A plastid DNA phylogeny of tribe Miliuseae: Insights into relationships and character evolution in one of the most recalcitrant major clades of Annonaceae

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- *Premise of the study:* Tribe Miliuseae (~25 genera and ~510 species) includes a substantial part of the species and generic diversity in the pantropical flowering-plant family Annonaceae (~108 genera and ~2400 species). Previous molecular phylogenetic analyses have failed to resolve the backbone phylogeny of the tribe, impeding biogeographical and evolutionary studies. We use a dense generic taxon sample (~89% of generic diversity in Miliuseae) and plastid DNA sequence data (~7 kb) to clarify the phylogenetic relationships of and within the tribe.
- *Methods:* Parsimony and Bayesian phylogenetic reconstructions and ancestral character-state reconstructions of several reproductive characters were performed.
- *Key results:* Dendrokingstonieae, Monocarpieae, and Miliuseae are recovered in a strongly supported clade, and each tribe is strongly supported as monophyletic. Miliuseae are characterized by a synapomorphic cryptoaperturate/disulculate pollen apertural system. *Stenanona* is shown to be nested within the paraphyletic genus *Desmopsis*. The only Neotropical clade (*Sapranthus*, *Tridimeris*, *Desmopsis*, and *Stenanona*) in the predominantly Asian Miliuseae is shown to be closely related to an undescribed genus from continental Southeast Asia and the Indo-Malayan and Austral-Pacific genus *Meiogyne*. Ancestral character-state reconstructions of several reproductive characters that are diagnostically important at the generic level indicate a considerable degree of homoplasy.
- *Conclusions:* The results improve our understanding of the relationships of and within Miliuseae, but parts of the backbone of the phylogeny remain poorly supported. Additional data from variable nuclear markers or reduced-genome-representation approaches seem to be required to further resolve relationships within this recalcitrant clade.

   Key words: Annonaceae; character evolution; chloroplast markers; Miliuseae; morphology; palynology; phylogenetic analyses.

   The flowering-plant family Annonaceae comprises ~108 genera and ~2400 species of trees, shrubs, and woody lianas (Rainer and Chatrou, 2006; Chatrou et al., 2012) that predominantly inhabit lowland rainforests throughout the tropics. It is the most species-rich family in the early divergent order Magnoliales (Sauquet et al., 2003). Annonaceae are characterized by a suite of features, such as vessel elements with simple perforations, distichous leaf arrangement, a trimerous perianth differentiated into calyx and corolla, and ruminate endosperm (e.g., Keßler, 1993; Sauquet et al., 2003).

   On the basis of recent phylogenetic analyses of a supermatrix containing up to eight plastid markers, Chatrou et al. (2012) identified major clades in the family and classified these at the subfamilial and tribal levels. The family is now classified into four subfamilies: Anaxagoreoideae, Ambavioideae, Annonoideae, and Malmeoideae. The latter two subfamilies together constitute a large clade containing >95% of the species in the family (Rainer and Chatrou, 2006; Chatrou et al., 2012). The study by Chatrou et al. (2012) and previous molecular phylogenetic studies (e.g., Mols et al., 2004a, 2004b; Richardson et al., 2004; Pirie et al., 2006; Couvreur et al., 2008) have brought much of the backbone phylogeny of the Annonaceae into focus, providing a framework to address evolutionary questions regarding morphological character evolution (e.g., Saunders, 2010, 2012; Doyle and Le Thomas, 2012; Koek-Noorman and Westra, 2012), historical biogeography of the family (Couvreur et al., 2011), and patterns and timing of diversification (Erkens et al., 2012; Pirie and Doyle, 2012). Despite this considerable progress, parts of the family phylogeny, especially of and within the largely paleotropical tribe Miliuseae, are still unsatisfactorily resolved (e.g., Chatrou et al., 2012).

