	0.01	1	0.01	0.01	1	1	0.5
Au	0.01	1	0.01	0.01	0.01	0.01	0.5	1

Third window of time: 60-80 Ma.

	NA	SA	EA	Af	Md	In	Se	Au
NA	1	1	1	0.01	0.01	0.01	0.01	0.01
SA	1	1	0.01	0.01	0.01	0.01	0.01	1
EA	1	0.01	1	0.01	0.01	0.01	1	0.01
Af	0.01	0.01	0.01	1	1	1	0.01	1
Md	0.01	0.01	0.01	1	1	1	0.01	0.01
In	0.01	0.01	0.01	1	1	1	0.01	0.01
Se	0.01	0.01	1	0.01	0.01	0.01	1	0.01
Au	0.01	1	0.01	1	0.01	0.01	0.01	1

Fourth Window of time: before 80 Ma

	NA	SA	EA	Af	Md	In	Se	Au
NA	1	0.01	1	0.01	0.01	0.01	0	0.01
SA	0.01	1	0.01	1	1	1	0	1
EA	1	0.01	1	0.01	0.01	0.01	0	0.01
Af	0.01	1	0.01	1	1	1	0	1
Md	0.01	1	0.01	1	1	1	0	1
In	0.01	1	0.01	1	1	1	0	1
Se	0	0	0	0	0	0	1	0
Au	0.01	1	0.01	1	1	1	0	1

Fig A3.1. Maximum clade credibility tree of the clusioid clade based on the analysis of a combined four-gene data set (BC placement of *Paleoclusia*; see text for details). The phylogeny and divergence times were simultaneously estimated using BEAST. Divergence time estimates were obtained by using three fossil constraints and assigning a uniform distribution to the root node between 89.9 and 125 Ma based on the youngest age possible for the *Paleoclusia* fossil and the oldest occurrence of tricolpate pollen grains representing the eudicot clade, respectively. Fossil names and arrows indicate the placement of fossil constraints. Posterior probabilities converted to percentages are given above the branches; only nodes receiving > 50% supported are annotated. Error bars at each node represent the 95% highest posterior distributions of divergence times. Scale bar represents the major Cretaceous and Cenozoic intervals. Bon. = Bonnetiaceae, Crat. = Cratoxyleae, End.= Endodesmieae, P.= Pleistocene, Pl. = Pliocene, Out.= outgroups, Tr. = Tristichoideae, W.= Weddellinoideae.

Fig A3.1 (Continued).

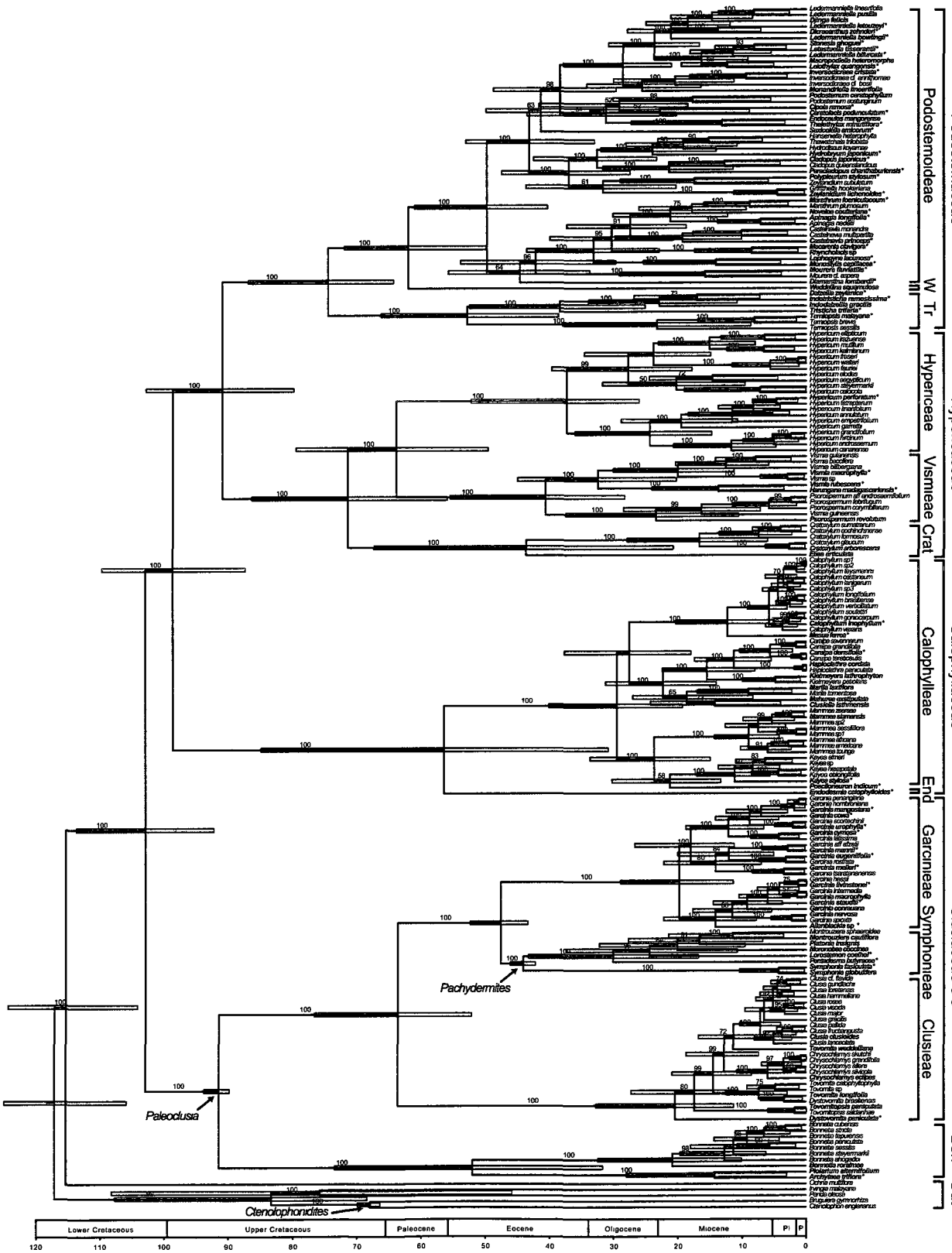
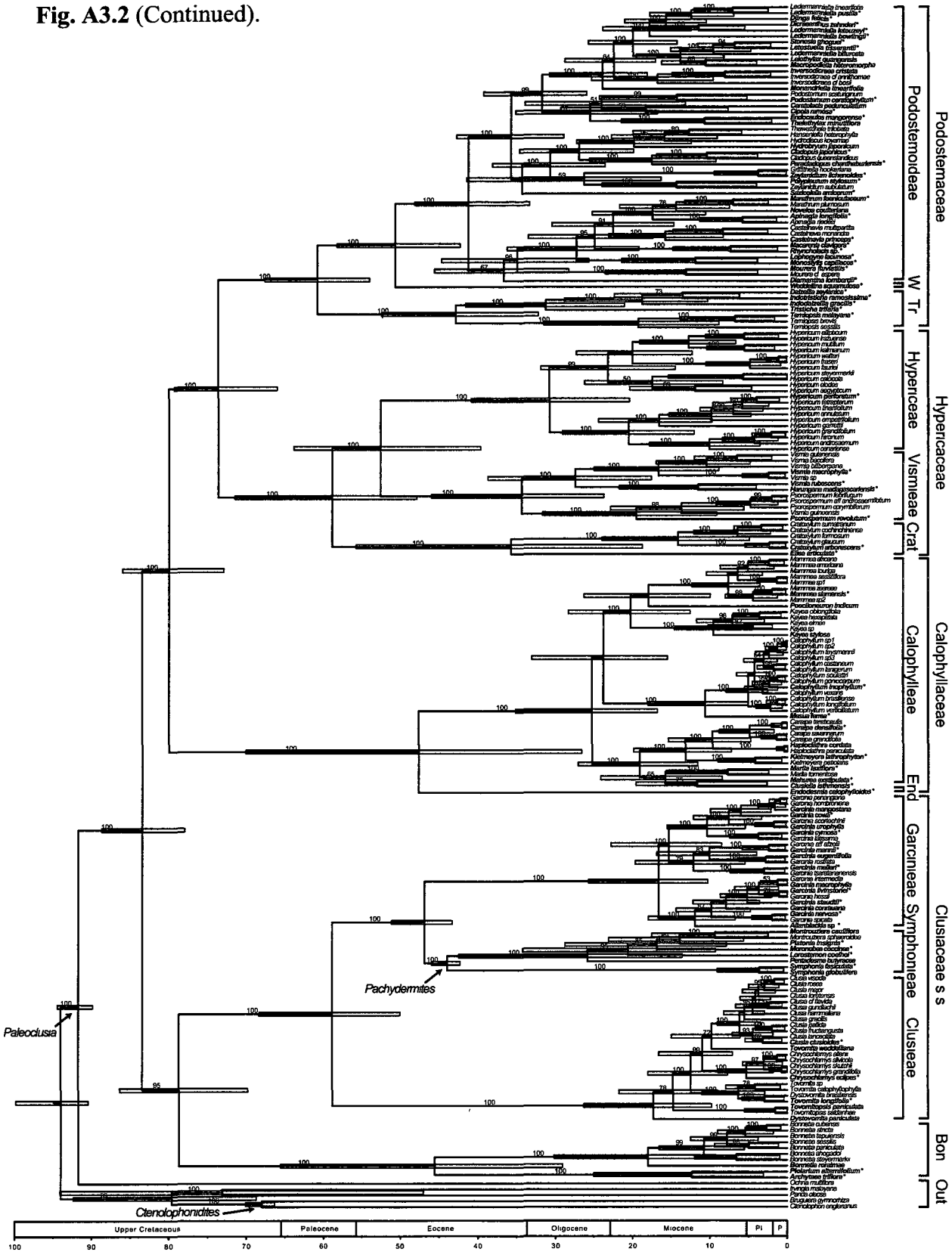


Fig. A3.2. Maximum clade credibility tree of the clusioid clade based on the analysis of a combined four-gene data set (OC placement of *Paleoclusia*; see text for details). The phylogeny and divergence times were simultaneously estimated using BEAST. Divergence time estimates were obtained by using three fossil constraints and assigning a uniform distribution to the root node between 89.9 and 125 Ma based on the youngest age possible for the *Paleoclusia* fossil and the oldest occurrence of tricolpate pollen grains representing the eudicot clade, respectively. Fossil names and arrows indicate the placement of fossil constraints. Posterior probabilities converted to percentages are given above the branches; only nodes receiving > 50% supported are annotated. Error bars at each node represent the 95% highest posterior distributions of divergence times. Scale bar represents the major Cretaceous and Cenozoic intervals. Bon. = Bonnetiaceae, Crat. = Cratoxyleae, End.= Endodesmieae, P.= Pleistocene, Pl. = Pliocene, Out.= outgroups, Tr. = Tristichoideae, W.= Weddellinoideae.

