

## COMBINED MORPHOLOGICAL AND MOLECULAR PHYLOGENY OF THE CLUSIOID CLADE (MALPIGHIALES) AND THE PLACEMENT OF THE ANCIENT ROSID MACROFOSSIL *PALEOCLUSIA*

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**Premise of research.** The clusioid clade is a member of the large rosid order Malpighiales and contains ~1900 species in five families: Bonnetiaceae, Calophyllaceae, Clusiaceae sensu stricto (s.s.), Hypericaceae, and Podostemaceae. Despite recent efforts to clarify their phylogenetic relationships using molecular data, no such data are available for several critical taxa, including especially *Hypericum ellipticifolium* (previously recognized in *Lianthus*), *Lebrunia*, *Neotatea*, *Thysanostemon*, and the second-oldest rosid fossil (~90 Ma), *Paleoclusia chevalieri*. Here, we (i) assess congruence between phylogenies inferred from morphological and molecular data, (ii) analyze morphological and molecular data simultaneously to place taxa lacking molecular data, and (iii) use ancestral state reconstructions (ASRs) to examine the evolution of traits that have been important for circumscribing clusioid taxa and to explore the placement of *Paleoclusia*.

**Methodology.** We constructed a morphological data set including 69 characters and 81 clusioid species (or species groups). These data were analyzed individually and in combination with a previously published molecular data set of four genes (plastid *matK*, *ndhF*, and *rbcL* and mitochondrial *matR*) using parsimony, maximum likelihood (ML), and Bayesian inference. We used ML ASRs to infer the evolution of morphological characters.

**Pivotal results.** Our phylogeny inferred from morphology alone was poorly supported but largely in agreement with molecular data. Moreover, our combined analyses were much better supported and largely confirm taxonomic hypotheses regarding relationships of extant taxa newly included here. The extinct *Paleoclusia* was placed as a member of stem group Clusiaceae s.s. or within crown group Clusiaceae s.s. as sister to one of its two major subclades.

**Conclusions.** Despite poor overall bootstrap support for the placement of *Paleoclusia*, ancestral character state reconstructions are generally in agreement with our placements. Our recommendation is that *Paleoclusia* be treated as either a minimum stem group or a crown group age constraint of Clusiaceae s.s.

**Keywords:** Clusiaceae, combined analysis, Guttiferae, morphology, *Paleoclusia*, rosids.

**Online enhancements:** appendixes, figures, supplementary table.

### Introduction

The clusioid clade belongs to the large angiosperm order Malpighiales (Ruhfel et al. 2011). It includes five families (Bonnetiaceae, Calophyllaceae, Clusiaceae sensu stricto [s.s.], Hypericaceae, and Podostemaceae; APG III 2009; Wurdack and Davis 2009; Xi et al. 2012) representing 89 genera (Ruhfel et al. 2011) and ~1900 species (Stevens 2001–). Habitats and growth forms in the clusioid clade show extreme variation, from large tropical rainforest trees to diminutive aquatic plants of swift-flowing waterways. 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 (Davis et al. 2005; 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, and timber production and in the pharmaceutical industry (Ernst 2003; Stevens 2007a, 2007b; Ruhfel et al. 2011).

Recent molecular studies have sought to clarify relationships within the clusioid clade (Gustafsson et al. 2002; Wurdack and Davis 2009; Ruhfel et al. 2011; Xi et al. 2012). 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 in-

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Manuscript received May 2012; revised manuscript received February 2013; electronically published June 7, 2013.

licated 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 because (i) specimens were unavailable for investigation, (ii) genomic DNA extractions from available material were unsuccessful, or (iii) the taxon is a fossil. These taxa include especially *Hypericum ellipticifolium* H.L. Li (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 an 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 current molecular-based phylogeny and, when analyzed in combination with molecular data, may allow us to place these missing 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 and do not exhibit strong conflict with molecular data and when the overall number of characters scored is large (Wiens 2003, 2009; Wiens and Moen 2008). 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 important for circumscribing taxa within the group. 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 can be readily assigned to an extant rosoid clade (Crepet et al. 2004; Schönenberger and von Balthazar 2006) as well as the oldest fossil in Malpighiales (Davis et al. 2005). As such, it has been used as a fossil constraint in numerous 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; Arakaki et al. 2011; Clarke et al. 2011; Xi et al. 2012). 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; they previously included members of Calophyllaceae, Clusiaceae s.s., and Hypericaceae (Wurdack and Davis 2009; Ruhfel et al. 2011). Additionally, the aquatic Podostemaceae are now also 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.). For these reasons, a more up-to-date analysis with improved taxon and character sampling is needed to reexamine the placement of this critical fossil rosoid taxon.

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 rosoids, which contain more than one-quarter 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 rosoid 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 aspect of understanding angiosperm diversification, including biome and genome evolution.

In this study we present phylogenetic hypotheses of the clusioid clade derived from morphological, molecular, and combined morphological and molecular data. 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*.

## Material 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). Within Podostemaceae three taxa representing well-supported subclades (Kita and Kato 2001; Moline et al. 2007; Ruhfel et al. 2011) were included to represent the subfamilies Podostemoideae, Weddellinoideae, and Tristichoideae. In many cases we included more than one representative from morphologically diverse genera (e.g., *Clusia*, *Garcinia*, *Hypericum*; see table 1) to better encompass their diversity. The molecular phylogeny of Ruhfel et al. (2011) revealed that the genera *Santomasia*, *Thornea*, and *Triadenum* were well supported as nested within *Hypericum* (cf. Nürk et al. 2013 for *Thornea*). It is 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 previ-

Table 1

Taxonomic Sampling Scheme for Morphological and Molecular Data (*matK*, *ndhF*, *rbcl*, and *matR*)

| Morphological data   | Molecular data  |
|--|---|
| <i>Paleoclusia chevalieri</i> Crepet & Nixon                                 | ...   |
| Bonnetiaceae:  |   |
| <i>Archytaea</i> <sup>a</sup>  | <i>Archytaea triflora</i> Mart.                               |
| <i>Bonnetia</i> <sup>a</sup>   | <i>Bonnetia sessilis</i> Benth.                               |
| <i>Ploiarium</i> <sup>a</sup>  | <i>Ploiarium alternifolium</i> Melchior                       |
| Calophyllaceae:  |   |
| <i>Calophyllum</i> <sup>a</sup>  | <i>Calophyllum inophyllum</i> L.                              |
| <i>Caraipa</i> <sup>a</sup>  | <i>Caraipa savannarum</i> Kubitzki                            |
| <i>Clusiella</i> <sup>a</sup>  | <i>Clusiella isthmensis</i> Hammel                            |
| <i>Endodesmia calophylloides</i> Benth.                                      | <i>Endodesmia calophylloides</i> Benth.                       |
| <i>Haploclathra</i> <sup>a</sup>   | <i>Haploclathra paniculata</i> Benth.                         |
| <i>Kayea</i> <sup>a</sup>  | <i>Kayea oblongifolia</i> Ridl.                               |
| <i>Kielmeyera</i> <sup>a</sup>   | <i>Kielmeyera petiolaris</i> Mart.                            |
| <i>Lebrunia busbaie</i> Staner   | ...   |
| <i>Maburea</i> <sup>a</sup>  | <i>Maburea exstipulata</i> Benth.                             |
| <i>Mammea americana</i> group <sup>a</sup>                                   | <i>Mammea americana</i> L.                                    |
| <i>Mammea bongo</i> group <sup>a</sup>                                       | ...   |
| <i>Mammea siamensis</i> group <sup>a</sup>                                   | <i>Mammea siamensis</i> T. Anderson                           |
| <i>Mammea touriga</i> (C.T. White & W.D. Francis) L.S. Sm.                   | <i>Mammea touriga</i> (C.T. White & W.D. Francis) L.S. Sm.    |
| <i>Marila grandiflora</i> group <sup>a</sup>                                 | ...   |
| <i>Marila tomentosa</i> group <sup>a</sup>                                   | <i>Marila tomentosa</i> Poepp. & Endl.                        |
| <i>Mesua ferrea</i> group <sup>a</sup>                                       | <i>Mesua ferrea</i> L.  |
| <i>Mesua thwaitesii</i> group <sup>a</sup>                                   | ...   |
| <i>Neotatea</i> <sup>a</sup>   | ...   |
| <i>Poeciloneuron indicum</i> Bedd.   | <i>Poeciloneuron indicum</i> Bedd.                            |
| <i>Poeciloneuron pauciflorum</i> Bedd.                                       | ...   |
| Clusiaceae s.s.:   |   |
| <i>Allanblackia</i> <sup>a</sup>   | <i>Allanblackia</i> sp.                                       |
| <i>Chrysochlamys</i> <sup>a</sup>  | <i>Chrysochlamys allenii</i> (Maguire) Hammel                 |
| <i>Clusia alata</i> Planch. & Triana   | ...   |
| <i>Clusia caudatum</i> (Planch. & Triana) Pipoly                             | ...   |
| <i>Clusia columbiana</i> Pipoly  | ...   |
| <i>Clusia comans</i> (Meisn.) Pipoly   | ...   |
| <i>Clusia flavida</i> (Benth.) Pipoly  | <i>Clusia</i> cf. <i>flavida</i> (Benth.) Pipoly              |
| <i>Clusia gundlachii</i> Stahl   | <i>Clusia gundlachii</i> Stahl                                |
| <i>Clusia major</i> L.   | <i>Clusia major</i> L.  |
| <i>Clusia panapanari</i> (Aubl.) Choisy                                      | ...   |
| <i>Clusia</i> p.p. ( <i>Oedematopus</i> spp.) <sup>a</sup>                   | ...   |
| <i>Clusia</i> p.p. ( <i>Quapoya</i> spp.) <sup>a</sup>                       | <i>Clusia hammeliana</i> Pipoly                               |
| <i>Decaphalangium peruvianum</i> Melch.                                      | ...   |
| <i>Dystovomita</i> <sup>a</sup>  | <i>Dystovomita paniculata</i> (Donn. Sm.) Hammel              |
| <i>Garcinia cymosa</i> (K. Schum.) I.M. Turner & P.F. Stevens                | <i>Garcinia cymosa</i> (K. Schum.) I.M. Turner & P.F. Stevens |
| <i>Garcinia dulcis</i> (Roxb.) Kurz  | <i>Garcinia spicata</i> Hook. f.                              |
| <i>Garcinia morella</i> Desr.  | <i>Garcinia urophylla</i> Scort. ex King                      |
| <i>Garcinia</i> p.p. ( <i>Pentaphalangium</i> spp.) <sup>a</sup>             | <i>Garcinia latissima</i> Miq.                                |
| <i>Garcinia</i> p.p. ( <i>Rheedea</i> spp.) <sup>a</sup>                     | <i>Garcinia macrophylla</i> Mart.                             |
| <i>Lorostemon bombaciflorum</i> group  | ...   |
| <i>Lorostemon coelhoi</i> Paula  | <i>Lorostemon coelhoi</i> Paula                               |
| <i>Montrouzeria</i> <sup>a</sup>   | <i>Montrouzeria cauliflora</i> Planch. & Triana               |
| <i>Moronobea</i> <sup>a</sup>  | <i>Moronobea coccinea</i> Aubl.                               |
| <i>Pentadesma</i> <sup>a</sup>   | <i>Pentadesma butyracea</i> Sabine                            |
| <i>Platonia insignis</i> Mart.   | <i>Platonia insignis</i> Mart.                                |
| <i>Septogarcinia sumbawaensis</i> Kosterm.                                   | <i>Garcinia cowa</i> Roxb.                                    |
| <i>Symphonia</i> <sup>a</sup>  | <i>Symphonia globulifera</i> L. f.                            |
| <i>Thysanostemon pakaraimae</i> Maguire                                      | ...   |
| <i>Tovomita</i> <sup>a</sup>   | <i>Tovomita calophyllophylla</i> García-Villacorta & Hammel   |
| <i>Tovomita weddelliana</i> Planch. & Triana                                 | <i>Tovomita weddelliana</i> Planch. & Triana                  |
| <i>Tovomitopsis</i> <sup>a</sup>   | <i>Tovomitopsis saldanhae</i> Engl.                           |
| Hypericaceae:  |   |
| <i>Cratoxylum</i> sects. <i>Cratoxylum</i> and <i>Tridesmos</i> <sup>a</sup> | <i>Cratoxylum cochinchinense</i> (Lour.) Blume                |
| <i>Cratoxylum</i> sect. <i>Isopterygium</i> <sup>a</sup>                     | <i>Cratoxylum arborescens</i> (Vahl) Blume                    |
| <i>Eliea articulata</i>  | <i>Eliea articulata</i> Cambess.                              |
| <i>Harungana madagascariensis</i> Poir.                                      | <i>Harungana madagascariensis</i> Poir.                       |
| <i>Hypericum</i> <i>Ascyreia</i> s.l. <sup>a</sup>                           | ...   |
| <i>Hypericum calcicola</i> Standl. & Steyerl.                                | <i>Hypericum calcicola</i> Standl. & Steyerl.                 |
| <i>Hypericum ellipticifolium</i> H.L. Li                                     | ...   |