Tribe Miliuseae consisted traditionally of only six genera, *Alphonsea* Hook.f. & Thomson, *Mezzettia* Becc. (tentatively included), *Miliusa* Lesch. ex A.DC., *Orophea* Blume, *Phoenicanthus* Alston, and *Platymitra* Boerl. (Keßler, 1993), which are characterized by "miliusoid" stamens (i.e., stamens without connective prolongations or with short connective prolongations not extending over the pollen sacs). Analyses of plastid DNA sequence data indicated, however, that these genera do not form a clade, but fall in various positions within a clade comprising ~25 genera (Mols et al., 2004a, 2004b; Chatrou et al., 2012). Tribe Miliuseae has recently been recircumscribed to accommodate all genera of this clade, making it the largest tribe in the subfamily Malmeoideae, comprising a substantial part of the species diversity in Annonaceae (~510 spp.: Chatrou et al., 2012). Members of Miliuseae are predominantly distributed in tropical and subtropical Asia, Australasia, and Oceania (India, across continental Southeast Asia and Malesia to Australia and Pacific islands such as New Caledonia and Fiji), but the tribe also includes a clade of four Neotropical genera (*Desmopsis* Saff., *Sapranthus* Seem., *Stenanona* Standl., and *Tridimeris* Baill.) and an Afro-Malagasy clade of species within *Hubera* Chaowasku (Chaowasku et al., 2012a). The tribe is morphologically highly diverse with regard to inflorescence architecture and position, petal morphology, endosperm rumination type, and pollen morphology (see Mols et al., 2004a). At present, the only synapomorphies of the Miliuseae thus far identified are palynological features (Doyle and Le Thomas, 2012), the most obvious of which is apertures. Miliuseae pollen has been considered cryptoaperturate/disulculate (Chaowasku et al., 2012b).

Previous phylogenetic analyses based on varying taxon sampling and up to eight plastid DNA regions have clarified several generic circumscriptions within Miliuseae, including disintegration of the previously highly polyphyletic genus *Polyalthia* Blume and realignment of its segregates (Mols et al., 2008; Saunders et al., 2011; Xue et al., 2011, 2012; Chaowasku et al., 2012a), and identification of the paraphyly of *Meiogyne* Miq. (Chaowasku et al., 2011b; Thomas et al., 2012; Xue et al., 2014) and *Desmopsis* (Mols et al., 2004a). The phylogenetic relationships of and within Miliuseae, however, remain mostly uncertain in these studies. For example, although Miliuseae have consistently been recovered as sister group of the monogeneric tribe Monocarpieae (e.g., Chatrou et al., 2012), the exact relationship between the two tribes is still somewhat obscure, as the monogeneric tribe Dendrokingstonieae, which has been hypothesized to be closely related to Monocarpieae on the basis of macromorphology and palynology (Chaowasku et al., 2012b), has not been included in previous molecular phylogenetic analyses.

Mols et al. (2004a) performed ancestral character-state reconstructions using parsimony to understand character evolution within the morphologically highly diverse Miliuseae. They reconstructed the ancestral states of 13 vegetative and reproductive characters using a phylogenetic tree based on a combination of DNA sequence data and morphology (~3 kb plus 42 morphological characters). Several genera (e.g., *Tridimeris* and *Trivalvaria* [Miq.] Miq.) were not sampled, however, and the results were inconclusive because of the poorly resolved relationships within Miliuseae.

The aims of this study, therefore, are to clarify relationships within Miliuseae, and to investigate the evolution of diagnostically important reproductive characters within this recalcitrant and morphologically diverse clade. To achieve these aims, a molecular phylogeny of Miliuseae using seven plastid markers

(~7 kb) and covering ~89% of generic diversity was reconstructed. In addition, accessions of tribes previously inferred or hypothesized to be related to Miliuseae were included to assess the intertribal relationships of Miliuseae.