Fig. A3.2 (Continued).



APPENDIX 1:

Phylogenetic placement of *Rheopteris* and the polyphyly of *Monogramma* (Pteridaceae s.l.): Evidence from *rbcL* sequence data

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Phylogenetic Placement of *Rheopteris* and the Polyphyly of *Monogramma* (Pteridaceae s.l.): Evidence from *rbcL* Sequence Data

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Abstract—Recent molecular investigations have elucidated the generic and subgeneric relationships of most vittarioid genera (Pteridaceae sensu lato pro parte) However, the phylogenetic placement of *Monogramma* and *Rheopteris* remains to be examined The inclusion of the monotypic *Rheopteris* in the vittarioids has been questioned since its description half a century ago, and although the placement of *Monogramma* within the vittarioids is well supported with nonmolecular characters, its relationship to other members of the vittarioid clade is unknown We present new phylogenetic evidence from plastid *rbcL* sequence data indicating that *Rheopteris cheesmaniae* is well supported as a member of the vittarioid clade, and that *Monogramma* is polyphyletic Data from molecular and nonmolecular characters suggest that a clade containing *Rheopteris* and part of *Monogramma* (i e those species sometimes recognized in the genus *Vaginularia*) represents the earliest diverging lineage within the vittarioids, and that remaining members of *Monogramma* are derived from within *Haplopteris* Our study supports the separation of *Vaginularia* from *Monogramma* sensu stricto

Keywords—ferns, *Haplopteris*, *Monogramma*, *Rheopteris*, *Vaginularia* Vittariaceae

The vittarioids [i e Pteridaceae sensu lato (s l) pro parte, sensu Smith et al (2006)] are a clade (Crane et al 1995, Hasebe et al 1995) of approximately 100–130 species of mostly epiphytic or lithophytic ferns, the majority of which are found in the damp forests of the New and Old World tropics (Lindsay 2003) Vegetative features for the group include the lack of sclerenchyma in their stems, the presence of spicule cells in the epidermis of their fronds, simple petiolar structure, and clathrate scales borne on their stems While most species have simple fronds with reticulate venation, some have extremely reduced laminae consisting either of only a costal vein or of a costal vein plus a small number of lateral veins Reproductively, members of the vittarioids possess smooth spores, no true indusium, often have soral paraphyses, and in most genera the sporangia are arranged in parallel or reticulate soral lines (Kramer 1990, Lindsay 2003) Their gametophytes have a ribbon-shaped, perennial thallus with fusiform gemmae on the margin, which aid in asexual reproduction (Goebel 1888, Goebel 1896, Farrar 1974) These characteristics contrast with the typical heart-shaped, short-lived, non-gemmae producing gametophytes of most ferns (Atkinson and Stokey 1964, Nayar and Kaur 1969, Farrar 1974) Vittarioid gametophytes have only been observed for 18 species (Lindsay 2003) and as a result most workers have based their classification primarily on morphological characteristics of the sporophyte

Vittarioid sporophytes are highly simplified, a condition that has been suggested as an adaptation to their epiphytic and lithophytic lifestyle (Kramer 1990) This simplification offers little in the way of morphological and anatomical characters to discern phylogenetic relationships within the group (Crane et al 1995, Lindsay 2003) Additionally, this simplification has confounded the elucidation of relationships between major vittarioid subclades and hampered the placement of the rarely collected and narrowly endemic *Rheopteris* *Rheopteris* is monotypic and has sometimes been associated with the vittarioids but does not exhibit the simplified morphology of most vittarioids, making it difficult to compare with these species on nonmolecular grounds

Rheopteris cheesmaniae is a climbing epiphyte known from only three collections from the mountains of West Sepik Province, Papua New Guinea (Lindsay 2003) Its phylogenetic position within pteridophytes has been uncertain since its description over a half century ago (Alston 1956) Alston refrained from assigning the genus to any family, and most current workers have tentatively placed it with the vittarioids on the basis of morphology, anatomy, and unpublished molecular data (Kramer 1990, Tryon and Lugardon 1991, Brummitt 1992, Lindsay 2003, Smith et al 2006) The ambiguity of its placement is due to its possession of some features that characterize the vittarioids, while also having unusual characters that are rare or absent within the group Shared features supporting its inclusion in the vittarioids include the presence of spicule cells in the upper epidermis of the fronds, clathrate scales, paraphyses, smooth spores, and the absence of indusia However, its stiff, erect, simply pinnate fronds with free veins and round sori are highly atypical of the vittarioids Gametophytes of *R cheesmaniae* have not been described (Lindsay 2003)

Monogramma (Poir) Commerson ex Schkuhr is among the most simplified of the vittarioid genera, with some species being little over 1 mm wide and 1 cm long While its placement as a member of the vittarioids is not in question due to its many anatomical and morphological features shared with the group (Kramer 1990, Crane 1997, Lindsay 2003), its relationship to other vittarioids is unclear (Crane 1997) *Monogramma* is most often treated as a single genus (Benedict 1911, Williams 1927, Kramer 1990, Tryon and Lugardon 1991, Smith et al 2006), but other classifications (Copeland 1947, Crabbe et al 1975, Tagawa and Iwatsuki 1985, Andrews and Pedley 1990, Parris et al 1992) have segregated the genus *Vaginularia* Fée from *Monogramma* sensu stricto (s s) on morphological grounds *Monogramma* s s contains taxa in which the fronds have only a costal vein, while *Vaginularia* has fronds with a costa and a few lateral veins Other differences between these two groups are presented by Benedict (1911) and Copeland (1947) They note that members of *Monogramma* s s have paraphyses with funnel-shaped apical cells and an annulus of approximately 20 cells Members of

Vaginularia, on the other hand, have paraphyses with noncapitate apical cells and an annulus of 14–16 cells

A recent molecular investigation of the vittarioids has clarified relationships among many of the major subclades within the group (Crane et al 1995), and accompanying taxonomic revisions (Crane 1997) have been made to reflect these insights However, due to its rarity and the lack of adequate material, *Rheopteris* has yet to be placed phylogenetically There are also no published phylogenetic studies that have included *Monogramma*

The purpose of our study is to i) assess the phylogenetic placement of *Rheopteris* to determine if molecular evidence supports its inclusion in the vittarioids, and ii) to determine the phylogenetic placement of *Monogramma* s l within the vittarioids To accomplish these objectives we assembled a phylogeny of the vittarioids using the plastid gene *rbcL*, which included *R cheesmaniae*, four representatives of *Monogramma* s l , and several other previously unsampled vittarioid species *rbcL* has been especially effective in elucidating relationships in the vittarioids (Crane et al 1995) and more broadly across ferns (Crane et al 1995, Hasebe et al 1995) We also gathered new morphological data from these taxa to conduct character-state optimizations to aid in the interpretation of our molecular results

Materials and Methods

Taxonomic Sampling—We included 109 *rbcL* sequences in this study spanning all major fern lineages sensu Smith et al (2006, Appendix 1), including representatives from all genera of the vittarioids sensu Crane (1997) *Ananthacorus*, *Anetium*, *Antrophyum*, *Haplopteris*, *Hecistopteris*, *Monogramma* s l , *Polytaenium*, *Radiovittaria*, *Scoliosorus*, and *Vittaria* We obtained 13 new *rbcL* sequences (Appendix 1) from the vittarioids, including accessions of *Rheopteris cheesmaniae*, *Monogramma acrocarpa*, *M angustissima*, *M dareicarpa*, and *M trichoidea* Additional sequences not generated by us were acquired from GenBank (Appendix 1) Genomic DNA of *Rheopteris cheesmaniae* was extracted from a 24-yr-old herbarium specimen at the Harvard University Herbaria (*Croft 1716 [A]*) This specimen can be viewed online at http //asaweb huh harvard edu 8080/databases/specimens?barcode=219538 Our sampling of *Monogramma* s l included taxa from each of the two major subgroups of the genus, which are sometimes segregated as *Monogramma* s s (*M dareicarpa*) and *Vaginularia* Fée (*M acrocarpa*, *M angustissima*, and *M trichoidea*, Kramer 1990, Crane 1997, Lindsay 2003) The remaining additions have not been included in previous molecular phylogenetic studies and were added for an ongoing project on the taxonomy and biogeography of the vittarioids *Lycopodium digitatum* and *Cycas circinalis* were used as outgroups following Pryer et al (2001)

DNA Sequencing—Total cellular DNA was prepared with the DNAesy Plant Mini Kit Protocol (Qiagen, Valencia, California) Amplification and sequencing protocols for *rbcL* followed Little and Barrington (2003, see also P Wolf's website at http //bioweb usu edu/wolf/rbcL%20primer%20map htm) using primers F1F (5'-ATGTCACCACAAACAGAAAC-TAAAGCAAGT-3'), 26F (5'-ATGTCACCACAAACAGAGACTAAAGC-3') and F1379R (5'-TCACAAGCAGCAGCTAGTTCAGGACTC-3') Internal primers 656F (5'- CTGCAGGTACATGYGAAGARATG-3'), and 382R (5'-CACYTGAATCCCRTGAGG-3') were also used when necessary

Phylogenetic Analyses—Nucleotide sequences were aligned by eye The ends of sequences were trimmed from each data set to maintain complementary data among taxa Missing data accounted for 0 9% of the data matrix The data matrix, trees, and voucher information are available in TreeBASE (study number S1833) or GenBank (Appendix 1)

Maximum-parsimony (MP) analyses were implemented with PAUP* ver 4 0b10 (Swofford 2003) A heuristic search of 100 random taxon addition replicates was conducted with tree-bisection-reconnection (TBR) branch swapping and MulTrees on Characters were weighted equally and character states were unordered Gaps were treated as missing and included in the analyses Bootstrap support (Felsenstein 1985) for each clade was estimated from 1,000 heuristic search replicates as above with random taxon addition holding no more than ten trees per replicate