**Table 1**  
(Continued)

| Morphological data   | Molecular data                                     |
|--|--|
| <i>Hypericum</i> Euhypericum <sup>a</sup>                                    | <i>Hypericum perforatum</i> L.                     |
| <i>Hypericum</i> p.p. ( <i>Triadenum</i> spp.) <sup>a</sup>                  | <i>Hypericum fraseri</i> (Spach) Steudel           |
| <i>Hypericum</i> sect. <i>Adenotrias</i> <sup>a</sup>                        | <i>Hypericum aegypticum</i> L.                     |
| <i>Hypericum</i> sects. <i>Brathys</i> and <i>Trignobrathys</i> <sup>a</sup> | <i>Hypericum irazuense</i> Kuntze ex N. Robson     |
| <i>Hypericum</i> sect. <i>Elodes</i> <sup>a</sup>                            | <i>Hypericum elodes</i> L.                         |
| <i>Hypericum</i> sect. <i>Myriandra</i> <sup>a</sup>                         | <i>Hypericum kalmianum</i> L.                      |
| <i>Hypericum steyermarkii</i> Standl.  | <i>Hypericum steyermarkii</i> Standl.              |
| <i>Psorospermum cerasifolium</i> group <sup>a</sup>                          | ...  |
| <i>Psorospermum febrifugum</i> group <sup>a</sup>                            | <i>Psorospermum febrifugum</i> Spach               |
| <i>Psorospermum lamianum</i> H. Perrier                                      | ...  |
| <i>Psorospermum staudtii</i> group <sup>a</sup>                              | ...  |
| <i>Vismia affinis</i> Oliv.  | ...  |
| <i>Vismia cayennensis</i> group <sup>a</sup>                                 | <i>Vismia billbergiana</i> Beurl.                  |
| <i>Vismia laurentii</i> De Wild.   | <i>Vismia guineensis</i> (L.) Choisy               |
| <i>Vismia orientalis</i> Engl.   | ...  |
| <i>Vismia rubescens</i> Oliv.  | <i>Vismia rubescens</i> Oliv.                      |
| Podostemaceae:   |  |
| <i>Podostemoideae</i> <sup>a</sup>   | <i>Podostemum ceratophyllum</i> Michx.             |
| <i>Weddellinoideae</i> <sup>a</sup>  | <i>Weddellina squamulosa</i> Tul.                  |
| <i>Tristichoidae</i> <sup>a</sup>  | <i>Tristicha trifaria</i> (Bory ex Willd.) Spreng. |

Note. An ellipsis indicates that molecular data were 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). Following Ruhfel et al. (2011), species in the Hypericaceae genera *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* are treated here as species of *Hypericum*. For composite morphological terminals representing segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum*, former names are indicated in parentheses.

<sup>a</sup> Composite terminal.

ously been described as members of *Hypericum*, and we treat them as such here following Ruhfel et al. (2011; see table 1).

Terminals scored for morphology included a mixture of single species and composite placeholders (see table 1). Composite terminals encompass several species and were mostly defined based on well-supported clades identified by Ruhfel et al. (2011). In addition, some composite terminals for *Hypericum* (*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 terminals *Hypericum* *Ascyreia* s.l. and *Hypericum* Euhypericum are based on informal names given to well-supported clades in the latter study. Composite terminals not previously identified in molecular phylogenetic analyses were based on recent circumscriptions by Stevens (2007a, 2007b; P. F. Stevens, unpublished manuscript) and are assumed to represent monophyletic groups. Well-known, smaller genera were grouped into multiple terminals if they appeared to be morphologically and/or anatomically heterogeneous. For instance, species of *Symphonia* show great uniformity in flower and fruit, while *Marila* breaks into two groups delimited by characters of inflorescence, flower, and seed. For large genera such as *Clusia* and *Garcinia* our knowledge was much poorer, and we focused on individual species or groups of species that are members of morphologically distinct, and presumably monophyletic, groups. We provide further information about our assumptions in table 1 and appendix A.

Molecular data from Ruhfel et al. (2011) were selected to

match our morphological sampling (table 1); however, data for the genes used in our study are unavailable for 23 of our 81 ingroup terminals. Each species scored for morphology was analyzed in combination with molecular data from the same species, except for three Clusiaceae s.s. (*Garcinia dulcis* [Roxb.] Kurz, *Garcinia morella* Desr., and *Septogarcinia sambawaensis* Kosterm.) and two Hypericaceae (*Vismia cayennensis* [Jacq.] Pers. and *Vismia laurentii* De Wild.) species. Morphological data from these species were paired with molecular data from species that are closely related based on morphology or molecular data (Bamps 1966; Sweeney 2008; Ruhfel et al. 2011; P. Sweeney, personal communication). For composite terminals we included molecular data from a single representative species that is known to be included in that clade (table 1). For example, the genus *Bonnetia* is scored as a morphological composite. Thus, in the combined analyses we paired morphological data from the composite terminal *Bonnetia* with molecular data from *Bonnetia sessilis* Benth.

A recent analysis using complete plastid genomes to resolve broad Malpighiales relationships (Xi et al. 2012) has identified a strongly supported clade containing the clusioids plus Ochneaceae s.l. (including Medusagynaceae and Quiinaceae), Ctenolophonaceae + Erythroxylaceae + Rhizophoraceae, and Pandaceae + Irvingiaceae (family designations follow Xi et al. 2012). 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 mor-

phological topologies, we rooted these trees in a position similar to the ingroup rooting inferred from molecular data (i.e., along the branch connecting Bonnetiaceae + Clusiaceae s.s. with Calophyllaceae + Hypericaceae + Podostemaceae; Ruhfel et al. 2011; Xi et al. 2012).

Crepet and Nixon (1998) placed *Paleoclusia* as a member of Clusiaceae by analyzing morphological data in a phylogenetic framework. They also indicated that the fossil has several distinctive characters that suggest a placement in the clusioid clade, including an aril, a fasciculate androecium, the presence of resin canals, a short style, and possible dioecy. We further verified its placement as a member of the clusioid clade using two interactive keys: Watson and Dallwitz (1992–) and Nixon (<http://www.plantsystematics.org>). Both keys identified *Paleoclusia* as a member of Clusiaceae s.l.: Watson and Dallwitz included all five clusioid families; Bonnetiaceae and Podostemaceae, however, were absent from the Nixon key. 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 recognize the presence of an aril (see below). A broad phylogenetic analysis including morphological data from all major groups of angiosperms (Nandi et al. 1998) could be the ultimate test of *Paleoclusia*'s phylogenetic placement; however, this is outside the scope of our study. Given the previous phylogenetic placement of *Paleoclusia* with Clusiaceae s.l. and the results of our keying exercise, we have confidence in the assignment of *Paleoclusia* to the clusioid clade.

#### Morphological Data

Sixty-nine discrete (binary or multistate) morphological characters (characters 1–69 in app. C, available online) representing vegetative and reproductive structures were scored for 81 clusioid taxa, including *Paleoclusia* (see table 1; app. A; see also app. C; table C1). An additional 57 characters were initially examined but not included for various reasons (see app. C for details). Crepet and Nixon (1998) scored 61 characters in their morphological matrix; however, only 16 of those characters are similar to those used here. This discrepancy is partly due to their selection of characters that could be scored broadly across asterid (e.g., Ericaceae and Theaceae) and rosid (e.g., Hypericaceae and Clusiaceae s.s.) lineages. In contrast, our study focuses on the five closely related clusioid families, which necessitates a different set of characters. Scoring of taxa was in all cases based on direct observations unless otherwise indicated. Morphological data for the composite *Hypericum* terminals 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 Rutschauer 2007).

Problems with the delimitation of character states have been discussed by Gift and Stevens (1997) and Stevens (1991, 1996, 2000). For a study like this, whether to include the all-too-often “unrepresentative” character states of single species presents a difficult choice. Individual species in composite terminals, such as *Calophyllum*, or species in parts of genera that are not incorporated in this study may show variation that seems to be at odds with our scoring. Thus, abaxial palisade

layers of mesophyll tissue (character 21) occur sporadically, as in some species of *Clusia* (Vesque 1892), in *Garcinia aristata* Griseb. (the *Rheedea* group), and in *Calophyllum ardens* PF Stevens. For such taxa that are members of composite terminals, such sporadic variation is not represented in our scoring since as best as can be estimated, this variation is likely to represent a derived state.

*Paleoclusia* was scored for ~45% of our morphological characters (i.e., 31 of 69). We were unable to score most vegetative and anatomical characters for this taxon because only fossilized flowers have been found. Our character scoring was largely similar to that of Crepet and Nixon (1998) in those characters that were overlapping. The lone exception is that we scored *Paleoclusia* as lacking an aril. In all extant Clusiaceae the aril surrounds the seed (fig. 1), but in *Paleoclusia* it appears to be adjacent to the seed (figs. 28, 29 in Crepet and Nixon 1998). In addition, the structure in question in *Paleoclusia* has a cell wall pattern that is very similar to that found on the seeds (figs. 28, 30 in Crepet and Nixon 1998). Thus, it seems more likely that this structure is an aborted seed rather than an aril (Stevens 2001–; see discussion published August 2010).

Dioecy is known to occur in several clusioid clades (*Calophyllum*, Clusiaceae, *Clusiella*, Garcinieae, and *Mammea*; Dunthorn 2004; Martins et al. 2007; Stevens 2007a; Sweeney 2008; Leal et al. 2012) and may have evolved multiple times in *Calophyllum* (Stevens 1980; Vamosi 2006; Vela 2010). Our



**Fig. 1** Seed of *Tovomitopsis saldanhae* Engl. (Clusiaceae, Clusiaceae s.s.). Note the brightly colored aril surrounding the seed. Photograph by Volker Bittrich.

scoring of *Calophyllum* as dioecious thus provides a minimum bound on the number of origins of dioecy in the clusioid clade. The presence/absence of dioecy was scored and used for ASRs but not in phylogenetic reconstruction. This decision was made for two reasons. First, it is likely that dioecy is homoplasious across the clusioids. Second, it is unclear whether *Paleoclusia* is dioecious; stamens of *Paleoclusia* mostly lack pollen, but in some anthers, pollen is present (Crepet and Nixon 1998). We thus felt that its inclusion might have a biased effect on the placement of *Paleoclusia*.

Finally, the vegetative morphology of Podostemaceae has been difficult to interpret and has complicated their comparison to other angiosperms (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; C. T. Philbrick, unpublished data), which makes them difficult to compare with other clusioids. Because it is unclear which vegetative characters are homologous with other clusioids (Katayama et al. 2008), very few vegetative characters were scored for Podostemaceae. In total, 34 of the 69 characters used in this study were scored for at least one representative of 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\*, version 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](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. Inapplicable characters were treated as missing data and included in our analyses. Characters coded with multiple states for a single taxon were treated as polymorphic. 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 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, version 7.2.6 (Stamatakis 2006; available at <http://www.exelixis-lab.org>). In the Mk model, transitions among all character states are equally probable. 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, version 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 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 the following three ways: (i) using Tracer, version 1.5 (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) 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 (first 10% of trees) 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*

Our molecular data set included four genes, three plastid (*matK*, *ndhF*, and *rbcL*) and one mitochondrial (*matR*), sampled from 58 clusioid taxa, plus three outgroups (table 1; app. B). These data were from Ruhfel et al. (2011; TreeBase [<http://www.treebase.org>] accession S10995); the alignment was unmodified except to remove indels that were no longer applicable following our taxon adjustments for this study. Prior to analyzing our four genes in a combined analysis, we conducted separate tree searches in the ML framework described below on each single-gene data set. These analyses were conducted to detect potential problems for analyzing these genes simultaneously. We considered two topologies to be at odds if they contained conflicting nodes supported by  $\geq 70$  BP (Hillis and Bull 1993).

#### *Phylogenetic Analyses of Molecular Data*

MP, ML, and BI analyses were conducted as described above with the following differences. In the ML and BI analyses, the data set was partitioned by gene 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 with MrModelTest, version 2.3 (distributed by J. A. A. Nylander at <http://www.abc.se/~nylander/>), using the Akaike Information Criterion (table 2). 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) and molecular (fig. 3) phylogenies for conflicting nodes, i.e., those nodes that disagreed with support greater than 70 BP or 95 PP. Two areas of conflict between the molecular and morphological data sets were detected (see “Results”). To determine whether the morphological and molecular data sets could reject the topology derived from the

**Table 2**  
Data Set Characteristics

| Data set                           | <i>matK</i>        | <i>ndhF</i>        | <i>rbcL</i>        | <i>matR</i>    | 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 + $\Gamma$ | GTR + I + $\Gamma$ | GTR + I + $\Gamma$ | GTR + $\Gamma$ | NA                 | Mk          | NA                              |

Note. Percent missing data was 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 maximum likelihood and Bayesian analyses. Models of sequence evolution for the molecular data were chosen by the Akaike Information Criterion using MrModelTest, version 2.3. NA = not applicable.

rival data set, we performed alternative topology tests using the approximately unbiased test (Shimodaira 2002) as implemented in the R software package, scaleboot version 0.3-2 (Shimodaira 2008; distributed by CRAN at <http://www.r-project.org>). Constraint searches were conducted using ML 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* Mart. and *Garcinia urophylla* Scott. ex King as sister taxa. Using the morphological data set we also conducted two constraint searches. The first constrained *Allanblackia* to be sister to *Garcinia* p.p. (*Rheedia* spp.), the second constrained *Septogarcinia sumbawaensis* (the taxon scored for morphology only that was paired with *Garcinia cowa* Roxb.) as sister to *G. morella*. 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.