MATERIALS AND METHODS

Taxon and character sampling (Appendix 1)—All genera of the Miliuseae were sampled, except for *Oncodostigma* Diels, *Phoenicanthus*, and the recently described genus *Wangia* X.Guo & R.M.K.Saunders, for which leaf material suitable for DNA extraction was not available. When possible, at least two species per genus were sampled, including a putatively new genus within Miliuseae. Accessions of tribes Fenerivieae, Maasieae, Malmeeae, and Monocarpieae, representing other major clades of Malmeoideae, were also included. Accessions of *Dendrokingstonia* (the only genus of Dendrokingstonieae) were included to elucidate its position within Malmeoideae. A species of *Annickia* Setten & Maas and one of *Greenwayodendron* Verdc., both from the tribe Piptostigmateae, were selected as outgroups. Seven plastid markers (*rbcL* exon, *trnL* intron, *trnL-F* spacer, *matK* exon, *ndhF* exon, *psbA-trnH* spacer, and *ycf1* exon) were amplified. Sequences were obtained from previous studies (Mols et al., 2004a, 2004b; Pirie et al., 2006; Su et al., 2008; Chaowasku et al., 2012a, 2013a, 2013b) or newly generated for this study (32 sequences; see Appendix 1).

The *rbcL* and *ycf1* exon sequences are missing for 24 and 8 accessions, respectively (see Appendix 1), because of failure in DNA amplification or unavailability of leaf material. In addition to the DNA sequence data (7027 characters included), 11 indels were coded as binary characters using the simple indel coding method of Simmons and Ochoterena (2000). An inversion of a 15-nucleotide stretch in the *psbA-trnH* spacer was present in roughly half of the accessions sequenced. This inversion was reverse-complemented to make the analyzed sequences comparable throughout the data matrix (see Pirie et al., 2006). Taxon names and voucher information for molecular phylogenetic (including GenBank accession numbers), macromorphological, and palynological (with applied techniques indicated) studies are given in Appendices 1, 2, and 3, respectively.

Mols et al. (2004a) adopted a total-evidence approach and included 42 morphological characters in their phylogenetic analyses. We did not follow this approach in the present study because (1) the morphological data partition of Mols et al. (2004a) had very limited phylogenetic utility at the generic and deeper levels and (2) coding of several characters is highly problematic, as detailed below (see Reconstructions of ancestral character states).

DNA extraction, amplification, and sequencing—All methods and reagents used for DNA extraction, amplification, and sequencing follow Chaowasku et al. (2012a).

Phylogenetic analyses—Sequences were edited using Staden (http://staden. sourceforge.net/) and, subsequently, were manually aligned on the basis of homology assessment using the similarity criterion (see Simmons, 2004). Parsimony analysis was performed in TNT version 1.1 (Goloboff et al., 2008). All characters were equally weighted and unordered. Incongruence among markers was assessed by analyzing each marker individually, to see if there was any significant conflict in clade support (Seelanan et al., 1997; Wiens, 1998). Multiple most parsimonious trees were generated by a heuristic search of the combined data, with 6000 replicates of random sequence addition, saving 10 trees per replicate, and using the tree bisection and reconnection (TBR) branch-swapping algorithm. Clade support was measured by symmetric resampling (SR), which is not affected by a distortion (resulting in incorrectly estimated percentages) associated with some bootstrap and jackknife methods (Goloboff et al., 2003). A default change probability was used. Four hundred thousand replicates were run, each with two replicates of random sequence addition, saving one tree per replicate. A clade with SR ≥85%, 70–84%, and ≤69% was considered strongly, moderately, and weakly supported, respectively.