Maximum likelihood (ML) analyses were implemented with TREEFINDER ver June 2007 (Jobb et al 2004, Jobb 2007) under the GTR + I + Γ model with all parameters estimated from the data We used four starting trees to avoid getting trapped in local optima Three of these starting trees were obtained using the "Generate Start Trees" option in TREEFINDER with an initial neighbor-joining tree specified as the user defined "center tree " The fourth starting tree was a randomly selected tree (of twelve) recovered using parsimony To select the optimal model of sequence evolution for the data set we performed a series of hierarchical likelihood ratio tests (Felsenstein 1981, Huelsenbeck and Rannala 1997) and calculated the Akaike information criteria (Akaike 1974) using Modeltest ver 3 7 (Posada and Crandall 1998) Both tests resulted in the same optimal model of evolution Bootstrap support was estimated in TREEFINDER from 100 replicates using the default settings and the same four starting trees listed above

Hypothesis Testing—To assess alternate topological placements of *Rheopteris* and to test the monophyly of *Monogramma* s l we employed the Shimodaira-Hasegawa (SH, Kadowaki et al 1996) and Approximately Unbiased (AU, Shimodaira 2002) tests using ML, and the Templeton test (Templeton 1983, Larson 1994, Mason-Gamer and Kellogg 1996) using MP To do this we first conducted searches using ML and MP enforcing a number of less optimal topological constraints First, we examined the robustness of the placement of *Rheopteris* as a member of the vittarioids in which *Rheopteris* was i) excluded from crown group vittarioids, and ii) excluded from stem group vittarioids (i e the vittarioids plus the next well-supported node outside of this clade, the vittarioids plus *Adiantum*) Second, we examined the robustness of conflicting placements of *Rheopteris* within the vittarioids between analyses using MP and ML Since *Rheopteris* was placed as sister to the clade containing *Monogramma trichoidea*, *M acrocarpa*, and *M angustissima* in all analyses, we constrained this entire clade either as sister to the core vittarioids (as inferred using MP), or as sister to a subclade containing *Haplopteris*, *Hecistopteris*, *Monogramma dareicarpa*, and *Radiovittaria* (as inferred using ML) A third constraint was conducted to test the monophyly of *Monogramma* s l In this constraint, all species of *Monogramma* were held to be monophyletic All resulting topologies were then tested against the most optimal topologies as stated above

Character-State Optimization—To determine if nonmolecular data could be used to distinguish between alternative placements of *Rheopteris*, we mapped morphological and anatomical characters onto conflicting molecular-based topologies with MacClade version 4 08 using parsimony (Maddison and Maddison 2005) The topologies used for inferring patterns of morphological evolution were reduced from the full taxonomic sampling (i e 109 accessions) to include the vittarioids (including *Rheopteris* and *Monogramma* s l) plus their outgroup, *Adiantum* We scored seven morphological and anatomical characters for 36 vittarioids and three *Adiantum* species (Table 1), including clathrate scales (present or absent), soral paraphyses (present or absent), frond morphology (simple or compound), sclerenchyma (present or absent), spore shape (bilateral or tetrahedral), and paraphysis apical cell type (slender, spherical, or funnel-shaped) These characters and their associated states have been previously described in morphological and phylogenetic studies of the vittarioids (Nayar 1962, Kramer 1990, Farrar 1993, Crane 1997, Lindsay 2003), and were selected on the basis of their utility in distinguishing major subgroups of vittarioids The absence of sclerenchyma in the roots of *Rheopteris cheesmaniae* has previously been reported by Schneider (1996) To investigate the presence of sclerenchyma in the remaining tissues, we stained cross-sections of a pinnule, stipe, and rhizome of this species with phloroglucinol, a test for lignin (Johansen 1940) If lignin is present the cells become red-violet We use the term sclerenchyma as defined by Esau (1965), i e "complexes of thick-walled cells, often lignified, whose primary function is mechanical "

The literature is conflicting in describing the venation patterns in species of *Monogramma* s l with lateral veins arising from the costal vein (i e those species sometimes segregated as *Vaginularia*) Some sources indicate that these species have free venation (Copeland 1947, Kramer 1990), while others indicate that the same species have anastomosing venation (Benedict 1911, Crane et al 1995) Similarly, Crane (1997) describes the venation in members of *Monogramma* s l as free, but in his key to the vittarioid genera in that same paper he uses "vein single or veins anastomosing" in the couplet leading to *Monogramma* s l

To investigate venation patterns in *Monogramma* s l we rehydrated fronds of herbarium specimens, cleared them with bleach, and examined them under a dissecting microscope Sporangia and paraphyses were carefully removed to trace venation when branching was obscured To observe general surface morphology, we then stained all cleared fronds with Safranin O, a stain which highlights cutinized, lignified, and suber-

TABLE 1 Characters and character-states used for character-state optimization. Characters are 1) clathrate scales, 2) soral paraphyses, 3) frond morphology, 4) sclerenchyma, 5) venation, 6) spore shape, and 7) paraphysis apical cell type For the characters clathrate scales, soral paraphyses, and sclerenchyma, "0" indicates absence while "1" indicates presence For frond morphology, "0" indicates simple fronds and "1" indicates compound fronds, for venation, "0" indicates reticulate venation and "1" indicates free venation, for spore shape "0" indicates tetrahedral spores and "1" indicates bilateral spores, for paraphysis apical cell type "0" indicates slender apical cells, "1" indicates spherical apical cells, and "2" indicates funnel-shaped apical cells Unknown character-states are denoted with a "?", inapplicable characters are denoted by a "—"

	Characters and character-states						
Taxon	1	2	3	4	5	6	7
Adiantum capillus-veneris L	0	0	1	1	1	0	—
Adiantum pedatum L	0	0	1	1	1	0	—
Adiantum raddianum C Presl	0	0	1	1	1	0	—
Ananthacorus angustifolius (Sw) Underw & Maxon	1	1	0	0	0	1	0
Anetium citrifolium (L) Splitg	1	0	0	0	0	0	—
Antrophyum callifolium Blume (sample 1)	1	1	0	0	0	0	0
Antrophyum callifolium Blume (sample 2)	1	1	0	0	0	0	0
Antrophyum callifolium Blume (sample 3)	1	1	0	0	0	0	0
Antrophyum plantagineum (Cav) Kaulf	1	1	0	0	0	0	1
Antrophyum reticulatum (G Forst) Kaulf	1	1	0	0	0	0	0
Haplopteris anguste elongata (Hayata) E H Crane	1	1	0	0	0	1	2
Haplopteris ensiformis (Sw) E H Crane	1	1	0	0	0	1	2
Haplopteris flexuosa (Fee) E H Crane	1	1	0	0	0	1	2
Haplopteris fudzinoi (Makino) E H Crane	1	1	0	0	0	1	2
Haplopteris scolopendrina (Bory) C Presl	1	1	0	0	0	1	2
Haplopteris sp (sample 1)	1	1	0	0	0	1	2
Haplopteris sp (sample 2)	1	1	0	0	0	1	2
Haplopteris zosterifolia (Willd) E H Crane	1	1	0	0	0	1	2
Hecistopteris pumila (Spreng) J Sm	1	1	0	0	1	0	2
Monogramma acrocarpa (Holttum) D L Jones	1	1	0	0	1	0	0
Monogramma angustissima (Brack) comb ined	1	1	0	0	1	0	0
Monogramma dareicarpa (sample 1) Hook	1	1	0	0	1	1	2
Monogramma dareicarpa (sample 2) Hook	1	1	0	0	1	1	2
Monogramma trichoidea (Fee) J Sm ex Hook	1	1	0	0	1	0	0
Polytaenium cajenense (Desv) Benedict	1	0	0	0	0	0	—
Polytaenium lanceolatum (L) Benedict (non Desv)	1	0	0	0	0	0	—
Polytaenium lineatum (Sw) J Sm	1	0	0	0	0	0	—
Radiovittaria gardneriana (Fee) E H Crane	1	1	0	0	0	1	2
Radiovittaria minima (Baker) E H Crane	1	1	0	0	0	1	2
Radiovittaria remota (Fee) E H Crane	1	1	0	0	0	1	2
Radiovittaria stipitata (Kunze) E H Crane	1	1	0	0	0	1	2
Rheopteris cheesmaniae Alston	1	1	1	0	1	0	1
Scoliosorus boryanus (Willd) E H Crane	1	1	0	0	0	1	1
Scoliosorus ensiformis (Hook) T Moore	1	1	0	0	0	1	1
Vittaria appalachiana Farrar & Mickel	1	?	0	0	?	?	?
Vittaria dimorpha Mull Berol	1	1	0	0	0	0	0
Vittaria graminifolia Kaulf	1	1	0	0	0	0	0
Vittaria isoetifolia Bory	1	1	0	0	0	1	0
Vittaria lineata (L) Sm	1	1	0	0	0	1	0

ized cell walls (Ruzin 1999) We recorded frond venation and surface morphology in those *Monogramma* species reported as having lateral veins (*M acrocarpa*, *M emarginata*, *M paradoxa*, *M paradoxa* var *angustissima*, *M subfalcata*, and *M trichoidea*) and in those species reported to possess only a costal vein (i e *Monogramma* s s , *M dareicarpa* and *M graminea*)

RESULTS

Sequences/Matrices—Our nucleotide sequence alignment was 1205 base pairs in length and required no indels Five hundred fifty-one of the characters were parsimony-informative (46% of the total data)

Phylogenetic Analyses—The MP and ML topologies (Figs 1, 2, full trees reduced to vittarioids plus their closest relative *Adiantum*) were very similar with respect to relationships of most major fern lineages sensu Hasebe et al (1995) Similarly, relationships within the vittarioids were largely consistent with Crane et al (1995)