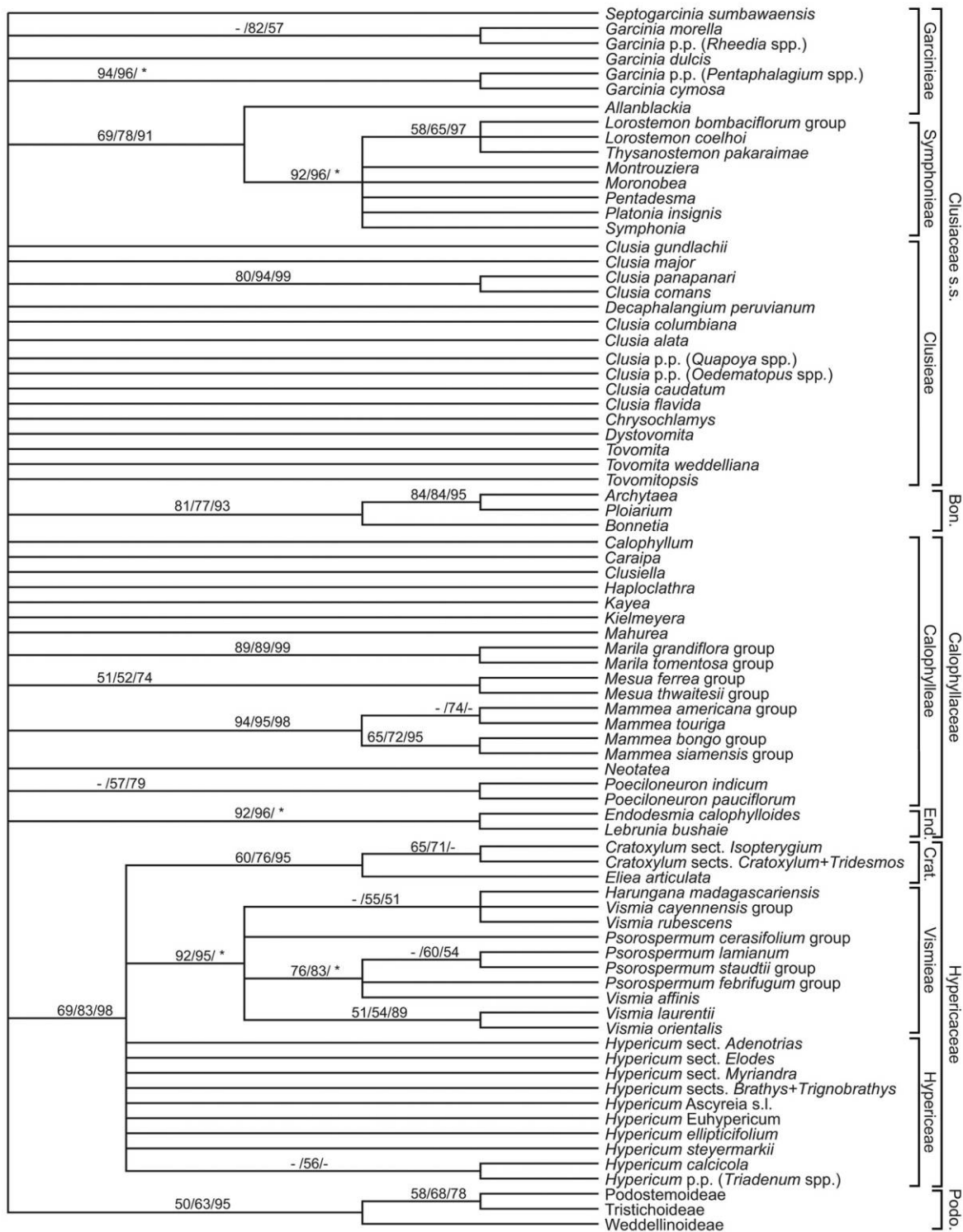
MP and ML analyses of the combined molecular and morphological data were conducted as described above. ML and BI analyses each had five partitions, one for each gene and one for the morphological data. BI analyses of the combined data using the parameters listed above, however, did not reach convergence in many cases (especially when *Paleoclusia* was included). To achieve convergence we implemented two changes to our BI search strategy. First, for each Markov chain Monte Carlo search we supplied an optimal ML starting topology 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 topology for each chain. Using this strategy, searches reached convergence in most 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 ac-

ceptable levels of convergence when *Paleoclusia* was included. For consistency, these two changes were implemented in all BI analyses.

#### Ancestral State Reconstructions

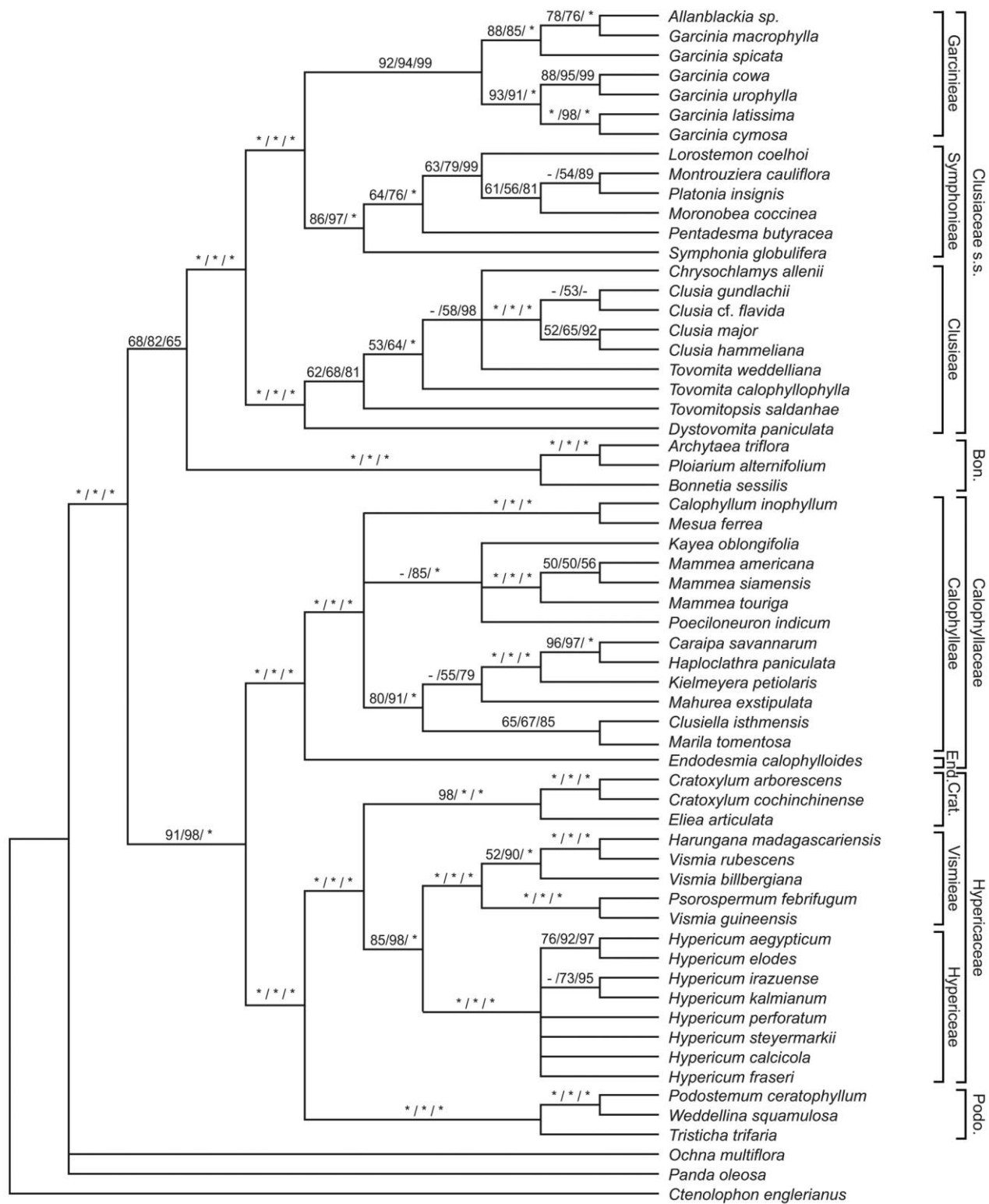
We used ML ASRs as implemented in Mesquite, version 2.74 (Maddison and Maddison 2010), to infer the evolution of the 69 morphological characters scored for this study. We examined all characters scored for two reasons. First, we sought to examine characters that have been historically important for determining 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), androecium arrangement, fascicledia presence/absence, carpel number, and breeding system (Cronquist 1981; Stevens 2007a, 2007b; Weitzman et al. 2007). Second, we sought to determine which characters might shed light on the alternative placements of *Paleoclusia*.

Ancestral state reconstruction using ML was chosen for two reasons. First, ML reconstructions consider branch lengths; i.e., the longer a branch is, 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). Data were analyzed using the Mk model with rate parameters estimated from the data. The likelihood decision threshold of 2 was selected (Pagel 1999) to determine the optimal ASRs at each node. Characters were treated as unordered and reconstructed onto the ML topology inferred from the combined data (fig. 4). This allowed us to include the 22 extant taxa that were scored only for morphology. We chose to exclude *Paleoclusia* from ASRs given its phylogenetic uncertainty (fig. 5; see below). Instead, we evaluated the alternate placements of this taxon in light of the ASRs inferred from extant taxa. Care should be taken in interpreting our ASRs because 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). Potential implications of this limitation

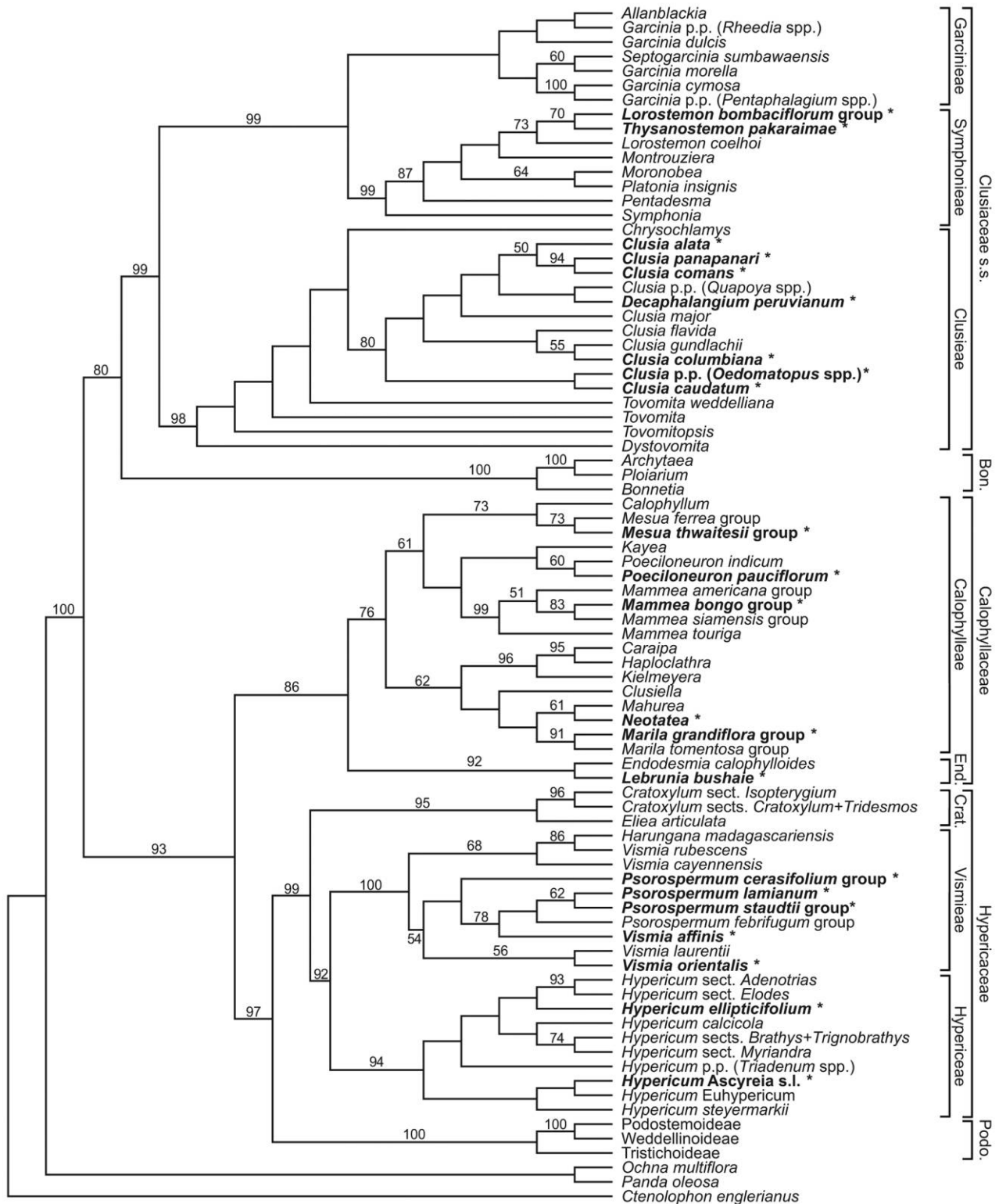


**Fig. 2** Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on the morphological data set not including *Paleoclusia*. Support values  $\geq 50\%$  are indicated accordingly: maximum parsimony bootstrap percentages (BP)/ML BP/Bayesian posterior probabilities (PP) converted to percentages. An asterisk indicates maximum support (100 BP or 100 PP). A hyphen indicates that the node was not present in a particular analysis. 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). Following Ruhfel et al. (2011), species in the Hypericeae genera *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* are treated here as species of *Hypericum* (see also table 1). For composite morphological terminals representing segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum*, former names are indicated in parentheses. Bon. = Bonnetiaceae; Crat. = Cratoxyleae; End. = Endodesmieae; Podo. = Podostemaceae.