Bayesian Markov chain Monte Carlo (MCMC; Yang and Rannala, 1997) phylogenetic analysis was performed in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). The data matrix was divided into seven partitions on the basis of DNA region identity (the *trnL* intron and the adjacent *trnL-F* spacer were combined as a single partition) and a binary indel-code partition. The most appropriate model of sequence evolution for each partition was selected by AIC (Akaike, 1974) scores, using FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). The general time reversible (GTR;

Tavaré, 1986) nucleotide substitution model with among-site rate variation modeled with a gamma distribution was selected for four partitions (*rbcL*, *matK*, *ndhF*, *ycf*1), and the Hasegawa-Kishino-Yano (HKY; Hasegawa et al., 1985) substitution model with among-site rate variation modeled with a gamma distribution was selected for the *trnLF* (= *trnL* intron + *trnL-F* spacer) and *psbA-trnH* partitions. The "coding=variable" setting and a F81-like binary model were selected for the binary indel partition as recommended in the MrBayes 3.1 manual (http://mrbayes.sourceforge.net/wiki/index.php/Manual). Four independent analyses, each using four MCMC chains, were simultaneously run; each run was set for 10 million generations. The default prior settings were used except for the prior parameter of rate multiplier ("ratepr" [=variable]) and the prior probability distribution on branch lengths ("brlenspr" [=unconstrained:exp(100)]). The latter prior setting is to avoid the MCMC chains from being trapped in the areas of parameter space with unrealistically high values for the tree length parameter, resulting in a false convergence or a failure to reach convergence after hundreds of millions of generations (Marshall, 2010). The temperature parameter was set to 0.05. Trees and all parameter values were sampled every 1000th generation. Convergence was assessed by checking the standard deviation of split frequencies of the runs with values <0.01 interpreted as indicating good convergence, by checking for adequate effective sample sizes (ESS > 200) using Tracer version 1.5 (Rambaut and Drummond, 2009), and by checking the stationarity of posterior probabilities of splits within runs and the convergence of posterior probabilities of splits between different runs using AWTY (Nylander et al., 2008). The initial 25% of samples were discarded as the burn-in, and a 50% majority-rule consensus tree was generated from the remaining samples. A clade with posterior probabilities (PP) ≥0.96, 0.91–0.95, and ≤0.90 was considered strongly, moderately, and weakly supported, respectively.

Reconstructions of ancestral character states—Ancestral character states of nine characters, which have historically been proved to be diagnostically important in Annonaceae systematics, and which have been used in previous analyses (e.g., Doyle and Le Thomas, 1996; Mols et al., 2004a), including six macromorphological and three palynological characters, were reconstructed. Character states (Appendix 4) were scored using published descriptions and/or observations based on living and herbarium material (see Appendix 4 for references; specimens studied are indicated in Appendices 2 and 3).

Macromorphological characters—(1) Outer petal appearance: (0) = showy (outer petals much larger than sepals [>2 times longer and wider than sepals] and/or similar to inner petals in size); (1) = ± sepaloid (outer petals approaching sepals in size [≤2 times longer and wider than sepals] and considerably smaller than inner petals [≥2 times shorter and narrower than inner petals]).

(2) Inner petal base: (0) = not clawed. (1) = distinctly clawed.

(3) Maximum ovule number per ovary: (0) = 1. (1) ≥ 2. In previous studies, two-ovuled ovaries have been treated as a separate character state (Doyle and Le Thomas, 1996; Mols et al., 2004a), but none of the genera in Miliuseae invariably exhibit two-ovuled ovaries, so we differentiated only uniovulate and multiovuled ovaries.

(4) Endosperm rumination type: (0) = spiniform to flattened pegs. (1) = lamelliform.

(5) Flower sexuality: (0) = bisexual flowers. (1) = unisexual flowers (in the same or different individuals). (2) = bisexual and staminate flowers (in the same or different individuals).

(6) Inflorescence position: (0) = axillary. (1) = terminal including its derived forms (internodal: extra-axillary, leaf-opposed, supra-axillary).

Pollen characters—(7) Dispersal unit: (0) = monad. (1) = tetrad.

(8) Apertural system: (0) = monosulcate. (1) = cryptoaperturate or disulculate.