MP analyses yielded 12 most parsimonious trees (Fig 1), which were very similar in regard to relationships within the vittarioid clade, and all topologies placed a monophyletic *Adiantum* as sister to the vittarioids The vittarioids, including *Rheopteris*, were strongly supported [bootstrap percentage (BP) 100] All vittarioid genera were monophyletic and received strong support (BP ≥ 95) except *Haplopteris* and *Monogramma* s l Relationships between major vittarioid subclades, however, were poorly supported *Monogramma trichoidea*, *M acrocarpa*, and *M angustissima* (hereafter referred to as *Vaginularia trichoidea*, *V acrocarpa*, and *V angustissima* or the "*Vaginularia* clade" to aid in the interpretation of the results) formed a strongly supported clade (BP 100), which was moderately placed (BP 72) as sister to *Rheopteris*, thus entire clade was in turn weakly placed (BP ≤ 50) as sister to the remaining vittarioids The remaining vittarioids belonged to two major clades The first was strongly supported (BP 100) and contained two well-supported subclades (BP 100) The first subclade included *Monogramma dareicarpa* strongly nested (BP 97) in *Haplopteris*, and the second subclade contained

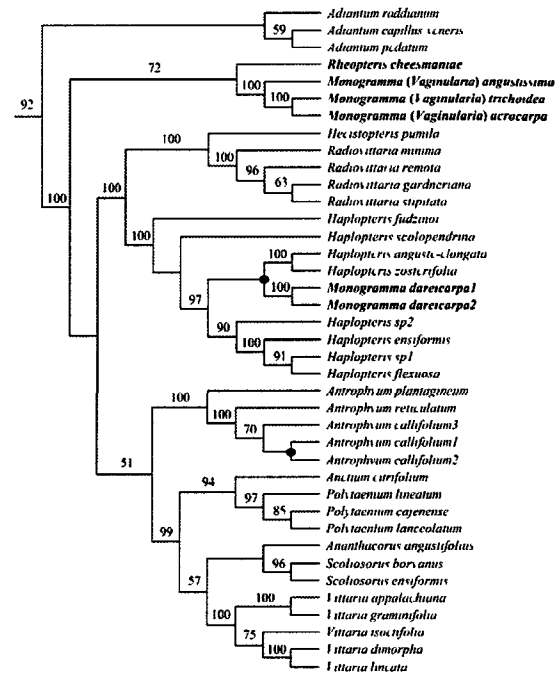

FIG 1 One of 12 most parsimonious trees based on plastid *rbcL* sequence data Figure reduced from 109 taxa spanning all major fern lineages to show only the vittarioids [cf Vittariaceae of Crane (1997) including *Rheopteris*] plus their outgroup, *Adiantum* Bootstrap values are given for clades supported at > 50% Length = 5841, CI = 0 202, RI = 0 629 Black dots indicate nodes that collapse in the strict consensus tree

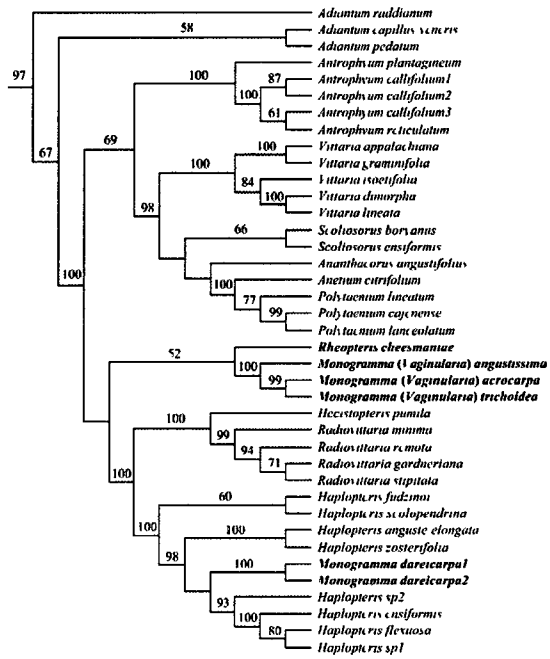

FIG 2 Maximum likelihood tree topology (–ln L = –27185 16) based on plastid *rbcL* sequence data Figure reduced from 109 taxa spanning all major fern lineages to show only the vittarioids [cf Vittariaceae of Crane (1997) including *Rheopteris*] plus their outgroup, *Adiantum* Bootstrap values are given for clades supported at > 50%

Radiovittaria and *Hecistopteris* The second major clade was poorly supported (BP 51) Within this clade, *Antrophyum* was sister to a strongly supported (99 BP) clade containing *Anetium, Ananthacorus, Polytaenium, Scoliosorus,* and *Vittaria Anetium* and *Polytaenium* formed a strongly supported clade (94 BP), which was sister to a weakly supported (BP 57) clade containing *Ananthacorus, Scoliosorus,* and *Vittaria* Within the latter clade, *Vittaria* was sister to a poorly supported clade (BP ≤ 50) containing *Ananthacorus* and *Scoliosorus*

The ML topology (Fig 2) was very similar to the MP topology and no clades conflicted at ≥ 70 BP We detected seven poorly supported differences between results from ML and MP First, *Adiantum* was not monophyletic a weakly supported (BP 58) clade containing *A capillus-veneris* and *A pedatum* was weakly supported (BP 67) as the sister taxon to the vittarioids Second, the clade containing *Rheopteris, Vaginularia acrocarpa, V angustissima,* and *V trichoidea* was weakly placed (BP ≤ 50) as sister to the clade containing *Haplopteris, Hecistopteris, Monogramma dareicarpa,* and *Radiovittaria* Third, *Antrophyum callifolium* was not monophyletic *A callifolium* (accession 3) was weakly placed (BP 61) as sister to *A reticulatum* rather than with the two other accessions of *A callifolium* Fourth, *Vittaria* and the clade containing *Anetium* and *Polytaenium* switched positions relative to the MP result *Vittaria* was instead placed sister to a clade containing *Ananthacorus, Anetium, Polytaenium,* and *Scoliosorus* Fifth, *Ananthacorus* was weakly placed (BP ≤ 50) as sister to the *Anetium/Polytaenium* clade rather than sister to *Scoliosorus* Sixth, *Haplopteris scolopendrina* was placed as sister to *H fudzinoi* (BP 60) Seventh, the two *M dareicarpa* samples were weakly placed (BP ≤ 50) as sister to a clade with *Haplopteris* sp 1, *H* sp 2, *H ensiformis,* and *H flexuosa,* rather than sister to *H anguste-elongata* and *H zosterifolia* as in the MP results

Given the weak support for the nonmonophyly of *A callifolium* combined with better evidence from the MP analyses supporting its monophyly (BP 70), we will not discuss the implications of this result further

Hypothesis Testing—We rejected the hypothesis that *Rheopteris* is not a member of the stem group vittarioids (Templeton $p \leq 0\ 01$, SH $p < 0\ 01$, AU $p < 0\ 01$) and were unable to reject the hypothesis that *Rheopteris* is not a member of the crown group vittarioids (Templeton $0\ 19 < p < 0\ 46$, SH $p = 0\ 71$, AU $p = 0\ 33$) Conflicting placements of the clade containing *Rheopteris, Vaginularia trichoidea, V acrocarpa,* and *V angustissima* within the vittarioid clade could not be rejected (Templeton $0\ 56 < p < 0\ 83$, SH $p = 0\ 81$, AU $p = 0\ 56$) We also rejected the hypothesis that *Monogramma* s l is monophyletic (Templeton $p < 0\ 01$, SH $p < 0\ 01$, AU $p < 0\ 01$)

Character-State Optimization—No sclerenchyma was evident in the pinnule, stipe, or rhizome of *Rheopteris* Cells in the sectioned material, including parenchyma and tracheids, did stain red-violet, indicating the presence of lignin, but none appeared thick-walled We observed free venation in all species of *Monogramma* s l with lateral veins (i e species sometimes assigned to *Vaginularia*) In these species the lateral veins run parallel with and very close to the costal vein and it is on these lateral branches, not the vein representing the continuation of the costal vein, that the sori develop Safranin O staining also revealed tiny two or three-celled, rigid hairs scattered over the frond surfaces of *Monogramma dareicarpa* and *M graminea,* putative members of *Monogramma* s s These hairs were not present in *Monogramma* species with branched venation, i e putative members of *Vaginularia* Two sources list *Monogramma* s l as having tetrahedral spores (Kramer 1990, Crane 1997) We examined many specimens of *Monogramma dareicarpa* and all unequivocally had bilateral spores, so we scored this species as having bilateral spores

Total tree length was most optimal when nonmolecular characters were mapped onto the MP topologies (length = 18 steps) rather than the ML topology (length = 20 steps) Character-state optimizations were identical for five of the seven characters we examined (i e clathrate scales, soral paraphyses, frond morphology, sclerenchyma, and paraphysis apical cell type), but were more optimal on the MP topologies for venation and spore shape (Fig 3) Each of these latter two characters was a single step longer when optimized onto the ML topology

DISCUSSION

The phylogenetic placement of *Rheopteris cheesmaniae* has been uncertain since its description (Alston 1956, Kramer 1990, Tryon and Lugardon 1991, Brummitt 1992, Lindsay 2003) Molecular and nonmolecular data presented here clearly support its inclusion in the vittarioids, perhaps as sister to *Vaginularia* However, the infrafamilial placement of the *Rheopteris/Vaginularia* clade remains unclear MP places it sister to the remaining vittarioids (Fig 1), while ML places it sister to a clade containing *Haplopteris, Monogramma dareicarpa, Hecistopteris,* and *Radiovittaria* (Fig 2)

Putative synapomorphies for the vittarioids, including *Rheopteris,* consist of the presence of spicule cells in the epi-