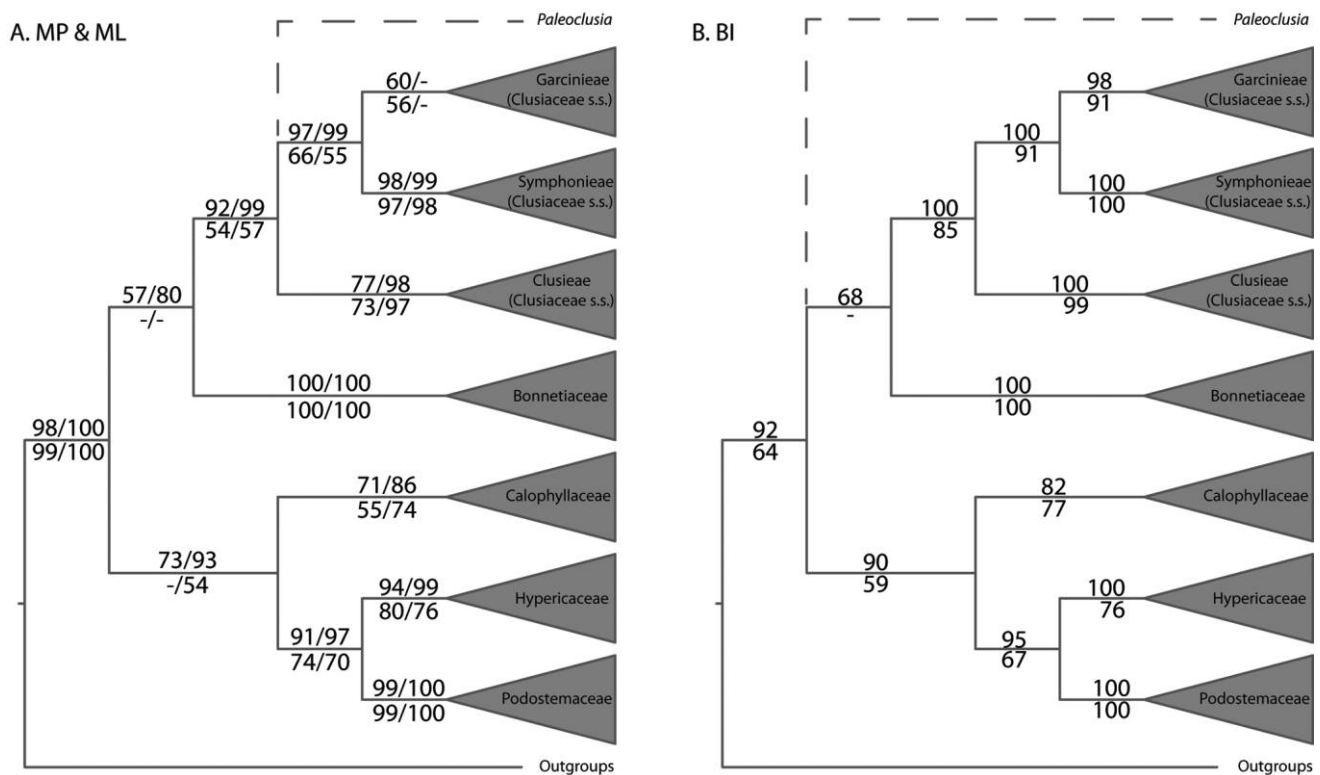




**Fig. 3** Fifty percent maximum likelihood (ML) majority-rule consensus tree of the clusioid clade based on a four-gene (*matK*, *ndbF*, *rbcL*, and *matR*) molecular data set. Support values  $\geq 50\%$  are indicated: maximum parsimony bootstrap percentages (BP)/ML BP/Bayesian posterior probabilities (PP) converted to percentages. An asterisk indicates maximum support (100 BP or 100 PP). A hyphen indicates that the node was not present in a particular analysis. Following Ruhfel et al. (2011), species in the Hypericaceae genera *Santomasia*, *Thornea*, and *Triadenum* are treated here as species of *Hypericum* (see also table 1). For composite morphological terminals representing segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum*, former names are indicated in parentheses. Bon. = Bonnetiaceae; Crat. = Cratoxyleae; End. = Endodesmiaceae; Podost. = Podostemaceae.



**Fig. 4** Optimal maximum likelihood (ML) topology of the clusioid clade based on the combined morphological and molecular data sets not including *Paleoclusia*. Support values  $\geq 50\%$  are indicated. Taxa scored for morphology only are in bold and marked with an asterisk. Taxon names used in figure are from the morphological data source (table 1). Following Ruhfel et al. (2011), species in the Hypericaceae genera *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* are treated here as species of *Hypericum* (see also table 1). For composite morphological terminals representing segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum*, former names are indicated in parentheses. Bon. = Bonnetiaceae; Crat. = Cratoxyleae; End. = Endodesmieae; Podo. = Podostemoideae.



**Fig. 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). Support values  $\geq 50\%$  are indicated; in A, maximum parsimony bootstrap percentages (BP)/ML BP; in B, Bayesian posterior probabilities converted to percentages. Support values above and below branches represent analyses excluding and including the *Paleoclusia* fossil, respectively. Clade size is not drawn proportional to species number.

will be addressed in the “Discussion.” We also calculated the consistency index (CI; Kluge and Farris 1969; Farris 1989), retention index (RI; Archie 1989a, 1989b; Farris 1989), and the rescaled consistency index (RC; Farris 1989) for each character as calculated by the program MacClade, version 4.08 (Maddison and Maddison 2005).

## Results

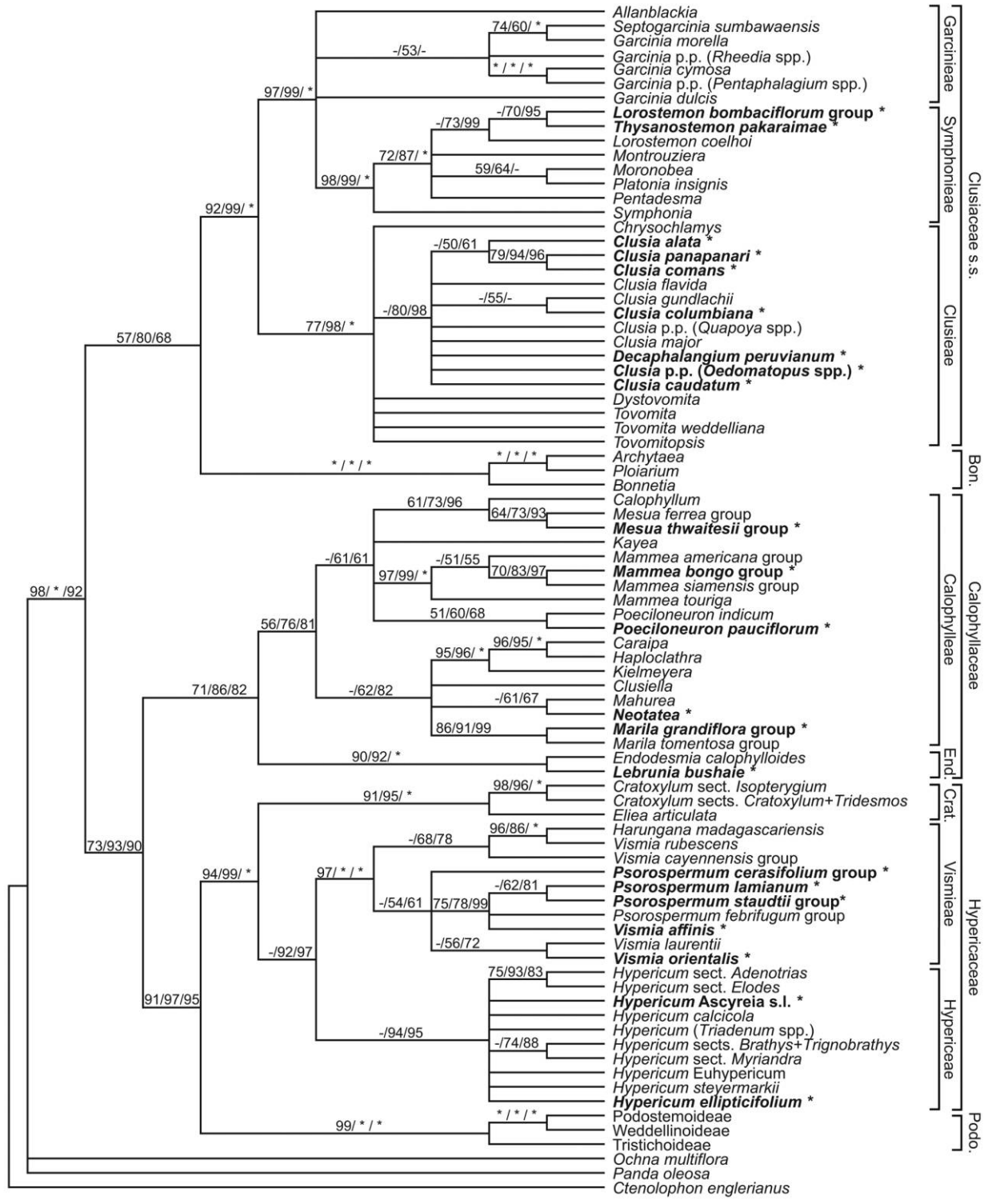
Our analysis of each data set (morphological, molecular, and combined morphological + molecular) resulted in similar topologies with few strongly conflicting nodes (see below). When including *Paleoclusia*, however, topologies were similar but resulted in a decline in support along the backbone of the tree (fig. 5). Relevant characteristics for each data set are listed in table 2. Unless otherwise indicated, we focus our discussion from here forward on the 50% ML majority rule consensus tree from the morphological (fig. 2), molecular (fig. 3), and combined morphological + molecular (fig. 6) data sets. We will also discuss the optimal ML topology inferred from the combined analysis (fig. 4), especially when support is low.

### Morphological Data and Phylogenetic Analyses

The morphological matrix is available in table C1. 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 3). Missing data for each taxon ranged from 0% to ~55%. Seventeen of the 81 taxa scored for morphology were missing data for >10% of the characters scored (table C1). Only four taxa had greater than 50% missing data: *Paleoclusia* (55%), Podostemoideae (53%), Weddellinoideae (53%), and Tristichoideae (53%).

The phylogeny inferred from our morphological data was less resolved but identified numerous clades in common with molecular phylogenies. 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). MP tree searches resulted in 163 topologies of 398 steps (CI = 0.60, RI = 0.81, RC = 0.49). Taxa not previously included in molecular phylogenetic studies were placed with varying levels of support. The position of *Neotatea* (Calophyllaceae) was unresolved, but it 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 elliptifolium* (Hypericaceae) was well placed (83 BP) as a member



**Fig. 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  $\geq 50\%$  are indicated: maximum parsimony bootstrap percentages (BP)/ML BP/Bayesian posterior probabilities (PP) converted to percentages. 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 for morphology only are in bold and marked with an asterisk. Taxon names used in figure are from the morphological data source (table 1). Following Ruhfel et al. (2011), species in the Hypericaceae genera *Liantbus*, *Santomasia*, *Thornea*, and *Triadenum* are treated here as species of *Hypericum* (see also table 1). For composite morphological terminals representing segregate genera now considered to be included in *Clusia*, *Garcinia*, and *Hypericum*, former names are indicated in parentheses. Bon. = Bonnetiaceae; Crat. = Cratoxyleae; End. = Endodesmieae; Podo. = Podostemoaceae.

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 with two *Lorostemon* species.

The placement of *Paleoclusia* was poorly supported (<50 BP; <95 PP, not shown) when it was included in our analyses. MP tree searches resulted in 39 equally parsimonious topologies of 400 steps (CI = 0.60, RI = 0.81, RC = 0.49). In the most parsimonious trees, *Paleoclusia* was 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 again 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. With two exceptions, support values in the morphological analysis (fig. 2) generally remained unchanged with the inclusion of *Paleoclusia*: support for *Allanblackia* + Symphonieae dropped from 78 to 54 BP; support for Hypericaceae dropped from 83 to 75 BP.

#### Molecular Data and Phylogenetic Analyses

Topologies derived from the single-gene analyses were generally in agreement, except for two instances of strong conflict in Calophyllaceae. The first involved *Mammea*; *Mammea americana* L., *Mammea siamensis* T. Anderson, and *Mammea touriga* (C.T. White & W.D. Francis) L.S. Sm. formed a clade in all analyses, but the relationships between these closely related species differed between each data set. Using *matK*, *M. touriga* was sister to *M. americana* with 99 BP; using *ndhF*, *M. touriga* was instead more closely related to *M. siamensis* with 77 BP; and using *rbcl*, *M. americana* was most closely related to *M. siamensis* with 77 BP. These *Mammea* taxa also formed a clade in the *matR* topology, but none of its internal branches were supported with >70 BP. The second conflict involved the placements of *Clusiella isthmensis* Hammel and *Kielmeyera petiolaris* Mart. Using *matR*, *Clusiella* was sister to *Haploclathra paniculata* Benth. + *Caraipa savannarum* Kubitzi with 91 BP; using *ndhF*, *K. petiolaris* was sister to *H. paniculata* + *C. savannarum* with 100 BP. These conflicts appear to be related to insufficient taxon sampling in Calophyllaceae for these genes. None of these conflicts were present in Ruhfel et al. (2011), which included many more Calophyllaceae. Given the relatively few instances of conflict and because our combined topology was similar to Ruhfel et al. (2011), we analyzed these four genes simultaneously.

The aligned molecular data set of all four genes included 5988 nucleotide bases (1412 of which were parsimony informative) 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 50% majority rule ML topology is very similar to Ruhfel et al. (2011). The clusioid clade and all five families received strong support (100 BP; fig. 3). Interfamilial relationships were the same as reported previously (Wurdack and Davis 2009; Ruhfel et al. 2011; Xi et al. 2012). In addition, support for some areas in our topology improved from Ruhfel et al. (2011). In particular, we recovered a strongly supported (94 BP) Garcinieae and increased support along the backbone of Symphonieae. There were also areas of the phy-

logeny where support values declined, but this was pronounced only within *Hypericum*.