(9) Infratectum type: (0) = columellate to coarsely granular. (1) = finely and densely granular. (2) = exine atectate (i.e., exine not to very weakly differentiated into tectum, infratectum, and basal layer).

Some characters that have previously been considered diagnostically important at the generic level were not analyzed, because distinct character states were difficult to distinguish or the characters were highly polymorphic at the generic level.

Two main types of tertiary leaf venation, reticulate and percurrent, have traditionally been differentiated in the Annonaceae, and this character has been used in phylogenetic analyses (Doyle and Le Thomas, 1996) and for generic circumscription (e.g., Chaowasku et al., 2011a, 2012b; Xue et al., 2012). Extensive observations indicate, however, that a number of genera in Miliuseae do not show discrete distributions of these character states and that intermediate types are sometimes present (e.g., *Monoon* Miq.: Chaowasku et al., 2011a, under *Enicosanthum* Becc.; *Polyalthia*: Xue et al., 2012; *Meiogyne* and *Pseuduvaria* Miq.: T. Chaowasku, personal observation). The intermediate form was treated as an additional character state in Doyle and Le Thomas (1996), but we did not follow this approach in the present study because many genera would be scored as polymorphic with either reticulate and intermediate or percurrent and intermediate tertiary leaf venation.

The shape and configuration of stamen connective tissue found in the Miliuseae are variable, and two discrete states, so-called "uvarioid" stamens characterized by a peltate-truncate connective extending over the pollen sacs, and so-called "miliusoid" stamens without connective prolongations or with short connective prolongations not extending over the pollen sacs, have been recognized and used for generic delimitation (Keßler, 1993; Mols et al., 2004a). We did not include this character in the analyses, however, because intermediate forms are often present (i.e., sometimes the stamen connective tissue is reduced or elongated; van Heusden, 1994 and Jessup, 2007: *Meiogyne*; Mols and Keßler, 2000a: *Phaeanthus* Hook.f. & Thomson; Schatz and Maas, 2010: *Stenanona*; Xue et al., 2011: *Marsypopetalum* Scheff.), and discrete types are difficult to differentiate.

Regarding the texture of the endosperm (glass-like vs. soft), Doyle and Le Thomas (1996) and Mols et al. (2004a) included this character in their analyses and found some phylogenetic signal. We reinvestigated this character, however, and found that character-state determination is subjective; for example, van Setten and Koek-Noorman (1992) described the endosperm texture of *Neouvaria* Airy Shaw as glass-like, whereas Mols et al. (2004a) and Xue et al. (2012) stated that it is soft. These inconsistencies prompted us to exclude this character from the analyses.

The trees remaining after the initial 50% of trees sampled in the Bayesian phylogenetic reconstructions had been discarded were included as input trees for Bayesian and parsimony ancestral character-state reconstructions in BayesTraits (Pagel et al., 2004) and Mesquite (Maddison and Maddison, 2010), respectively. The outgroups (accessions of Piptostigmateae) plus Malmeeae, Maasieae, and Fenerivieae were excluded, and the taxon set was pruned in Mesquite so that it included only a single representative (accession) per genus. We adopted this approach because molecular data on the basis of a dense taxon sampling representative of morphological variability was not available for most Miliuseae genera.

In the absence of densely sampled molecular phylogenies in combination with ancestral character-state reconstructions for most genera in Miliuseae, characters were scored as polymorphic when more than one character state was observed within a genus. For *Pseuduvaria* (Su et al., 2008, 2010), *Meiogyne* (Thomas et al., 2012; Xue et al., 2014), and *Miliusa* (Chaowasku et al., 2013a), for which extensively sampled molecular phylogenies are available, only character states inferred to be ancestral for the respective genera on the basis of parsimony reconstructions (using the methods outlined below; results not shown) were scored.