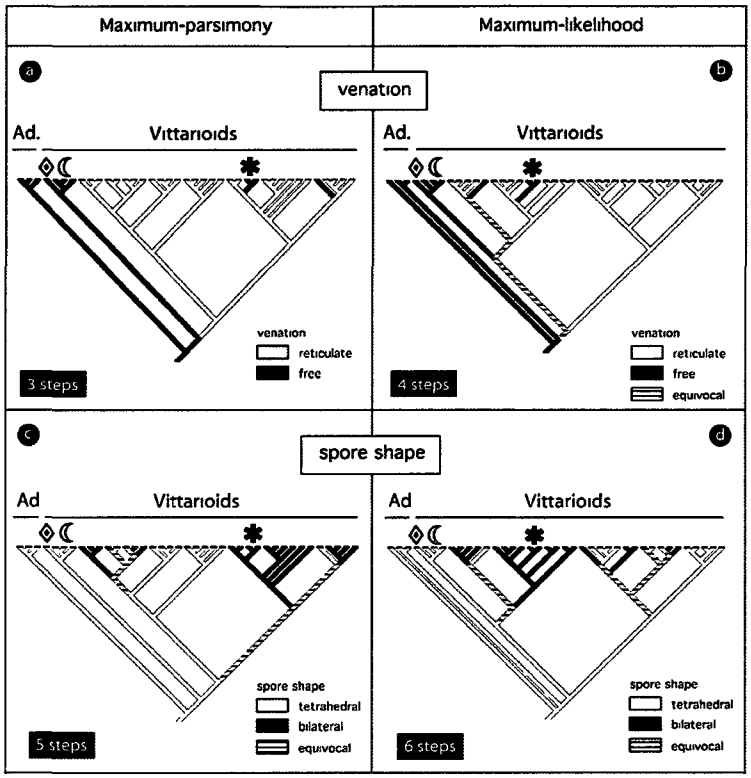

FIG 3 Most parsimonious character-state optimizations of venation and spore shape when reconstructed on the maximum parsimony (MP, a, c) and maximum likelihood (ML, b, d) topologies Topologies reduced from 109 taxa spanning all major fern lineages to show only vittarioids [cf Vittariaceae of Crane (1997) including *Rheopteris*] plus their outgroup, *Adiantum* (Ad) MP topology shown is one of 12 randomly selected MP trees, character-state optimizations do not change across this set of trees Each character undergoes fewer character-state changes when optimized on the MP topology compared to optimization on the ML topology Symbols indicate the placement of *Monogramma dareicarpa* (*), *Rheopteris cheesmaniae* (◊), and the clade containing *Monogramma* (*Vaginularia*) *acrocarpa, M* (*V*) *angustissima, and M* (*V*) *trichoidea* (☾)

dermis of their fronds and clathrate scales borne on their stems Lack of sclerenchyma has also been reported as putatively synapomorphic for the vittarioids (Bower 1923, Kramer 1990, Lindsay 2003) Expanding on the results of Schneider (1996), who concluded that the roots of *Rheopteris* lack sclerenchyma, our study revealed that *Rheopteris* also lacks sclerenchyma in the pinnule, stipe, and rhizome While these anatomical and morphological features support the placement of *Rheopteris* with the vittarioids, this taxon also possesses characters that are rare or absent in the vittarioids, but which are common in members of the outgroup *Adiantum* (e g stiff, erect, simply pinnate fronds with free venation) The combination of putatively synapomorphic and symplesiomorphic traits in *Rheopteris* suggest that it may be better placed as sister to the vittarioids rather than nested within them Given this set of factors, *Rheopteris* has been suggested as a transitional link bridging members of Pteridaceae s l with the vittarioids (Kramer 1990) Since the vittarioids are nested within Pteridaceae s l (Hasebe et al 1995, Smith et al 2006), a phylogenetic placement of *Rheopteris* as sister to the vittarioids, rather than nested within them, might provide support for the assertion by Kramer (1990) Our data suggest that this is not the case, however, and instead indicate that *Rheopteris* along with part of *Monogramma* (i e the *Vaginularia* clade) belong to an early diverging lineage that is sister to the remaining vittarioids (Fig 1) or alternatively placed as a nested member of the vittarioids (Fig 2) We favor the first scenario slightly (see below), which suggests either the loss of stiff, erect, simply pinnate fronds early in the vittarioids followed by the reversal of these traits in *Rheopteris*, or the retention of these traits in the lineage leading to the *Rheopteris/Vaginularia* clade and then their subsequent loss in *Vaginularia*

Our character-state optimizations of morphology and anatomy support the MP topology in which the *Rheopteris/Vaginularia* clade represents an early diverging lineage of the vittarioids (Fig 3) Evolutionary reconstructions of venation pattern and spore shape are each a single step longer when reconstructed onto the ML topology, in which the *Rheopteris/Vaginularia* clade is placed as a more nested member of the vittarioids Of these two reconstructions, however, only the reduction in step-length of venation pattern is tied to the placement of the *Rheopteris/Vaginularia* clade And while both the ML and MP topologies indicate that *Rheopteris* is sister to *Vaginularia* and that this clade is in turn sister to either the rest of the vittarioids (MP) or one of its major subclades (ML), these associations are not strong and only more and better data may clarify these relationships Nevertheless, the data at

hand, albeit weakly supported, favor the MP over the ML topology

Our data also indicate that the current circumscription of *Monogramma* s l is not warranted and that the recognition of *Monogramma* s s and *Vaginularia* is a better representative of the evolutionary history of the vittarioids In all of our analyses *M dareicarpa* is strongly supported as a nested member of *Haplopteris* while the *Vaginularia* clade appears to be more closely related to *Rheopteris* The polyphyly of *Monogramma* s l is also supported by nonmolecular data Fronds of *Monogramma* s s possess only a costal vein and have paraphyses with a funnel-shaped apical cell, while fronds of *Vaginularia* have a costal vein with one to three free lateral veins and paraphyses with slender apical cells The number of annulus cells between *Monogramma* s s and *Vaginularia* also differs, the former having 20 cells and the latter 14–16 (Copeland 1947) In addition, we determined that members of *Monogramma* s s (*M dareicarpa* and *M graminea*) have very short rigid hairs consisting of two or three cells scattered over the abaxial and adaxial frond surfaces Such hairs are not present in members of *Vaginularia*, but their presence in other vittarioid genera has yet to be investigated The phylogenetic distribution of these hairs in vittarioid taxa is part of a larger on-going investigation by one of us (S L) Paraphysis apical cell type also supports the placement of *M dareicarpa* within *Haplopteris* When this character is optimized onto the MP and ML topologies the funnel-shaped type has arisen only once and is synapomorphic for the clade containing *Monogramma dareicarpa, Haplopteris, Hecistopteris,* and *Radiovittaria* (Table 1) Although the presence of free venation in *M dareicarpa* does not fit this clade, it is easy to imagine that the reduction of fronds to such a small size in this species (i e they are typically less than 1mm wide and 10 mm long) may eliminate all but the costal vein

In light of these well-supported phylogenetic results, the present circumscription of *Monogramma* needs to be reconsidered Although the type species of the genus, *M graminea*, was not included in our study, the morphology of that species is similar to the included species *M dareicarpa*, and there is little doubt that the two species are closely related Since *Monogramma* is nested within *Haplopteris* and is the older of the two names (Crane 1997), *Haplopteris* may need to be synonymized with *Monogramma* in future classifications of the genus Similarly, the type species of *Vaginularia*, included in our study (*M trichoidea*), is more closely related to other vittarioids than to members of *Monogramma* s s , indicating that *Vaginularia* should be recognized as its own entity Under this scenario a number of names could be resurrected, such as *V acrocarpa* Holttum, *V angustissima* (Brack) Mett , *V emarginata* (Brause) Goebel, *V paradoxa* (Fée) Mett , *V subfalcata* (Hook) C Chr , and *V trichoidea* (J Sm) Fée However, any future recircumscription should be guided by increased phylogenetic sampling across the genus

In summary, our evidence from molecular and nonmolecular data firmly supports the inclusion of *Rheopteris cheesmanae* with the vittarioids While more data are needed to place this taxon definitively within the vittarioids, our data point toward the placement of *Rheopteris* as sister to a clade containing *Monogramma trichoidea, M acrocarpa, and M angustissima* (i e *Vaginularia* spp), with this *Rheopteris/Vaginularia* clade perhaps representing the earliest diverging lineage within the vittarioids Our study also reveals that *Monogramma* is not monophyletic and that previous circumscriptions recognizing *Monogramma* s s and *Vaginularia* better reflect the evolutionary history of the group Although it is clear that members of *Monogramma* s s are embedded in *Haplopteris*, more data are needed to better place *Vaginularia* within the vittarioids Future molecular phylogenetic analyses including additional taxa and molecular characters, as well as morphological study of the gametophytes of *Rheopteris, Monogramma* s s , and *Vaginularia* may be especially useful in resolving relationships within the vittarioids In particular, the development and arrangement of the gemmae (when present) have been shown to be phylogenetically informative within the group (Crane et al 1995, Crane 1997) Finally, one additional character that should be examined is the presence of short, rigid, two or three-celled hairs found on the fronds of *Monogramma* s s but not on *Vaginularia* The distribution of these hairs should be investigated in other vittarioid genera to determine their phylogenetic utility

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APPENDIX 1 Taxa, GenBank accession numbers, and voucher information (only for sequences generated in our laboratory) for *rbcL* sequences analyzed Taxa are listed in alphabetical order by genus and species

Vittarioids sequenced for this study—*Antrophyum callifolium* Blume (sample 1), *D J Middleton et al 1419* (A), EU024554 *Antrophyum callifolium* Blume (sample 2), *Lindsay & Middleton 1* (MICH), EU024555 *Antrophyum callifolium* Blume (sample 3), *Lindsay & Middleton 2* (MICH), EU024556 *Haplopteris fudzinoi* (Makino) E H Crane, *K Seto 31617* (A), EU024557 *Haplopteris scolopendrina* (Bory) C Presl, *D J Middleton et al 1400* (A), EU024558 *Haplopteris sp* (sample 1), *Takeuchi 15216* (MICH), EU024559 *Haplopteris sp* (sample 2), *F C How 73766* (GH), EU024560 *Monogramma acrocarpa* (Holttum) D L Jones, *T Ranker 1778* (COLO), EU024561 *Monogramma angustissima* (Brack) comb ined , *W A Sledge 1631* (L), EU024562 *Monogramma dareicarpa* Hook (sample 1), *A H G Alston 14599* (GH), EU024563 *Monogramma dareicarpa* Hook (sample 2), *P J Darbyshire & R D Hoogland 8032* (BM), EU024564 *Monogramma trichoidea* (Fée) J Sm ex Hook , *A C Jeremy 7831* (GH), EU024565 *Rheopteris cheesmaniae* Alston, *Croft 1716* (A), EU024566