#### Combined Morphological and Molecular Data and Phylogenetic Analyses

We observed two instances of conflict in Garcinieae + Symphonieae between the morphological (fig. 2) and molecular (fig. 3) phylogenies inferred using ML. The first involved the placement of *Garcinia macrophylla* and *Garcinia urophylla* in the molecular phylogeny and the associated representatives of these species in the morphological phylogeny, *Garcinia* p.p. (*Rheedia* spp.) and *Garcinia morella*, respectively (table 1). Using morphology (fig. 2), these taxa are sisters with high support (82 ML BP). In the molecular topology (fig. 3), *G. macrophylla* is instead sister to *Allanblackia* sp. with moderate support (76 ML BP). The second involved the placement of *Allanblackia*. Using morphology (fig. 2), *Allanblackia* is sister to Symphonieae with moderate support (78 ML BP). In the molecular topology (fig. 3) it is sister to *G. macrophylla* with moderate support (76 ML BP). The molecular data could not reject ( $P = 0.0697$ ) *Allanblackia* as a member of the Symphonieae clade. The molecular data, however, rejected ( $P = 0.0023$ ) *G. macrophylla* and *G. urophylla* as sister taxa. The morphological data rejected each of our constraint searches: *Allanblackia* sister to *Garcinia* p.p. (*Rheedia* spp.;  $P = 0.0216$ ) and *Septogarcinia sumbawaensis* sister to *G. morella* ( $P = 0.0468$ ).

The results of analyzing several variations of our morphological and combined data sets with different taxon and morphological character sampling were largely consistent with those presented below. Additional conflicts were evident only 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.

Our combined data matrix included 84 taxa and 6056 characters (~48% missing data; table 2). 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). The clusioid clade and each of its major subclades were generally strongly supported (>80 BP; figs. 4, 6), and results were largely consistent with the separate analyses of the morphological and molecular data sets (figs. 2, 3). The combined topology (fig. 6) was less resolved than the molecular topology (fig. 3) in several key areas, especially in Calophyllaceae, Clusiaceae, 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 extant taxa scored only for morphology were generally

well supported in phylogenetic positions consistent with their taxonomic circumscriptions (figs. 4, 6), regardless of the amount of missing data. Within Clusiaceae s.s., *Lorostemon coelhoi* Paula, the *Lorostemon bombaciflorum* group, and *Thysanostemon* formed a clade (73 BP) and were strongly placed (99 BP) within Symphonieae. Within this clade, the *L. bombaciflorum* group was more closely related to *Thysanostemon* (70 BP), indicating that *Lorostemon* may not be monophyletic. Within Clusiaceae, taxa representing the many segregate genera (e.g., *Decaphalangium*, *Havetia*, *Havetiopsis*, *Oedematopus*, *Pilosperma*, *Quapoya*, and *Renggeria*) that now belong in *Clusia* (Gustafsson et al. 2007) formed a well-supported (80 BP) clade with other *Clusia*. Most of these segregate genera have been included in previous molecular studies, except *Pilosperma*, represented here by *Clusia caudatum* (Planch. & Triana) Pipoly. Our results indicate that *Pilosperma* is properly treated in *Clusia* as has been proposed 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., *H. ellipticifolium*, the *Mammea bongo* group, 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).

Analyses including *Paleoclusia* produce a dramatic drop in support along the backbone of the tree (fig. 5), but relationships among extant taxa remain unchanged (fig. 6). MP tree searches resulted in 132 topologies of 5411 steps (CI = 0.63, RI = 0.81, RC = 0.51). In our 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 Clusiaceae. In the optimal ML topology, *Paleoclusia* was placed within Garcinieae 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. 5). BI analyses differed in the placement of *Paleoclusia* by weakly (64 PP) placing it in a trichotomy with the two main clusioid subclades (fig. 5).

#### Ancestral State Reconstructions

CI, RI, and RC values for each character are listed in table 3. For brevity, we present only those ASRs that have been historically important for determining relationships in the clusioid clade and for characters that shed light on the placement of *Paleoclusia* (app. D, figs. D1–D24, available online). Characters in the latter group can be further divided into two categories. The first includes characters that can be scored for the fossil with the available material. These characters 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. The second includes characters that cannot be scored but may be helpful in future studies if more complete material of this fossil is discovered.

## Discussion

### Comparison of the Morphological and Molecular Phylogenies

The topology inferred from morphological data (fig. 2) was much less resolved than the one inferred from molecular data (fig. 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, and Vismieae were all identified as monophyletic. Calophyllaceae and Clusiaceae s.s., however, were 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 (not shown). Analyses of the morphological data matrix that excluded these three clades, *Paleoclusia*, and the taxa involved in our strongly reported conflicts (*Allanblackia*, *Garcinia morella*, and *Garcinia* p.p. [*Rheedia* spp.]; see “Results”) resulted in monophyletic Clusiaceae s.s. and Calophyllaceae. However, when *Paleoclusia* is included, Calophyllaceae and Clusiaceae s.s. are once again recovered as nonmonophyletic.

*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, nonfasciculate androecium; and sessile stigmas. Reasons for the conflicting placement of the poorly known Endodesmieae are less clear but may result from their vegetative similarity to Clusiaceae s.s. and their possession of fruits similar to Calophyllaceae (Notis 2004; Stevens 2007a). Endodesmieae were placed either within Calophyllaceae 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 Calophyllaceae (the sister group of Endodesmieae). 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 were excluded was dramatic (data not shown), and many vegetative characters cannot easily be scored for Podostemaceae (see “Material and Methods”).

### 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 (fig. 6) than the morphological data alone, especially when *Paleoclusia* was excluded. However, the topology from the combined analysis is less resolved than the topology produced using molecular data

Table 3

Percent Missing Data, Number of State Changes, Consistency Index (CI), Retention Index (RI), and Rescaled Consistency Index (RC) for Each Morphological Character Scored in This Study

| Character  | % missing | States | Changes | Steps | CI  | RI  | RC  |
|--|-----------|--------|---------|-------|-----|-----|-----|
| 1. Conventional 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    | .29 | .63 | .18 |
| 5. Functional terminal buds  | 11.3      | 2      | 5       | 6     | .33 | .5  | .17 |
| 6. Terminal buds with scales                                       | 3.8       | 2      | 7       | 13    | .54 | .71 | .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     | .25 | .63 | .16 |
| 9. Leaf insertion  | 3.8       | 2      | 4       | 5     | .4  | .5  | .2  |
| 10. Colleters present  | 6.3       | 2      | 6       | 7     | .29 | .85 | .24 |
| 11. Stipuliform structures   | 3.8       | 2      | 5       | 6     | .33 | .56 | .19 |
| 12. Secondary veins arising from the length of the midrib          | 3.8       | 2      | 1       | 6     | 1   | 0   | 0   |
| 13. Intersecondary veins modified as resin canals                  | 11.3      | 2      | 2       | 2     | .5  | 0   | 0   |
| 14. Tertiary veins parallel and at right angles to secondary veins | 3.8       | 2      | 1       | 4     | 1   | 1   | 1   |
| 15. Exudate in plant body  | 0         | 2      | 2       | 3     | .67 | .67 | .44 |
| 16. Shape of exudate containing structures in mesophyll            | 3.8       | 3      | 3-4     | 16    | .88 | .94 | .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     | .5  | .82 | .41 |
| 19. Midrib structure   | 6.3       | 4      | 8       | 10    | .5  | .81 | .41 |
| 20. Lateral bundles in leaf transcurrent                           | 15        | 2      | 7       | 7     | .14 | .77 | .11 |
| 21. Abaxial palisade tissue present                                | 12.5      | 2      | 3       | 5     | .6  | .33 | .2  |
| 22. Stomatal type  | 6.3       | 2      | 1       | 1     | 1   | 1   | 1   |
| 23. Indumentum of unbranched unicellular hairs                     | 3.8       | 2      | 6       | 11    | .55 | .38 | .2  |
| 24. Indumentum of multicellular hairs                              | 3.8       | 3      | 4-5     | 11    | .73 | .75 | .55 |
| 25. Lamina with marginal setae                                     | 3.8       | 2      | 1       | 1     | 1   | 1   | 1   |
| 26. Lamina with marginal disciform glands                          | 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    | .47 | .5  | .23 |
| 30. Inflorescence type   | 3.8       | 3      | 9       | 10    | .3  | .59 | .18 |
| 31. Pattern of inflorescence internode elongation                  | 8.8       | 2      | 1       | 1     | 1   | 1   | 1   |
| 32. Terminal flowers on inflorescence present                      | 5         | 2      | 2       | 3     | .67 | .5  | .33 |
| 33. Bracteoles   | 6.3       | 3      | 3       | 5     | .8  | .89 | .71 |
| 34. Flower buds  | 0         | 2      | 1       | 2     | 1   | 1   | 1   |
| 35. Sepal number   | 3.8       | 4      | 12      | 15    | .4  | .55 | .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   |
| 38. Androecium arrangement   | 0         | 2      | 7       | 10    | .4  | .83 | .33 |
| 39. Androecium adnate to petals                                    | 3.8       | 2      | 1       | 1     | 1   | 1   | 1   |
| 40. Fascicledia present in staminate or perfect flowers            | 1.3       | 2      | 5       | 7     | .43 | .84 | .36 |
| 41. Filament attachment  | 1.3       | 2      | 1       | 5     | 1   | 1   | 1   |
| 42. Filament much thinner than anthers                             | 1.3       | 2      | 3       | 3     | .33 | .92 | .31 |
| 43. Filaments papillate  | 0         | 2      | 2       | 3     | .67 | .67 | .44 |
| 44. Anther orientation   | 7.5       | 2      | 3       | 3     | .33 | .82 | .27 |
| 45. Anthers locellate  | 0         | 2      | 5       | 6     | .33 | .2  | .07 |
| 46. Anther length  | 0         | 2      | 3       | 3     | .33 | .75 | .25 |
| 47. Anthers with crateriform glands                                | 1.3       | 2      | 2       | 6     | .83 | 0   | 0   |
| 48. Anther thecae with porose dehiscence                           | 0         | 2      | 2       | 2     | .5  | .5  | .25 |
| 49. Pollen aperture number   | 10        | 2      | 4       | 11    | .73 | .67 | .48 |
| 50. Pollen with suprategical elements                              | 12.5      | 2      | 5       | 6     | .33 | .5  | .17 |
| 51. Carpel number  | 0         | 6      | 12-14   | 50    | .82 | .68 | .56 |
| 52. Ovary septate  | 7.5       | 2      | 2       | 2     | .5  | 0   | 0   |
| 53. Ovules per carpel  | 0         | 2      | 7       | 12    | .5  | .57 | .29 |
| 54. Style length   | 6.3       | 2      | 6       | 13    | .62 | .82 | .51 |
| 55. Stylar fusion  | 10        | 2      | 5       | 8     | .5  | .86 | .43 |
| 56. Stigma exposure  | 1.3       | 2      | 1       | 1     | 1   | 1   | 1   |
| 57. Stigma type  | 1.3       | 3      | 8       | 12    | .5  | .6  | .3  |
| 58. Stigma surface   | 2.5       | 3      | 6       | 7     | .43 | .76 | .33 |
| 59. Fruit type   | 0         | 3      | 8       | 12    | .5  | .81 | .4  |
| 60. Seeds with aril  | 0         | 2      | 1       | 1     | 1   | 1   | 1   |
| 61. Seeds winged   | 0         | 2      | 3       | 4     | .5  | .67 | .33 |

**Table 3**  
(Continued)

| Character                                     | % missing | States | Changes | Steps | CI  | RI  | RC  |
|---|-----------|--------|---------|-------|-----|-----|-----|
| 62. Seeds with surface glands                 | 3.8       | 2      | 2       | 3     | .67 | .75 | .5  |
| 63. Seed coat complex                         | 5         | 2      | 5       | 6     | .33 | .88 | .29 |
| 64. Lignified exotegmen                       | 7.5       | 2      | 10      | 11    | .18 | .64 | .12 |
| 65. Ratio of cotyledon to hypocotyl + radicle | 8.8       | 3      | 4       | 4     | .5  | .95 | .48 |
| 66. Cotyledons cordate at the base            | 27.5      | 2      | 1       | 1     | 1   | 1   | 1   |
| 67. Germination type                          | 65        | 2      | 5       | 6     | .33 | .6  | .2  |
| 68. Seedling with accessory roots             | 63.8      | 2      | 5       | 5     | .2  | .2  | .04 |
| 69. Dioecy                                    | 0         | 2      | 5       | 5     | .2  | .86 | .17 |

Note. *Paleoclusia* was not included in the calculation of these values.

alone (fig. 3). 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 because extant taxa scored only for morphology are generally well placed with their closest relatives as proposed in earlier taxonomic classifications (see below; table 1; fig. 6). We will focus our discussion on the placements of four taxa that have received little previous 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 Neotropical genus, is supported as a member of Calophylleae (76 BP) and is placed within a Neotropical clade including the genera *Caraiipa*, *Clusiella*, *Haploclathra*, *Kielmeyera*, *Mahurea*, and *Marila*. This clade is not well supported (62 BP; fig. 6) in our combined analysis but receives strong support in our molecular analyses (91 BP; fig. 3). In addition to the biogeographic support for this placement, *Neotatea* is a good fit morphologically with members of this 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*) mostly possess 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 seeds with

a vascularized wing that does not completely encircle the seed. In our optimal ML topology (fig. 4), and in 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 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 seed wing of the *Kielmeyera* + *Haploclathra* + *Caraiipa* clade is independently derived from the seed wing found in the *Neotatea* + *Mahurea* clade. In the former clade the wing lacks vascular tissue and completely surrounds the seed while in the latter the wing is vascularized but does not completely surround the seed (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 our 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-carpellate gynoecium, the latter of which was found to be a synapomorphy for this clade (fig. D1).