For the reconstructions in BayesTraits, the MCMC mode and the "multistate" model of evolution were selected. We used the reversible-jump (RJ) MCMC (Pagel and Meade, 2006) with a hyperprior approach (see Pagel et al., 2004) as recommended in the BayesTraits manual (http://www.evolution.reading.ac.uk/Files/BayesTraits-V1.0-Manual.pdf). The interval of 0–30 for the RJ-hyperprior implementing an exponential distribution was applied. The "addMRCA" command was used to calculate the posterior distribution of ancestral character states at selected nodes of interest of the pruned 50% majority-rule consensus tree. A total of 5 million iterations were run, with sampling every 100th iteration, and discarding a burn-in of 500000 iterations. To get optimal ranges for acceptance rates (20–40%), we adjusted the "ratedev" parameter for each character. Results of the MCMC runs including the ESS values were checked in Tracer (Rambaut and Drummond, 2009).

For parsimony ancestral character-state reconstructions in Mesquite, character state changes were treated as unordered. The "trace over trees" option was selected, and reconstructions across the input trees were summarized at each node of the pruned 50% majority-rule consensus tree using the "Uniquely Best State" option.

Pollen morphology—Pollen samples were taken from dried herbarium specimens or spirit material (see voucher information in Appendix 3). Following Chaowasku et al. (2008) and Couvreur et al. (2009), the pollen was not acetolysed for scanning electron microscopy (SEM). For transmission electron microscopy (TEM), all material was prepared following van der Ham (1990).

The general pollen terminology used follows Punt et al. (2007). The exine subdivision into tectum, infractectum, and basal layer, following Le Thomas (1980), is used.

RESULTS

Phylogenetic analyses—General descriptive statistics of sequence data, including the number of characters in each partition and the number and percentage of variable and parsimony informative characters (PICs), are given in Table 1. The *psbA-trnH* spacer shows the highest percentage of PICs. The *ycf1* region shows the highest percentage of PICs among all coding regions sequenced (*rbcL* exon, *matK* exon, *ndhF* exon, and *ycf1* exon).

Parsimony analysis of the combined data resulted in 45 most parsimonious trees with 2246 steps. The ensemble consistency and retention indices were 0.74 and 0.72, respectively. There was no strong conflict (SR ≥ 85%) in the analyses of individual markers (results not shown).

Figure 1 shows the 50% majority-rule consensus tree of the Bayesian phylogenetic analysis with PP and corresponding parsimony SR support values. Results of both parsimony and Bayesian analyses were largely congruent; clades present in the Bayesian 50% majority-rule consensus tree, but not in the strict consensus tree of the parsimony analysis, are indicated in Figure 1.

The ingroup, comprising the strongly supported tribes Miliuseae (SR 100%; PP 1), Monocarpieae (monogeneric; SR 100%; PP 1), Dendrokingstonieae (monogeneric; SR 99%; PP 1), Fenerivieae (monogeneric; SR 100%; PP1), Maasieae (monogeneric; SR 100%; PP1), and Malmeeae (SR 92%; PP 1), was monophyletic with strong support (SR 100%; PP 1). The first three tribes were strongly supported as a monophyletic group (SR 99%; PP 1), which forms a polytomy with Fenerivieae, Maasieae, and Malmeeae. The Miliuseae and Monocarpieae, together, were recovered as a monophyletic group with weak to moderate support (SR < 50%; PP 0.93).