Sequences downloaded from GenBank—*Acrostichum aureum* L , U05601 1 *Actinostachys digitata* (L) Wall , U05650 1 *Adiantum capillus-veneris* L , D14880 1 *Adiantum pedatum* L , U05602 1 *Adiantum raddianum* C Presl, U05906 1 *Ananthacorus angustifolius* (Sw) Underw & Maxon, U20932 1 *Anemia mexicana* Klotzsch, U05603 1 *Anetium citrifolium* (L) Splitg , U21284 1 *Angiopteris evecta* (G Forst) Hoffman, L11052 1 *Antrophyum plantagineum* (Cav) Kaulf , U21285 1 *Antrophyum reticulatum* (G Forst) Kaulf , U05604 1 *Arthropteris beckleri* (Hook) Mett , U05605 1 *Asplenium adiantum-nigrum* L , AF318600 1 *Asplenium filipes* Copel , U30605 1 *Athyrium filix-femina* (L) Roth ex Mert , U05908 1 *Azolla caroliniana* Willd , U24185 1 *Blechnum occidentale* L , U05909 1 *Blotiella pubescens* (Kaulf) R M Tryon, U05911 1 *Botrychium strictum* Underw , D14881 1 *Calochlaena dubia* (R Br) M D Turner & R A White, U05615 1 *Cephalomanes thysanostomum* (Makino) K Iwats , U05608 1 *Ceratopteris thalictroides* (L) Brongn , U05609 1 *Cheiropleuria bicuspis* (Blume) C Presl, U05607 1 *Cibotium barometz* (L) J Sm , U05610 1 *Coniogramme japonica* (Thunb) Diels, U05611 1 *Culcita macrocarpa* C Presl, AM177334 1 *Cyathea lepifera* (J Sm ex Hook) Copel , U05616 1 *Cycas circinalis* L , L12674 1 *Davallia mariesii* T Moore ex Baker, U05617 1 *Dennstaedtia punctilobula* (Michx) T Moore, U05918 1 *Dicksonia antarctica* Labill , U05618 1 *Dipteris conjugata* Reinw , U05620 1 *Doryopteris concolor* (Langsd & Fisch) Kuhn, U05621 1 *Elaphoglossum hybridum* (Bory) T Moore, U05924 1 *Equisetum arvense* L , L11053 1 *Gleichenia japonica* Spreng , U05624 1 *Haplopteris anguste-elongata* (Hayata) E H Crane, U21291 1 *Haplopteris ensiformis* (Sw) E H Crane, U21290 1 *Haplopteris flexuosa* (Fée) E H Crane, U05656 1 *Haplopteris zosterifolia* (Willd) E H Crane, U21296 1 *Hecistopteris pumila* (Spreng) J Sm , U21286 1 *Histiopteris incisa* (Thunb) J Sm , U05627 1 *Lindsaea odorata* Roxb , U05630 1 *Lonchitis hirsuta* L , U05929 1 *Loxogramme grammitoides* (Baker) C Chr , U05631 1 *Loxsoma cunninghamii* R Br ex A Cunn , U30834 1 *Lycopodium digitatum* Dill ex A Braun, L11055 1 *Lygodium japonicum* (Thunb) Sw , U05632 1 *Marsilea quadrifolia* L , U05633 1 *Matonia pectinata* R Br , U05634 1 *Metaxya rostrata* (Kunth) C Presl, U05635 1 *Microlepia strigosa* (Thunb) C Presl, U05931 1 *Micropolypodium okuboi* (Yatabe) Hayata, U05658 1 *Monachosorum henryi* Christ, U05932 1 *Nephrolepis cordifolia* (L) C Presl, U05637 1 *Notholaena delicatula* Maxon & Weath , U19500 1 *Notholaena fendleri* Kunze, U27727 1 *Notholaena rosei* Maxon, U27728 1 *Notholaena sulphurea* (Cav) J Sm , U28254 1 *Oleandra pistillaris* (Sw) C Chr , U05639 1 *Onoclea sensibilis* L , U05640 1 *Onychium japonicum* (Thunb) Kunze, U05641 1 *Osmunda cinnamomea* L , D14882 1 *Pellaea andromedifolia* (Kaulf) Fée, U19501 1 *Pellaea boivinii* Hook , U29132 1 *Pellaea cordifolia* (Sessé & Moc) A R Sm , U28253 1 *Pellaea pringlei* Davenp , U28787 1 *Pellaea rotundifolia* (G Forst) Hook , U28788 1 *Plagiogyria japonica* Nakai, U05643 1 *Platyzoma microphyllum* R Br , U05644 1 *Polypodium australe* Fée, U21140 1 *Polytaenium cajenense* (Desv) Benedict, U20934 1 *Polytaenium lanceolatum* (L) Benedict, U21287 1 *Polytaenium lineatum* (Sw) J Sm , U20935 1 *Psilotum nudum* (L) P Beauv , U30835 1 *Pteridium aquilinum* (L) Kuhn, U05646 1 *Pteris fauriei* Hieron , U05647 1 *Pteris vittata* L , U05941 1 *Radiovittaria gardneriana* (Fée) E H Crane, U21294 1 *Radiovittaria minima* (Baker) E H Crane, U21288 1 *Radiovittaria remota* (Fée) E H Crane, U21289 1 *Radiovittaria stipitata* (Kunze) E H Crane, U21293 1 *Rumohra adiantiformis* (G Forst) Ching, U05648 1 *Saccoloma inaequale* (Kunze) Mett , AY612682 1 *Salvinia cucullata* Roxb ex Bory, U05649 1 *Scoliosorus boryanus* (Willd) E H Crane, U20930 1 *Scoliosorus ensiformis* (Hook) T Moore, U20931 1 *Stromatopteris moniliformis* Mett , U05653 1 *Taenitis blechnoides* (Willd) Sw , U05654 1 *Thelypteris beddomei* (Baker) Ching, U05655 1 *Thyrsopteris elegans* Kunze, AM177353 1 *Vittaria appalachiana* Farrar & Mickel, U88961 1 *Vittaria dimorpha* Mull Berol , U21292 1 *Vittaria graminifolia* Kaulf , U21295 1 *Vittaria isoetifolia* Bory, U20936 1 *Vittaria lineata* (L) Sm , U20937 1

APPENDIX 2:

Phylogenetic patterns of species loss in Thoreau'swoods are driven by climate change

(as published in Proceedings of the National Academy of Sciences)

Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change

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Climate change has led to major changes in the phenology (the timing of seasonal activities, such as flowering) of some species but not others The extent to which flowering-time response to temperature is shared among closely related species might have important consequences for community-wide patterns of species loss under rapid climate change Henry David Thoreau initiated a dataset of the Concord, Massachusetts, flora that spans ≈150 years and provides information on changes in species abundance and flowering time When these data are analyzed in a phylogenetic context, they indicate that change in abundance is strongly correlated with flowering-time response Species that do not respond to temperature have decreased greatly in abundance, and include among others anemones and buttercups [Ranunculaceae *pro parte* (*p p*)], asters and campanulas (Asterales), bluets (Rubiaceae *p p*), bladderworts (Lentibulariaceae), dogwoods (Cornaceae), lilies (Liliales), mints (Lamiaceae *p p*), orchids (Orchidaceae), roses (Rosaceae *p p*), saxifrages (Saxifragales), and violets (Malpighiales) Because flowering-time response traits are shared among closely related species, our findings suggest that climate change has affected and will likely continue to shape the phylogenetically biased pattern of species loss in Thoreau's woods

conservation | extinction | phenology | phylogenetic conservatism | phylogeny

The impact of climate change on species and communities has been well documented Arctic forests are shifting poleward and alpine tree lines are shifting upward (1–3), spring flowering time is advancing rapidly (4–7), pest outbreaks are spreading (8), and numerous species are declining in abundance and risk extinction (9) However, despite these generalized trends, species vary dramatically in their responses to climate change For example, although the spring flowering times of many temperate plants are advancing, some are not changing and others are flowering later in the season (5, 10, 11) Understanding the evolutionary (i e , phylogenetic) history of traits that are influenced by climate (e g , flowering phenology) has been an underexplored area of climate change biology, despite the fact that it could prove especially useful in predicting how species and communities will respond to future climate change Closely related species often share similar traits, a pattern known as phylogenetic conservatism (12–16, 17) If closely related species share similar traits that make them more susceptible to climate change (14, 17), species loss may not be random or uniform, but rather biased against certain lineages in the Tree of Life (i e , phylogenetic selectivity, see ref 18) However, a deeper inquiry into these patterns has been hampered largely because adequate datasets documenting community-wide responses to climate change are exceedingly rare

During the mid-19th century, the naturalist and conservationist Henry David Thoreau spent decades exploring the temperate fields, wetlands, and deciduous forests of Concord, Massachusetts, in the northeastern United States He wrote extensively about the natural history of the area (19) and kept meticulous notes on plant species occurrences and flowering times (11, 20) Several botanists have since resurveyed the Concord area, thus providing a unique community-level perspective on changes in its floristic composition and flowering times during the past ≈150 years (11, 20) Despite the fact that ≈60% of all natural areas in Concord are undeveloped or have remained well protected, a striking number of species have become locally extinct 27% of the species documented by Thoreau have been lost, and 36% exist in such low population abundances that their extirpation may be imminent (20) Also, the species that have been lost are overly represented in particular plant families (20), suggesting that extinction risk may be phylogenetically biased

Although habitat loss due to succession and development (e g , loss of wetlands, abandonment of farms, reforestation, and construction of homes and roads) has contributed to decreases in abundance for some species in Thoreau's Concord (20), climate change may also help to explain the seemingly nonrandom pattern of species loss among certain plant groups It has been shown recently (11) that the mean annual temperature in the Concord area has risen by 2 4 °C over the past ≈100 years and that this temperature change is associated with shifts in flowering time species are now flowering an average of 7 days earlier than in Thoreau's time Along with changes in flowering phenology, species range is likely to be influenced by climate change (21) Thus, the Concord surveys provide a unique opportunity to examine the extent to which changes in abundance may be correlated with these climatologically sensitive traits Also, by incorporating phylogenetic history into our analyses, we can test whether species that share similar traits are closely related (i e , phylogenetic conservatism), and to what extent these traits correlate with decreases in abundance Such findings could identify groups of closely related species that are at higher risk of extinction (18, 22)

The data for the 473 species we analyzed were collected by Thoreau (1852–1858), Hosmer (1878, 1888–1902), and Miller-Rushing and Primack (2003–2007) (see *Materials and Methods*, see refs 20 and 23) Scorings include information on changes in species abundance, species habitat, and 2 separate measures of flowering-time response to temperature (i e , the ability of species flowering time to track short-term seasonal temperature changes, and the shift in species flowering time over long-term intervals) We further scored the current mean latitudinal range and native/introduced status of each species We constructed a composite phylogeny of all species to test for (*i*) the phylogenetic conservatism of each trait, and (*ii*) correlations between these traits and change in abundance when accounting for phylogeny