*Hypericum ellipticifolium* (Hypericeae), previously placed in the monotypic genus *Lianthus* from China, remains unplaced with molecular data. In our combined analyses *H. ellipticifolium* is strongly placed (94 BP; fig. 6) in the largely unresolved subclade Hypericeae. In Hypericaceae, staminodes are present in all members of Cratoxyleae and Vismieae. In Hypericeae, however, staminodes are present only in *Hypericum* sections *Adenotrias* and *Elodes* (represented in our study by *Hypericum aegypticum* L. and *Hypericum elodes* L., respectively; Robson 1996) and the former generic segregates *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum* (Ruhfel et al. 2011). All sampled Hypericeae taxa with staminodes were placed in the same nested subclade of *Hypericum* by 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 is the poorly known *Thysanostemon* (Symphonieae; Clusiaceae s.s.) 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*, the *Lorostemon bombaciflorum* group, and *Thysanostemon pakaraimae*. Furthermore, our results indicate that *Lorostemon* is not monophyletic because *Thysanostemon* is more closely related to the *L. bombaciflorum* group (70 BP) than the latter is to other members of *Lorostemon*. Both *Lorostemon* and *Thysanostemon* have pollen with suprategal 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 synapomorphy 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 that is otherwise observed only in the Calophyllaceae taxa *Neotatea* and *Poeciloneuron pauciflorum*. *Thysanostemon* also has papillate filaments, which is a trait found only in the Symphonieae taxa *Platonia*, *Moronobea*, *Montrouziera*, *Thysanostemon*, and *Lorostemon*. However, this character is not constant within these taxa; *L. 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 whether this character defines a clade. Any nomenclatural changes should be deferred until molecular data are available for the poorly known *Thysanostemon* (Stevens 2007a). Previous attempts to extract DNA from *Thysanostemon* using available herbarium vouchers have been unsuccessful (Ruhfel et al. 2011).

*Vismia* and *Psorospermum* are not monophyletic (fig. 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 the *Vismia cayennensis* group) 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 are in need of taxonomic revision with respect to current 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 here 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. (Baretta-Kuipers 1976; Cronquist 1981; Takhtajan 1997), but subsequent phylogenetic evidence placed Theaceae s.l. in the asterid order Ericales (see Stevens 2001–; APG III 2009 and references therein). ASRs of this trait (fig. D2) reveal that the clusioid clade possessed opposite/whorled leaves ancestrally and that alternate leaves evolved at least four times within the group: in Bonnetiaceae, in two subclades of Calophyllaceae (*Mahurea* + *Neotatea* and *Caraipa* + *Haploclathra* + *Kielmeyera*), and in the *Psorospermum febrifugum* group. The ASR of the most recent common ancestor of the *Caraipa* + *Haploclathra* + *Kielmeyera* clade is ambiguous for this character (alternate = 0.47, opposite or whorled = 0.53)—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 opposite/whorled leaves within the clade. Instead, alternate leaf insertion in Podostemaceae would represent another gain of alternate leaves. The *P. febrifugum* group is polymorphic for this character, and this variation could not be included in our ML reconstructions (polymorphic traits are not allowed). This composite terminal, however, is deeply embedded in a clade of opposite leaved terminals 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. D3) and that it has been lost independently in Bonnetiaceae, Podostemoideae, and Tristichioideae. 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 is needed in Podostemoideae to determine the number of gains and losses within the subfamily. Exudate has been reported only in Neotropical Podostemoideae to date (Cook and Rutishauser 2007). We also suggest a detailed chemical analysis of exudates 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 of this character (fig. D4) reveal that the crown clusioid clade possessed glands ancestrally. Bonnetiaceae + Clusiaceae s.s. are reconstructed as equivocal, but crown Clusiaceae s.s. are es-

timated to have possessed canals ancestrally. Glands are estimated to be the ancestral state in the Calophyllaceae + Hypericaceae + Podostemaceae clade, though Podostemaceae was not scored for this character. However, we explored the effect of all scorings for Podostemaceae. No matter which state is present in this terminal, glands still receive >90% 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 scored only sepal number because petal number is often similar. ASRs indicate that the crown clusioid clade as well as the two major clusioid subclades are ancestrally five merous (fig. D5). 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 two to 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 in merosity 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 otherwise reconstructed as being ancestrally five merous.

The clusioid androecium shows variation in two potentially informative characters: androecium arrangement (fasciculate vs. not) and the presence of staminodes or fasciculates in staminate or perfect flowers. The latter terms refer to sterile stamens or sterile fascicles of stamens. There may be some association between these two characters: taxa with fasciculate androecia often have fasciculates. Stamen arrangement is reconstructed as equivocal at the clusioid crown node (fig. D6), as well as at the other early-diverging nodes within the clusioid clade. The crown nodes of the following four clades are confidently reconstructed as having fascicled stamens: *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 at least one additional origin (fig. D6). Fasciculates or staminodes in staminate or perfect flowers appear to have arisen three times independently (fig. D7): in Hypericaceae, a subclade of Symphonieae (all Symphonieae, minus *Symphonia*), and 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. Furthermore, 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 perhaps staminodial 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 scored here as not possessing staminodes. Our ASRs suggest that these structures have arisen multiple times within the clusioid clade, and more work is needed to explore their developmental origins.

Carpel number is also of interest in the clusioid clade (fig. D1). The ancestral state at the crown node of the clusioid clade is ambiguous. The Clusiaceae s.s. + Bonnetiaceae clade is ancestrally five carpellate, as is Clusiaceae s.s. Bonnetiaceae are also possibly five carpellate ancestrally, but *Bonnetia* is polymorphic for this character (three to five carpels), so the ancestral state at this node could not be confidently determined. The Calophyllaceae + Hypericaceae + Podostemaceae clade and each family within this clade are reconstructed as ambiguous.

Dioecy appears to have evolved at least four times within the clusioid clade (fig. D8). 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 indicate crown Clusiaceae s.s. are ancestrally dioecious. Clusiaceae and Garcinieae are ancestrally dioecious (>0.99 in each), while crown Symphonieae are not.

#### 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. 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. 5). In these respects our MP and ML results agree with those of 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; but see Wiens and Morrill 2011).

Character states that support the placement of *Paleoclusia* with Clusiaceae s.s. include extrorse anthers; a five-carpellate gynoeceum; short, fused styles; and dioecy. Extrorse anthers (fig. D9) 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 gynoeceum is present in *Paleoclusia* and is also reconstructed as the ancestral condition in the Bonnetiaceae + Clusiaceae s.s. clade (fig. D1). Five carpels also occur in Hypericaceae and Vismieae, but these taxa are dissimilar to *Paleoclusia* in important ways. Hypericaceae often have stigmas with rounded papillae (fig. D18), and Vismieae have many characters not present in *Paleoclusia* including hairs on the adaxial surface of the petals, which is a synapomorphy of the

tribe. *Paleoclusia* also has very short, fused styles, a combination of states that occurs in very few taxa outside of Clusiaceae s.s. (figs. D10, D11): *Bonnetia* (Bonnetiaceae), *Clusiella* (Calophyllaceae), and *Marathrum* and *Weddellina* (Podostemaceae). Finally, as mentioned above, dioecy (fig. D8) occurs only in Calophylleae (Calophyllaceae), Clusiaceae, and Garcinieae (Clusiaceae s.s.). If *Paleoclusia* were indeed dioecious, its fasciculate androecium, five carpels, and short styles would make it a very poor fit in Calophylleae.

Two characters 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 known only in *Clusiella* (Calophyllaceae), *Chrysochlamys*, *Clusia* s.l., and *Tovomitopsis* (Clusiaceae s.s.; Hammel 1999a; Gustafsson and Bittrich 2002; Gustafsson et al. 2007). However, a number of species in *Caraiipa* and *Marila* (Calophyllaceae) and *Hypericum* (Hypericaceae) have “glands” of various morphologies between the anther thecae (e.g., our character 47), although what (if anything) they secrete is unknown. Within Clusiaceae it is likely that anther 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 Clusiaceae (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* most similar to the early-diverging members of Clusiaceae (*Dystovomita*, *Tovomita*, and *Tovomitopsis*; Y. N. Seetharam, personal communication).

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 occurs only in Bonnetiaceae, Calophyllaceae, and Hypericaceae (fig. D12). *Paleoclusia* also has an indumentum of unicellular hairs on its pedicle and receptacle (figs. 2–6 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, the *L. bombaciflorum* group (Symphonieae) and *Garcinia dulcis* (Garcinieae; fig. D13). Unicellular hairs arose independently in each of these groups, and it is unlikely that *Paleoclusia* is embedded within Garcinieae or Symphonieae for reasons 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. Clusiaceae are defined by the synapomorphy of an arillate seed (fig. D16). The original publication of this fossil indicates that the seed of *Paleoclusia* is arillate (Crepet and Nixon 1998); however, our in-

terpretation of this structure is that it is most likely an aborted seed (Stevens 2001–). Without an aril, *Paleoclusia* would be a bad fit in crown Clusiaceae. In addition, its indumentum of unicellular hairs, fasciculate androecium, and filaments that are much thinner than its anthers (fig. D17) suggest that a phylogenetic placement within extant Clusiaceae is unlikely. Clusiaceae, 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 synapomorphy 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, and 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), it would also be a bad fit in this tribe.

The fossil shares some 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 (more than three apertures; fig. D14); however, reversals to three apertures occur in this 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. D15). 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 *G. 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 nectariferous structures, but species in this clade usually have four (fig. D5) sepals, not five as in *Paleoclusia*.

Given the generally well-supported placements 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 strong support. The uncertainty in the placement of *Paleoclusia* could be due to the lack of better vegetative and anatomical data as well as its possession of a combination of floral characters unlike any taxon in the clusioid clade. Vegetative characters indeed do seem important in placing clusioid taxa using morphology. When these characters are excluded from analysis (see “Material and 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. Based on our ASRs, characters that would be especially helpful in clarifying the placement of *Paleoclusia* would be the position of phellogen initiation in the root and stem (figs. D19, D20, respectively), cortical sclereid presence and shape (fig. D21), shape of exudate containing structures in the mesophyll (i.e., glands or canals; fig. D4), inflorescence type (fig. D22), fruit type (fig. D23), and especially cotyledon to hypocotyl ratio (fig. D24). 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 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 farther 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. Here, we place the important rosid taxon *Paleoclusia* consistently with Clusiaceae s.s. but without strong support. Until *Paleoclusia* is placed more confidently, we have two recommendations for its placement as a fossil age constraint. The first would be to consider the fossil as a crown group minimum age constraint of Clusiaceae s.s. The second would place *Paleoclusia* as a stem group minimum age constraint of Clusiaceae s.s. The first approach would likely result in older age estimates within the clusioid clade; the second approach would likely result in younger ages. Preliminary divergence time estimates of the clusioid clade (Ruhfel 2011) using a Bayesian approach (Drummond and Rambaut 2007) and treating *Paleoclusia* as a member of the Clusiaceae s.s. stem lineage estimated crown group clusioids at 102.9 Ma (minimum = 92.3, maximum = 113.7).

Several previous studies have used *Paleoclusia* as an age constraint in dating analyses. Most of these studies (Davis et al. 2005; Wang et al. 2009; Bell et al. 2010; Arakaki et al. 2011; Clarke et al. 2011) have used constraints that agree with our recommendations given their taxon sampling. However, other studies have placed *Paleoclusia* in positions that differ from our recommendations (Crepet et al. 2004; Magallón and Castillo 2009; Xi et al. 2012). An additional issue with some of these previous studies regarding the placement of this fossil as a constraint is that the phylogenies used are not in agreement with more recent studies of Malpighiales. The phylogenetic history of Malpighiales is now much more resolved and much better supported (Xi et al. 2012). For example, in Bell et al. (2010), *Paleoclusia* is placed as a crown group member of a clade ([Malpighiaceae + Euphorbiaceae] + the clusioids) that we are now confident does not exist. Regardless of the exact placement of *Paleoclusia* within the clusioids, what is clear is that its nested position within the rosids reinforces the hypothesis that the radiation of angiosperms since the origin of the eudicots at ~125 Ma (Magallón et al. 1999; Sanderson and Doyle 2001) was exceptional.

#### *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 that are unavailable for molecular analysis can be placed with certainty using a combined analysis of molecules and morphology (Wiens 2009; Wiens et al. 2010). The place-

ment of *Paleoclusia* is perhaps along stem Clusiaceae s.s. or along the stem of one of its two major subclades. Our ASRs further corroborate this placement, but support is not strong.

Further clarifying the number of origins of dioecy in the clusioid clade, particularly in *Calophyllum*, 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 traits that are 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., Clusiaceae, ~387 spp.; Garcinieae, ~270 spp.) whereas those in Calophyllaceae are relatively species poor (e.g., *Clusiella*, 7 spp.; *Mammea*, ~75 spp.; Stevens 2007a). A comparative methods approach will assist in determining the evolutionary correlates of the seemingly different 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, terminals should be coded at the species level rather than as composites; however, choosing appropriate representative species will require a much better understanding of relationships in many large clusioid subclades (e.g., Clusiaceae, *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.

#### **Acknowledgments**

Funding for this research was provided by NSF DDEP grant OISE-0936076 to B. R. Ruhfel and C. C. Davis and the NSF Assembling the Tree of Life grant DEB-0622764 to C. C. Davis. B. R. Ruhfel was also supported by the American Society of Plant Taxonomists, the Arnold Arboretum at Harvard University, the David Rockefeller Center for Latin American Studies at Harvard University, the Department of Organismic and Evolutionary Biology at Harvard University, and the Garden Club of America. We are grateful to the herbaria cited here for permission to examine material from their collections. Finally, we thank the following individuals for invaluable discussions regarding aspects of clusioid biology, taxonomy, and data analyses or for helpful comments on the manuscript: V. Bitrich, C. P. Bove, W. L. Crepet, M. H. G. Gustafsson, S. Mathews, N. M. Nürk, C. T. Philbrick, R. Rutishauser, H. Schaefer, Y. N. See-tharam, P. W. Sweeney, J. J. Wiens, Z. Xi, and W. H. Zhang.