Author contributions C G W and C C D designed research, C G W, B R R B P A J M-R and C C D performed research C G W B R and C C D analyzed data and C G W, B R R B P A J M-R and C C D wrote the paper

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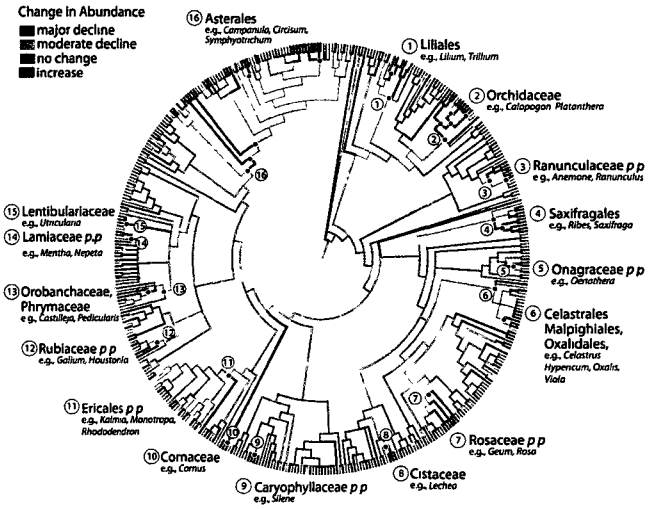

Fig 1 Composite phylogeny of 429 flowering plant species from the Concord flora depicting changes in abundance from 1900 to 2007 Change in abundance ranged on an integer scale from −5 to +4, and was calculated as the difference in abundance for each taxon in 1900 and 2007 based on 7 abundance categories (0 to 6, see *Materials and Methods*) Branch color indicates parsimony character state reconstruction of change in abundance For simplicity, we have indicated this reconstruction by using 4 colors red (major decline, −5 to −3), pink (moderate decline, −2), gray (little to no change, −1 to +1), and blue (increase, +2 to +4) For the complete character reconstruction and taxon labels see Fig S1 Average decline in abundance was calculated for all internal nodes as the mean change in abundance of descendant nodes weighted with branch length information ascertained from divergence time estimates An average decline of 2 5 or greater corresponds to a decline in abundance of 50% or greater, based on our most conservative scoring using 6 abundance categories (0 to 5, see *Materials in Methods*) Clades exhibiting these major declines are indicated with black dots Each of the most inclusive clades exhibiting these declines are indicated in pink and referenced numerically to their clade name Subclades in major decline that are nested within more widely recognized clades are labeled with the more familiar name followed by *pro parte* (*p p*) These clades include some of the most charismatic wildflower species in New England, such as anemones and buttercups (Ranunculaceae *p p*), asters, campanulas, goldenrods, pussytoes, and thistles (Asterales), bedstraws and bluets (Rubiaceae *p p*), bladderworts (Lentibulariaceae), dogwoods (Cornaceae), lilies (Liliales), louseworts and Indian paintbrushes (Orobanchaceae), mints (Lamiaceae *p p*), orchids (Orchidaceae), primroses (Onograceae *p p*), roses (Rosaceae *p p*), saxifrages (Saxifragales), Indian pipes (Ericales *p p*), and St John's worts and violets (Malpighiales)

Results and Discussion

Our results (Fig 1 and Table 1) indicate that change in abundance and flowering-time response traits were phylogenetically conserved, which indicates that species evolutionary history is important to understanding community response to climate change Species that are declining in abundance are more closely related than expected by chance Similarly, species that exhibit similar flowering-time responses to temperature are more closely related than expected by chance In contrast, latitudinal range was not phylogenetically conserved

Table 1 Statistical tests of phylogenetic conservatism and trait correlations with change in abundance

	Phylogenetic conservatism			Trait correlation								
				Model 1			Model 2			Model 3		
Trait	*n*	Observed rank		*n*	Estimate		*n*	Estimate		*n*	Estimate	
Flowering time tracking of seasonal temperature	175	19	**	175	−0 48	*	166	−0 62	*	140	−1 00	***
Shift in flowering time 1850–1900	319	2	***	319	−0 02	***	311	−0 01	*	140	0 03	***
Shift in flowering time 1900–2006	303	2,120	—	303	0 04	***	296	0 03	***	140	0 02	***
Shift in flowering time 1850–2006	271	340	†	271	0 04	***	253	0 03	***	140	—	—
Mean latitudinal range	414	3,705		414	−0 10	***	362	−0 08	***	140	−0 09	***
Change in abundance 1900–2006	429	1	***	—	—	—	—	—	—	—	—	—

Tests used a phylogeny with branch lengths adjusted for time The significance of phylogenetic conservatism was tested by comparing the rank of the observed standard deviation (SD) of descendent trait means to a null model based on 9,999 random iterations of trait distributions across the composite phylogeny The observed rank is compared with a 2-tail test of significance, i e , an observed rank of 250 equals a *P* value of 0 05 Trait correlations were tested by using the comparative methods of generalized estimating equations (GEE) Estimates describe the direction and magnitude of the correlation (e g , a negative estimate [−0 1] of mean latitude with change in abundance suggests that species from more southerly latitudes are increasing in abundance) Model 1 (univariate model), correlation of change in abundance with each trait, Model 2 (multivariate model), correlation of change in abundance with each trait and habitat, abundance (*ca* 1900), flowering season, and native/introduced status as covariates, Model 3 (multivariate model), correlation of change in abundance with all traits and habitat, abundance (*ca* 1900), flowering season, and native/introduced status as covariates (shift in flowering-time response 1850–2006 was excluded due to its high correlation with the other flowering-time shift traits) †, $P = 0\ 1$, *, $P = 0\ 05$, **, $P = 0\ 01$, ***, $P = 0\ 001$, n = sample size

(i e , phylogeny is not important in explaining the latitudinal distribution of species)

The ability of species to track seasonal temperatures was correlated with changes in abundance species whose flowering time does not track seasonal temperature have greatly declined in abundance over the past ≈100 years Similarly, shifts in species flowering time across all 3 long-term time intervals (1850–1900, 1900–2006, and 1850–2006) were correlated with change in abundance species that are not flowering earlier have declined in abundance Last, species range was correlated with change in abundance more northerly species have decreased in abundance in relation to southerly species Our results are robust (*i*) when controlling for multiple variables that may additionally affect decline in abundance [i e , initial abundance, habitat, native/introduced status, and flowering season (date of first flowering), see Table 1], (*ii*) to branch length information [supporting information (SI) Table S1], and (*iii*) to phylogenetic uncertainty (Table S2)

These results demonstrate that there is a phylogenetically *selective pattern of change in abundance* Decreases in abundance have been disproportionately high in certain clades, including asters, bladderworts, buttercups, dogwoods, lilies, louseworts, mints, orchids, saxifrages, and violets (see Fig 1) This result confirms previous floristic studies across similar time spans demonstrating that the risk of plant extinction (i e , occurring in low abundance, see ref 24) is taxonomically (20, 25–27) and phylogenetically (28) shared among close relatives However, to our knowledge our study is the first to report that the phylogenetic selectivity of extinction risk is correlated with traits directly influenced by climate change Species whose flowering times are not responsive to changes in temperature are decreasing in abundance Most strikingly, species with the ability to track short-term seasonal temperature variation have fared significantly better under recent warming trends In addition, species whose flowering times have shifted to be earlier in the year over the long-term have also fared significantly better under recent warming trends Based on our regression estimates (Table 1), change in abundance over the last ≈100 years is greatest when assessed against the ability of species to track short-term seasonal temperature versus long-term flowering shifts Thus, the association between flowering-time tracking and change in abundance is a better estimator of species response to rising temperatures Interestingly, these 2 flowering-time response traits are significantly, but weakly correlated This weak correlation raises the possibility of different mechanisms of phenological response to climate change (e g , plasticity, adaptation, see refs 29 and 30) Alternatively, confounding factors such as changes in population size may affect estimates of long-term shifts in first flowering dates, but would be less likely to influence estimates of tracking climate change over the short term (31)

Asynchronous phenological responses resulting from rapid climate change can have negative fitness effects on organisms, leading to dramatic declines in population sizes or local extinction (32) Selection on flowering phenology may be direct, for example, owing to a lack of available insect pollinators (33, 34) or due to increased flower-predation (35) Interestingly, phenological responses of insects also appear to be correlated with seasonal temperature (7), suggesting that plant species that respond to temperature change may better maintain important synchronous interactions, such as those between plants and pollinators (36), or better avoid negative interactions, such as predation Alternatively, selection on flowering phenology may be indirect by acting on phenological traits that are correlated with flowering time (e g , leafing out times, germination, see refs 37 and 38) For example, earlier snowmelt in the Rocky Mountains has been shown to induce early spring vegetative growth in certain species, exposing young buds and flowers to frost damage and causing declines in the sizes of some populations (39)

Last, the decline of more northerly distributed species suggests yet another impact of climate change shifting species ranges However, in our study species range was not phylogenetically conserved, meaning that it cannot explain the phylogenetic pattern of species loss Thus, our results suggest that flowering-time response, and not species range, better explain the phylogenetic nature of extinction risk among flowering plants experiencing rapid climate change in Concord For this reason, species range models that attempt to predict species response to climate change may be improved if they include species phenology, particularly the ability of species to track seasonal changes in climate

Climate change appears to have had a dramatic role in shaping the contemporary composition of the Concord flora Given that climate models predict at least a 1 1–6 4 °C increase in temperature during this century (22), changes in the Concord flora will likely continue to be shaped in a phylogenetically biased manner Although phylogenetic selectivity of extinction risk has been documented in animals (22) and plants (28), our study provides the strongest evidence to date that the phylogenetic pattern of extinction risk may be due to climate change

To the extent that local extinction of species underlies their global extinction (18, 40), these results represent a link between the impacts of climate change on local community composition and broader patterns of taxonomic selectivity observed in the fossil record during past mass extinction events (41, 42) Patterns of recent species loss under rapid global climate change can potentially illuminate the processes underlying past extinction events where the pattern of loss may be well characterized, but the process is less clear (e g , the Permian–Triassic mass extinction event) In the near term, this pattern of phylogenetic selectivity is likely to have an accelerated impact on the loss of species diversity groups of closely related species are being selectively trimmed from the Tree of Life, rather than individual species being randomly pruned from its tips Given that climate-influenced loss of phylodiversity has been so great in Concord, despite 60% of the area being well protected or undeveloped since the time of Thoreau, a more global approach to conservation prioritization is necessary to minimize future species loss Developing global conservation strategies will necessitate including information not only on species life history, but on their evolutionary history as well (43)