## Appendix A

## Representative Voucher Specimens for Clusioid Taxa Used to Score Morphology

All vouchers were used to score vegetative characters. Vouchers used to score anatomical (anat.), floral (fl.), or fruit (fr.) characters are labeled as such.

**BONNETIACEAE.** *Archytaea* – *A. angustifolia* Maguire: Maguire et al. 37566 (GH: anat.), Davidse et al. 16955 (MO: fl.); *A. multiflora* Benth.: Maguire & Fanshawe 23108 (A: anat., fr.), Tillett & Tillett 45521 (GH: anat.); Maguire 33052 (GH: fl.). *Bonnetia* – *B. abogadoi* (Steyermark) A.L. Weitzman & P.F. Stevens: Weitzman et al. 394 (A: anat.), Weitzman et al. 409 (A: anat., fl.), Huber et al. 10109 (K: anat.); *B. crassa* Gleason: Maguire & Maguire 35069 (GH: anat.); *B. cubensis* (Britton) R.A. Howard: Shafer 8232 (A: anat.); *B. fasciculata* P.F. Stevens & A.L. Weitzman: Steyermark et al. 128088 (MO: fl.); *B. holostyla* Huber: Schultes & Cabrera 14645 (GH: fl.); *B. kathleenae* Lasser: Maguire & Poletti 27929 (GH: fl.); *B. maguireorum* Steyermark: Maguire et al. 37093 (GH: fl., fr.). *B. martiana* Maguire var. *martiana*: Schultes & Cabrera 18401 (GH: fr.); *B. neblinae* Maguire: Maguire et al. 37111 (GH: anat.); *B. paniculata* Benth.: Maguire & Politi 28397 (GH: anat.); Maguire & Politi 28736 (GH: fr.); *B. roraimae* Oliv.: Wurdack 34184 (GH: fr.). *Ploiarium* – *P. alternifolium* Melchior: Stevens et al. 1074 (A: anat., fl.), SAN 80720 (A: fl.); Soepadmo & Mahmud 9072 (A: fr.); *P. sessile* (Scheff.) Hallier f.: van Royen 5557 (A: anat.), Kostermans & Soegeng 129 (K: anat.).

**CALOPHYLLACEAE.** *Calophyllum* – *C. banyengii* P.F. Stevens: Stevens et al. 296 (A: fr.); *C. brassii* A. C. Sm.: Stevens et al. 838 (A: fl.); *C. canum* Hook. f. ex T. Anderson: Stevens et al. 375 (A: fl.); *C. chapeleiri* Drake: Dorr et al. 4625 (A: anat.), Dorr 4628 (MO: anat.); *C. collinum* P.F. Stevens: Stevens et al. 921 (A: fr.); *C. griseum* P.F. Stevens: Stevens et al. 302 (A: fr.); *C. papuanum* Lauterb.: LAE 54734 (A: fl.); *C. rubiginosum* M.R. Hend. & Wyatt-Smith: FRI 16054 (A: anat.), Soepadmo 86 (A: fl.). *Caraipa* – *C. densifolia* Mart.: Pires et al. 50331 (GH: fl.), Davidse & Gonzalez 12592 (MO: fr.); *C. grandifolia* Mart.: Ducke 195 (GH: fl.); Brazil, Ducke 1694 (GH: fr.); *C. parviliptica* Cuatrec.: Schultes & Cabrera 16460 (GH: anat., fr.); *C. punctulata* Ducke: Maguire et al. 41694 (GH: anat., fl.), Prance & Silva 58808 (GH: anat., fr.); *C. richardiana* Cambess.: For. Dept. British Guiana 4065 (K: fr.); *C. sp. 1*: Kubitzki et al. 79:202 (US: fr.); *C. sp. 2*: Schultes & Liogier 9520 (GH: anat.); *C. tereticaulis* Tulasne: Lasser 1724 (GH: fr.). *Clusiella* – *C. axillaris* (Engl.) Cuatrec.: Kruckhoff 8933 (A: anat., fl.), Maguire et al. 37439 (GH: anat.), Schultes & Cabrera 16089 (GH: anat.); *C. elegans* Planch. & Triana: Soejarto et al. 2869 (GH: anat.), Killip & Cuatrecasas 39862 (GH: anat.), Antonio 3050 (MO: fr.); *Clusiella* sp.: Smith 109 (MO: fr.). *Endodesmia calophylloides* Benth.: Talbot & Talbot 3058 (BM: fr.), FHI 11922 (K: anat., fl.). *Haploclathra* – *H. leiantha* (Benth.) Benth.: Ducke 656 (GH: anat., fl.), Nelson 1244 (K: fr.), Ducke 656 (GH: fl.), Froes 22332 (fr.); *H. paniculata* Benth.: Campbell et al. 21833 (GH: anat., fl., fr.), Ducke 926 (GH: fr.), Riedel 1497 (GH: anat.). *Kayea* – *K. beccariana* Bail.: FRI 4099 (A: fr.); *K. daphnifolia* Ridl.: FRI 17389: (A: fl.); *K. ferruginea* Pierre: SFN 23900 (A: fr.); *K. kunstleri* King: FRI 23072 (A: fr.), Congdon & Hamilton 403 (A: anat., fl.); *K. macrantha* Baill.: S 23917 (A: fl.); *K. myrtifolia* Baill.: Stevens et al. 1185 (A: anat., fr.), Stevens et al. 1135 (A: fl.); *K. sp. 1*: Stevens et al. 1100 (A: anat.); *K. sp. 2*: SFN 29430 (A: fl.); *K. sp. 3*: bb 18908 (A: anat.); *K. sp. 4*: SAN 17441 (A: anat., fr.); *K. sp. 5*: S 18613 (A: anat., fr.); *K. wrayi* King: Chew-Wee-Lek 915 (A: fr.). *Kielmeyera* – *K. coriacea* Mart. & Zucc.: Irwin et al. 8576 (GH: fl.), Irwin et al. 11596 (US: fr.), Irwin et al. 17639 (GH: fl.), Irwin et al. 17823 (US: fr.), Solomon 7730 (fr.); *K. cuspidata* Saggi: SPF 18458 (K: fr.); *K. decipiens* Saggi: Kuhlmann 5029 (US: fr.); *K. excelsa* Cambess.: Riedel 1212 (GH: anat., fr.); *K. grandiflora* (Wawra) Saggi: Cuatrecasas 26604 (US: fr.); *K. neriifolia* Cambess.: Irwin et al. 11596 (US: fr.); *K. rizziniana* Saggi: Sucre et al. 5336 (US: fr.); *K. rubriflora* Cambess.: Irwin et al. 19989 (GH: anat.), Ratter & Ramos 292 (K: fr.); *K. sp.*: Eiten & Eiten 9465 (US: fr.); *K. speciosa* A. St.-Hil.: Irwin et al. 17639 (GH: anat., fl.); *K. variabilis* Mart. & Zucc.: Reitz & Klein 17853 (GH: fl.). *Lebrunia bushaie* Staner: Dubois 816 (A: anat., fl.), Michelson 794 (K: fl.), Evrard 5160 (K: anat.); A. Léonard 3480 (WAG: fr.). *Maburea* – *M. exstipulata* Benth.: Prance et al. 4546 (GH: anat., fr.), Tillett & Tillett 45520 (GH: fl.); *M. exstipulata* subsp. *duckei* (Huber) Kubitzki: Cowan & Wurdack 32037 (GH: fl.); *M. palustris* Aublet: Prance et al. 20017 (GH: anat.). *Mammea americana* group – *M. africana* Sabine: Hart 702 (MO: fl.), Kenfack & Issembe (MO: fr.), Small 558 (K, MO: anat.); *M. americana* L.: Matuda 3580 (A: anat., fl.); *M. immansueta* D. Arcy: Mori & Kallunki 4699 (MO: anat., fr.). *Mammea bongo* group – *M. bongo* (R. Vig. & Humbert) Kostermans: DeCary 5161 (US: fl.), Dorr et al. 4454 (A: anat.); *M. sp.* Madagascar, Dorr et al. 4430 (A: anat., fr.). *Mammea siamensis* group – *M. acuminata* (Kostermans) Kostermans: Stevens et al. 1191 (A: anat.); *M. odorata* (Raf.) Kostermans: BW 4641 (A: fr.), Kornassi 224 (A: fr.); *M. siamensis* Kostermans: Nanakorn 88142 (MO: anat.), Zimmermann s.n. 1899 (MO: fl.); *M. sp.*: McKee 4375 (A: anat., fl.). *Mammea touriga* (C.T. White & W.D. Francis) L.S. Sm.: Gray 943 (MO: anat., fr.), Gray 5776 (MO: fl.), O'Farrell 45 (BRI, L: anat.). *Marila grandiflora* group – *M. grandiflora* Griseb.: Broadway s.n. iii.1928 (GH: anat., fr.), Steyermark & Rabe 96154 (US: fr.); *M. magnifica* Linden & Planch. ex Triana & Planch.: Steyermark & Espinosa 123775 (GH: anat.), Steyermark et al. 95288 (US: fl.); *M. sp. 1*: Ancuash 288 (GH: anat., fl.), Wurdack 2336 (GH: fl.); *M. sp. 2*: Soejarto et al. 4071 (GH: anat., fl.). *Marila tomentosa* group – *M. biflora* Urb.: Ekman 4662 (A: anat.); *M. laxiflora* Rusby: Schunke V. 5003 (GH: anat., fr.); *M. macrophylla* Benth.: Johnston 685 (GH: anat., fr.); *M. pluricostata* Standl. & L.O. Williams: Allen 6538 (GH: anat.), Hammel et al. 18361 (CR: anat.); *M. tomentosa* Poepp. & Endl.: Kanehira 40 (GH: fl.), Schultes & Smith 3014 (GH: fl.), Soejarto et al. 1239 (GH: anat., fr.); *M. verapazensis* Donn. Sm.: Skutch 2495 (GH: fl.). *Mesua ferrea* group – *M. coromandeliana* Wight: Kostermans 25669 (A: fr.), *M. ferrea* L.: Kostermans 25012 (A: anat.), Comanor 1173 (GH: anat., fl.); Keenan et al. 3913 (A: fl.). *Mesua thwaitesii* group – *M. pulchella* Planch. & Triana: CP 3404 (GH: anat.), Anon s.n. (GH: fr.); *M. sp.*: Fernandes 369 (A: anat., fl.); *M. thwaitesii* Planch. & Triana: Anon s.n. (GH: anat., fr.); Ashton s.n. 10.20.89 (A: anat.). *Neotatea* – *N. colombiana*

Maguire: Schultes & Cabrera 14734 (GH: anat., fl.), Schultes & Cabrera 14484 (GH: fl.), Schultes et al. 15040 (GH: fr.); *N. duidae* (Kobuski & Steyermark) P.F. Stevens & A.L. Weitzman: Steyermark 58186 (A: fr.); *N. neblinae* Maguire: Maguire et al. 42243 (GH: fl.). *Poeciloneuron indicum* Bedd.: Ashton s.n. (A: fr.), B.S.I. Southern Circle 62852 (A: anat., fl.). *Poeciloneuron pauciflorum* Bedd.: Barber 4641 (MH: anat.), Broome 210 (K: fr.), Beddome 437 (BM: fl.).