Materials and Methods

Study Site Concord, Massachusetts (42°27′38″ N, 71°20′54″ W), is a small township encompassing 67 km^2 Although the town has undergone extensive development since the time of Thoreau, ≈60% of the total area has been undeveloped or remained well protected through the efforts of numerous national, state, local, and private parks, and land-trusts (20)

Floral Surveys Thoreau surveyed the Concord area for flowering times from 1851 to 1858, Hosmer surveyed the same area from 1888 to 1902, and Primack and Miller-Rushing performed the most recent survey between 2003 and 2007 (20) Thoreau and Hosmer did not generally census graminoids, wind-pollinated trees, and wind/water pollinated aquatics due to the difficulty of determining the start of flowering, Primack and Miller-Rushing also did not sample these groups These exclusions are not likely to affect our results for the following reasons First, the existing sampling includes the majority (≈70%) of species in Concord sensu the most comprehensive flora by Eaton (44) Second, this sampling represents all major branches of the angiosperm phylogeny (Fig S1, www huh harvard edu/research/staff/davis/Fig S1 pdf, references for composite phylogeny construction embedded therein) Third, the exclusion of predominately wind pollinated species is not likely to have an effect on the relationship between change in abundance and flowering-time response traits climate change appears to be much more likely to affect more conspicuously flowered, insect pollinated, species included in our dataset by means of the disruption of plant-pollinator fidelity (36)

Abundance Change The abundances of species were recorded for the 1888–1902 (Hosmer) and 2003–2007 (Primack and Miller-Rushing) inventories

Records from 1888 to 1902 included the following 6 abundance categories Very common, common, frequent, infrequent, uncommon, and rare Abundance categories from 2003 to 2007 were approximated to match the 1888–1902 survey by using Hosmer's journal records, and include very common (found throughout the area), common (occurring in >3 localities), frequent (occurring in 3 localities), infrequent (occurring in 2 localities), rare (occur in 1 locality), and very rare (10 or less individuals in a single locality) These 6 abundance categories were treated as a continuous trait scored from high (6) to low (1) abundance, with an additional scoring of zero for any species absent from a given survey We also analyzed these data with the categories very common and common combined (i e , states 5 to 0) This more conservative scoring did not significantly affect our results (results not shown)

Change in abundance was defined as the difference in abundance between the 1888–1902 and 2003–2007 surveys, 44 taxa that were indicated as rare in 1900 and extinct in 2007 were excluded Rare species are considerably more likely to go extinct by chance alone (24), and so might bias our results by inflating declines in abundance

Habitat Species were assigned to 1 of 5 habitat categories forest, grassland and field, roadside, wetland, and aquatic When species occurred in 2 or more habitats, they were assigned to the habitat where Eaton and Primack and Miller-Rushing saw the species most frequently (20) Habitat was included as a covariate in the models to control for the effect of habitat loss on extinction (see *Phylogenetic Conservatism and Trait Correlations* below) Importantly, species were lost from all habitats at approximately the same rate (20), which indicates that no habitat was particularly biased toward higher rates of extinction This result, especially when considering the protected nature of the Concord area, indicates that these patterns of local extirpation cannot be simply explained by human development or succession

Flowering-Time Response Flowering-Time Tracking of Seasonal Temperature The 15-year period between 1888 and 1902 provides the longest survey period to quantify the tracking of species flowering time with seasonal temperature Flowering-time tracking was determined with regard to seasonal variation in winter temperature (average temperature over January, April, and May, see ref 11) April and May represent monthly temperatures commonly associated with annual flowering in this region The month of January was also included because it was found to correlate with the flowering time of many species This correlation is presumably due to the severe cold of midwinter, which can damage plants and, thus, delay spring flowering (23) Flowering-time tracking was quantified as the correlation coefficient between annual first flowering day and winter temperature (11) Unlike flowering-time shift, our measure of flowering-time tracking from 1888 to 1902 is less likely to be affected by changes in abundance because population size was likely more stable during this shorter period (31) This trait provides an important measure of a species ability to respond to short-term temperature variation, allowing us to relate short-term temperature response with long-term changes in abundance from 1900 to 2006

Flowering-Time Response Shift in Flowering Time First day of flowering was recorded by Thoreau, Hosmer, and Miller-Rushing and Primack for 465, 461, and 478 species, respectively Observations were recorded annually for nearly all species over the duration of each botanists' survey (11) The timing of first flowering for each species was averaged over each botanists' survey period Shift in first flowering day was calculated as the difference in mean first flowering day from 1850–1900, 1850–2007, and 1900–2007 (11)

Name Standardization We standardized species names in the Concord flora by using the U S Department of Agriculture PLANTS Database (45) The most current accepted species name recognized in the database was used as our "correct" species name This standardized taxonomy was then used in all downstream applications including species range estimation and phylogentic tree construction (see below) In a small number of cases (18 species), sister species were identified as synonyms These sister taxa were collapsed into a single taxon

Species Latitudinal Range Estimation The latitudinal data of species were compiled from several online databases including the U S Department of Agriculture PLANTS Database, the National Herbarium of Canada, the Canadian Biodiversity Information Facility, the Royal Botanic Gardens Kew, Fairchild Tropical Botanic Garden, and the Missouri Botanical Garden (TROPICOS) Latitudinal data in these databases were derived from the literature, field-based observations, and herbarium specimens In total, 384,292 data points were obtained for 530 species with a median of 608 observations per species Three species with <20 observations were not included in the analysis due to the paucity of data The average latitude for each species was obtained across the contiguous United States and adjacent Canada The mean latitude for each species was weighted by the number of observations across the range, which more accurately represents the latitudinal affinity of each species

Local declines in species abundance could be due to populations occurring at the edge of their ranges, and thus their environmental tolerances Alternatively, if climate change is shifting environments northward, we would expect species with a range edge more north of Concord to be declining in abundance We tested for the effect of species range edge on decline in abundance, and found that species with range edges north of Concord, rather than near to Concord, were much more likely to have declined in abundance This finding supports the notion that species decline is likely associated with shifting environments resulting from climate change rather than to a local range edge effect Because species mean latitudinal range was found to be a much better predictor of decline in abundance when analyzed with species range edge, however, the latter was excluded from our analyses

Native/Introduced Status We obtained native/introduced status for each species from the U S Department of Agriculture PLANTS Database (45) Species were scored as "native" if they occurred in the continental United States or Canada at the time of Columbus, and "introduced" if they arrived from other regions since that time A small number of species (11 species) were coded ambiguously as "native and probably introduced" and were not included in our analyses

Phylogeny Construction A composite phylogeny of all species was constructed with Phylomatic version 1 (46) and was further resolved above the generic level by using recently published molecular phylogenies Studies using >1 gene were preferred, and bootstrap support >80% was required to resolve relationships Branch lengths were scaled to be approximately equal to time with divergence time estimates aggregated in Phylocom version 3 41 by using the 'BLADJ' function (47) Our composite phylogeny with branch lengths scaled for time (www huh harvard edu/research/staff/davis/Fig S2 pdf, references for composite phylogeny construction embedded in Fig S1) is available on TreeBASE (www treebase org) Species were pruned from this tree as necessary depending on data availability for each analysis To test the robustness of our results to uncertainties associated with divergence time estimation, we also ran our analyses on the same composite tree, but with branch lengths set to 1

Phylogenetic Conservatism and Trait Correlations The phylogenetic conservatism of each trait was evaluated separately by calculating the average magnitude of standard deviation (SD) of descendant nodes over the phylogeny, by using methods modified from Blomberg and Garland (48) as implemented in Phylocom by using the analysis of traits function (47)

Standard trait correlations can be biased by species relatedness (49, 50) To account for evolutionary history in trait correlations, we used the comparative *method of generalized estimating equations (GEE, ref 51), as implemented in* APE version 2 1-3 (52) GEE incorporates a phylogenetic distance matrix into the framework of a general linear model Importantly for this study, GEE also permits the simultaneous analysis of multiple categorical and continuous traits as covariates in the same model The inclusion of covariates allowed us to control for the effects of other factors that are likely to have an impact on change in abundance, including initial abundance, habitat, native/introduced status, and flowering season

We used 3 models to test for the correlation between change in abundance and our traits of interest (i e , flowering-time tracking, flowering-time shift, and species latitudinal range) Model 1 tested for the effect of each trait (e g , flowering-time tracking) on change in abundance

$$\text{change in abundance} = \text{flowering-time tracking}$$

Model 2 tested for the effect of each trait while accounting for the effects of a set of additional covariates that could also influence decline in abundance [i e , initial abundance (ca 1900), habitat, native/introduced status, and flowering season (date of first flowering)]

$$\begin{aligned}\text{change in abundance} = {} & \text{flowering-time tracking} \\ & + \text{initial abundance} \\ & + \text{habitat} \\ & + \text{native/introduced status} \\ & + \text{flowering season}\end{aligned}$$

Model 3 tested for the effect of all traits of interest (i e , in combination) while accounting for the effects of a set of additional covariates that could also influence decline in abundance [i e , initial abundance (*ca* 1900), habitat, *native/introduced status, and flowering season*)

change in abundance = flowering-time tracking
+ flowering-time shift
+ species latitudinal range
+ initial abundance + habitat
+ native/introduced status
+ flowering season

These analyses make the assumption that intraspecific variation is less than interspecific variation Given the phylogenetic scale at which we are comparing species (i e , across all angiosperms) this is a reasonable assumption and has been demonstrated empirically (53)

Sensitivity Analyses We tested the sensitivity of our results to branch length by setting all branch lengths to 1 Also, we tested the sensitivity of our results to phylogenetic uncertainty (54) All of our analyses were tested across a set of 50 trees where the polytomies were randomly resolved on each by using the program Mesquite (55) All results were robust to these sensitivity analyses (Table S1 and Table S2)

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APPENDIX 3:

Reply to McDonald et al.: Climate change, not deer herbivory, has shaped species decline in Concord, Massachusetts

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