CLUSIACEAE S.S. *Allanblackia* – *A. floribunda* Oliv.: Jongkind et al. 1513 (MO: fl.), LeBrun 1058 (A; anat.), Louis 9956 (A: anat.); *A. kisonghi* Vermoesen: Corbisier-Baland 1412: (A: anat., fl.); *A. marienii* Staner: LeBrun 1377 (A; anat.); *A. parviflora* A. Chev.: Klaine s.n. (A: fl.), Louis 197 (A: fl.); *A. stanerana* Exell & Mendonça: Gossweiler 8221 (BM: fr.). *Chrysochlamys* – *C. caribaea* Urb.: Beard 496 (A: fl., fr.), Beard 1136 (GH: fl.); *C. macrophylla* Pax: Kayap 987 (GH: fr.), Buchtein 5459 (GH: fl.); *C. sp.*: Schunke V 2497 (GH: fr.); *C. weberbaueri* Engl.: Klug 2746 (GH: fl.), Klug 3061 (GH: anat., fl.), Berlin 914 (GH: fr., anat.), Woytkowski 6166 (GH: fr.). *Clusia alata* Planch. & Triana: Lent 2092 (GH: anat., fl.), Lent 2033 (GH: fr.). *Clusia caudatum* (Planch. & Triana) Pipoly: Cuatrecasas 15185 (US: fr.), Cuatrecasas 16987 (US: anat.), Killip & Cuatrecasas 38720 (US: anat.), Gentry et al. 59606 (MO: fl.). *Clusia columbiana* Pipoly: Prance et al. 15527 (NY: anat., fl.), Lawrence 232 (MO: fr.). *Clusia comans* (Meisn.) Pipoly: Black 54–1626 (NY: anat., fl.); Black 54–1625 (NY: anat., fl., fr.). *Clusia flavida* (Benth.) Pipoly: Croat 20564 (GH: anat., fr.), Palacios et al. 8514 (MO: fl.). *Clusia gundlachii* Stahl: Hansen et al. 9271 (MO: fr.), Wagner 93 (A: anat., fr.), Wagner 1276 (A: anat., fl.). *Clusia major* L.: R.A. & E.S. Howard 19438 (A: anat., fr.), R.A. & E.S. Howard 15096 (A: anat., fl.), Beard 1143 (MO: fl.), Whitefoord 4386 (A: anat.). *Clusia panapanari* (Aubl.) Choisy: de la Cruz 3219 (GH: anat., fl.), Hitchcock 17634 (GH: anat., fr.), Maguire et al. 54027 (GH: fl.). *Clusia* p.p. (*Oedematopus* spp.) – *C. huberi* Pipoly: Maguire & Politi 28324A (GH: fl.); *C. obovata* (Planchon & Triana) Pipoly: Prance et al. 4233 (NY: anat., fr.), Maguire et al. 36049 (NY: anat.); *C. octandra* (Poepp.) Pipoly: Schultes & Lopez 10068 (GH: fr.), Maguire et al. 42628 (NY: anat.). *Clusia* p.p. (*Quapoya* spp.) – *C. hammeliana* Pipoly: Kayap 1317 (GH: anat.), Schunke V. 4646 (GH: fr.), Ancuash 671 (GH: anat.); *Quapoya longipes* (Ducke) Maguire: Schultes & Cabrera 15170 (GH: anat., fl.); *Q. scandens* Aubl.: Wachenheim 2929 (A: anat., fl.). *Decaphalangium peruvianum* Melch.: Revilla 468 (MO: fr.), Vasquez et al. 3405 (A: anat.); Schultes et al. 24109 (ECON: anat.), Soejarto 850 (ECON: fl.). *Dystovomita* – *D. clusiiifolia* (Maguire) D’Arcy: Maguire & Steyermark 60030 (GH: anat.), Maguire & Steyermark 60031 (GH: anat., fl.), Liesner & Gonzalez 9875 (MO: fr.), Gentry et al. 40433 (MO: fr.), Forero 4464 (MO: fr.); *D. paniculata* (Donn. Smith) Hammel: Mori & Kallunki 2612 (MO: fr.); Schubert et al. 812 (A: anat.); *D. sp.*: Schultes et al. 14755 (GH: anat.). *Garcinia cymosa* (K. Schum.) I.M. Turner & P.F. Stevens: Kanehira 3992 (A: anat.), NGF 41384 (A: fr.), NGF 43300 (A: anat., fl.). *Garcinia dulcis* (Roxb.) Kurz: Hartley 9943 (A: fr.), Moll 9622 (A: anat.), Takeuchi 4790 (A: fl.). *Garcinia morella* Desr.: Kostermans 24007 (MO: fl.), Kostermans 24854 (A: anat.), CP 372 (GH: fr.). *Garcinia* p.p. (*Pentaphalangium* spp.) – *Garcinia latissima* Miq.: Shaw-Meyer s.n. 11.xi.1963 (A: anat.), Hoogland 3821 (A: anat.); *G. pachycarpa* (A.C. Smith) Kostermans: Brass & Versteegh 13550 (A: fr.); *G. smithii* Kostermans: Brass 8206 (A: anat., fr.); *G. solomonensis* (A.C. Smith) Kostermans: BSIP 6714 (A; anat.), BSIP 14622 (A: anat.); *G. volkensii* (Lauterb.) Kostermans: Kanehira 1173 (A: anat., fl.). *Garcinia* p.p. (*Rheedea* spp.) – *G. madruno* Kunth: Davide & Gonzalez (MO: fr.), Rimachi Y. 3946 (MO: anat., fl.); *G. ruscifolia* (Griseb.) Lourteig: Clemente & Alain 4116 (A: anat.), Leon & Clemente 23246 (A: fr.). *Lorostemon bombaciflorum* group – *Lorostemon bombaciflorum* Ducke: Ducke 944 (GH: anat.), Ducke 1200 (fr.), Prance et al. 21628 (MO: fl.); *L. colombianus* Maguire: Schultes et al. 17619 (GH: fr.); *L. stipitatus* Maguire: Schultes et al. 15910 (GH: fr.). *Lorostemon coelhoi* Paula: Aluzio 245 (GH: anat.), Schultes & Cabrera 25898 (GH: fl.); Rodrigues & Loureira 78142 (NY: fr.). *Montrouziera* – *M. cauliflora* Planch. & Triana: McPherson 1557 (MO: anat.), McPherson 6284 (MO: fl.); *M. gabriellae* Baill.: Baumann-Bodenheim 15045 (A: anat.), Balansa 2364 (A: fr.), McPherson 1931 (MO: fr.); *M. sp.*: Balansa 3192 (A: fr.); *M. sphaeroidea* Planch. & Triana: Balansa s.n. (A: anat.), Balansa 3192 (A: fr.); *M. verticillata* Planch. & Triana: LeRat & LeRat 2483 (A: anat.). *Moronobea* – *M. coccinea* Aubl.: Schultes et al. 18221 (GH: anat.), Smith 2964 (GH: anat.); *M. intermedia* Engler: Maguire 33547 (NY: anat., fl.); *M. jennmannii* Engler var. *jennmannii*: Maguire & Fanshaw 23442 (A: anat.); *M. ptaritepuiana* Steyermark: Steyermark 59720 (GH: anat., fr.); *M. riparia* (Spruce) Planchon & Triana: Schultes et al. 18221 (GH: anat., fl.), Schultes & Lopez 9854 (GH: anat., fr.); *M. rupicola* R.E. Schultes: Schultes & Cabrera 15086 (GH: fr.). *Pentadesma* – *P. butyracea* Sabine: Baldwin 9698 (MO: fl.), Mayer 28 (US: fr.); Cooper 80 (US: fr.); Liberia, Cooper 273 (GH: anat.), Yale School of Forestry 13730 (A: anat.); *P. exelliana* Staner: Gilbert 553 (A: anat.); *P. reyndersii* Spirl.: Reynders 312 (A: anat.). *Platonia insignis* Martius: Schultes & Cabrera 19342 (MO: anat., fl.); Schultes et al. 18272 (GH: anat., fl.), BW 5588 (A: anat.); For. Dept. Brit. Guiana 5017 (K: fr.). *Septogarcinia sumbawaensis* Kostermans: Kostermans 18767 (A: fl.), Kostermans 19125 (A: fr.), Kostermans 18789 (A: anat., fl.). *Symphonia* – *S. globulifera* L. f.: Mori & Kallunki 2860 (GH: fl.), Prance et al. 16358 (MO: fl.), Krukoff 10722 (A: fr.), For. Dept. Brit. Guiana 4847 (K: fr.), Carrisso & Mendonca 436 (MO: fr.), de Wilde 3711 (A: fl.); *S. cf. louvelii* Jumelle & H. Perrier: Dorr & Barnett 4538 (A: anat.); *S. macrophylla* Vesque: Dorr & Barnett 4537 (A: anat.), Station Agric. de l’Alaotra 3482 (MO: fr.); *S. nectarifera* Jumelle & H. Perrier: Station Agric. de l’Alaotra 1901 (MO: anat.). *Thysanostemon pakaraimae* Maguire: Maguire et al. 44026 (GH: anat., fl., fr.). *Tovomita* – *T. brasiliensis* (Mart.) Walp.: Pires & Black 129 (GH: anat.); *T. calodictyos* Sandwith: Maguire & Fanshaw 22898 (A: anat., fl.); *T. macrophylla* Walpers: Spruce 3173 (GH: fl.); *T. plumieri* Griseb.: Howard & Howard 18992 (A: fl.); *T. sp.*: Schultes and Cabrera 15918 (GH: anat., fr.), Krukoff 7242 (A: anat., fr.), Madison et al. 3418 (GH: anat.); *T. cf. umbellata* Benth.: Krukoff 7019 (A: fr.). *Tovomita weddelliana* Planch. & Triana: Diaz et al. 7753A (MO: anat., fl.), Fonnegra et al. 4233 (MO: fr.), Huashikat (MO: fl.).

HYPERICACEAE. *Cratoxylum* sects. *Cratoxylum* + *Tridesmos* – *C. cochinchinense* (Lour.) Blume: KEP 93375 (A: anat.); *C. formosum* (Jack) Dyer ssp. *formosum*: BRUN 5555 (A: fl.), Kostermans & Anta 453 (A: anat.); *C. sumatranum* (Jack) Blume: Lorzing 12338 (A: anat.), van Balgooy 3469 (A: fr.). *Cratoxylum* sect. *Isopterygium* – *C. arborescens* (Vahl) Blume: Rahmat si Toroes 4859 (A; anat.), S 28155 (A: fl.), SAN 89495 (A: anat.); *C. glaucum* Korth.: S 16702 (A: anat., fl.), Hose 578 (A: anat.),

Sidiyasa & Arifin (A: fr.). *Eliea articulata* Cambess.: Humbert 5758 (A: anat., fl.), Areny & Rakotozafy 15350 (MO: fr), Fosberg 52577 (GH: anat.). *Harungana madagascariensis*: L.S. Smith 5321 (A: anat.), Leonard 1457 (A: anat.), Kersting 208A (fr.), Thomas 2315 (A: fl.), Brass 17379 (A: fr.). *Hypericum calcicola* Standl. & Steyermark: Steyermark 48946 (A: anat., fr.), Steyermark 50160 (A: anat., fl., fr.). *Hypericum ellipticifolium* H.L. Li: T.T. Yü 20125 (A: fl., fr.). *Hypericum p.p.* (*Triadenum* spp.) – *H. faurieri* R. Keller: Murata 19838 (A: anat., fr.), Suzuki 351 (A: fr.), Togasi & Isono 371 (A: fr.); *H. fraseri* (Spach) Steudel: Friesner 16304 (GH: anat.); *H. virginicum* L.: Boufford & Wood 17930 (A: anat., fl.), Long 29529 (anat., fr.). *Hypericum steyermarkii* Standl.: Matuda 2894 (A: anat., fl., fr.), Matuda S-228 (A: anat.). *Psorospermum cerasifolium* group – *P. cf. androsaemifolium* Baker: Dorr & Rakotozafy 4534 (A: fl., fr.); *P. cerasifolium* Baker: Perrier de la Bathie 1199 (P; fr.), Perrier de la Bathie 1162 (P; fr.), Kaudern s.n. ix.1912 (A: anat.); *P. fanerana* Baker: Bernardi 11054 (GH: fl.); *P. lanceolatum* (Choisy) Hochr.: Barnett & Dorr 248 (A: anat.), Dorr 3909 (A: fr.), de Cary 17703 (US: fr., anat.), Croat 32590 (MO: fr); *P. molluscum* (Pers.) Hochr.: Scott Elliot 2249 (K: fr.), *P. cf. revolutum* (Choisy) Hochr.: Dorr et al. 4433 (A: anat.). *Psorospermum febrifugum* group – *P. alternifolium* Hook.f.: Adam 5433–19 (MO: anat., fr.); *P. corymbiferum* Hochr.: Barter 1203 (BM: fl., fr.), Heudelot 768 (BM: fl.); *P. febrifugum* Spach: Breteler et al. 2338 (A: anat.), Teixeira & Figueira 5828 (A: fr.), Breteler 2793 (A: fr.), Wilson 188 (A: fr.), Becquaert 14 (anat., fr.); *P. glaberrimum* Hochr.: Scott-Elliot 5367 (A: anat., fl.); *P. mechowii* Engl.: Schmitz 5605 (BM: fl.), Teixeira et al. 9713 (BM: fr.). *Psorospermum lamianum* H. Perrier: Gentry 11297 (GH: anat.). *Psorospermum staudtii* group – *P. staudtii* Engl.: Zenker 4234 (K: fr.), FNI 3509–43 (K: fr.), Louis 8975 (A: anat., fl.); *P. senegalense* Spach: Nigeria, Dalziel s.n. 1912 (BM: fr.). *Vismia affinis* Oliv.: Toussaint 199 (A: anat.), Toussaint 85 (K: fl., fr.), Wagemans 2215 (K: anat., fr.), Gossweiler 6307 (BM: fr.), Gossweiler 9899 (BM: fl.). *Vismia cayennensis* group – *V. amazonica* Ewan: Simpson & Schunke V. 639 (US: fl., fr.); *V. cayennensis* (Jacq.) Pers.: Austin et al. 7202 (GH: anat.); *V. japurensis* Reichardt: Maguire & Stahel 25053 (GH: anat., fl.); *V. latifolia* (Aubl.) Choisy: B 2929 (GH: anat.); *V. macrophylla* Kunth: Dwyer & Kirkbride 7862 (GH: fl.); *V. micrantha* Mart. Ex A. At.-Hil.: Irwin et al. 29669 (NY: anat., fr.); *V. obtusa* Spruce ex Reichardt: Little 98387 (US: fr.); *V. rufescens* (Lam.) Pers.: Gleason 64 (US: fr.). *Vismia laurentii* De Wild.: Corbiseur-Balau 934 (A: anat., fr.). *Vismia orientalis* Engl.: Swynnerton s.n. 3.i.1922 (BM: anat.), Bruce 1058 (BM: fr.), R. M. Graham 2119 (BM: anat.), Mwasumbi & Mhoro 2591 (K: fr.), Lovett 503 (MO: fl.). *Vismia rubescens* Oliver: Louis 10247 (A: anat., fr.), Gossweiler 9169 (A: anat.), Gentry 33544 (MO: anat.), Le Testu 9265 (MO: fl.).

## Appendix B

### Voucher Information and GenBank Accessions for Sequences Used in This Study

Accessions in parentheses are from a different voucher source. A dash (—) indicates that the sequence was unavailable. Herbaria acronyms follow Thiers (2013). 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., Rubfel 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., Rubfel 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. Ndiva 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* Scott. 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; *Montrouzieria cauliflora* Planch. & Triana, *Loury 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; *Platonina 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), —, —, —; *Thornea calcicola* (Standl. & Steyermark) 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.

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