Most genera in Miliuseae represented by two or more accessions in the analyses were strongly supported as monophyletic. An exception is *Desmopsis*, which is paraphyletic because one species of *Stenanona* is nested within (SR 98%; PP 1). The clade comprising *Desmopsis* and *Stenanona* received strong support (SR 100%; PP 1). Within Miliuseae, clade A, which is composed of *Mitrephora* Hook.f. & Thomson, *Alphonsea*, and *Platymitra*, was moderately to strongly supported (SR 78%; PP 1). It is sister to the rest of the Miliuseae, which formed a weakly supported clade (clade B: SR < 50%; PP 0.79). Two major clades were recovered in clade B: clade C and clade D. Clade C was poorly supported (SR < 50%; PP 0.87). It is divided into two subclades (clades C1 and C2). Clade C1 includes two genera, *Hubera* and *Miliusa*, whose sister-group relationship was strongly supported (SR 90%; PP 0.98). Clade C2, which is the larger subclade of clade C and comprises six genera (*Orophea*, *Marsypopetalum*, *Trivalvaria*, *Pseuduvaria*, *Popowia* Endl., and *Polyalthia*), was weakly supported (SR < 50%; PP 0.83) and shows a poorly supported backbone. Two strongly supported sister relationships can be identified within this clade: *Marsypopetalum* and *Trivalvaria* (SR 99%; PP 1), and *Popowia* and *Polyalthia* (SR 97%; PP 1). Clade D, which is sister to clade C, is weakly supported in parsimony analysis (SR < 50%) but received strong support in the Bayesian analysis (PP 0.97). It comprises the moderately to strongly supported subclade D1 (SR 72%; PP 1) and subclade D2, which was strongly supported in Bayesian analysis (PP 0.99), but weakly supported in parsimony analysis (SR < 50%). Clade D1 comprises *Meiogyne*, *Sapranthus*, *Tridimeris*, *Desmopsis*, *Stenanona*, and an undescribed genus. Relationships among these six genera are well resolved and supported. Clade D2 contains *Phaeanthus*, *Neouvaria*, *Monoon*, *Stelechocarpus* Hook.f. & Thomson, *Winitia* Chaowasku, and *Sageraea* Dalzell. The last three genera form a strongly supported clade (SR 91%; PP 1); *Stelechocarpus* is sister to *Winitia* with moderate support (SR 82%; PP 0.91). *Monoon* and *Neo-uvaria* were strongly supported (SR 99%; PP 1) as sister genera. The clade composed of these two genera is sister to *Phaeanthus* with weak support (SR < 50%; PP 0.78).

Reconstructions of ancestral character states in tribe Miliuseae—The log-likelihood, RJ hyperprior parameter, acceptance rates, and posterior probabilities of each character state at nodes of interest (nodes Miliuseae, A, B, C, D, D1, and D2) all possessed ESS values (after burn-in was discarded) that were >1200, indicating adequate posterior sampling. Results of the parsimony and Bayesian ancestral character-state reconstructions were largely congruent and are illustrated in Figures 2 and 3 (for precise values of all characters reconstructed, see Appendices 5 and 6).

Outer petal appearance (character 1; Fig. 2A)—The derived state of outer petals being similar to the sepals in size (≤2 times longer and wider than sepals) and considerably smaller than the inner petals (≥2 times shorter and narrower than inner petals) (Fig. 4J) is inferred to have evolved multiple times from the ancestral state of showy outer petals (Fig. 4A–I, K, L): in *Miliusa* (clade C1), *Phaeanthus* (clade D2), and somewhere in each of several genera in clade C2 (*Orophea*, *Marsypopetalum*, *Trivalvaria*, *Pseuduvaria*, *Polyalthia*, *Popowia*).

TABLE 1. General descriptive statistics of sequence data included in the phylogenetic analyses. NA = not applicable.

DNA region	Number of included characters	Number of accessions (of all 65 accessions included) lacking sequence data (%)	Number of variable characters (%)	Number of parsimony-informative characters (%)
rbcL exon	1380	24 (36.9)	124 (9.0)	65 (4.7)
trnL intron + *trnL-F* spacer	926	0	201 (21.7)	101 (10.9)
matK exon	828	0	184 (22.2)	84 (10.1)
ndhF exon	2033	0	436 (21.4)	247 (12.2)
psbA-trnH spacer	431	0	137 (31.8)	94 (21.8)
ycf1 exon	1429	8 (12.3)	358 (25.1)	177 (12.4)
Combined data	7027	NA	1440 (20.5)	768 (10.9)
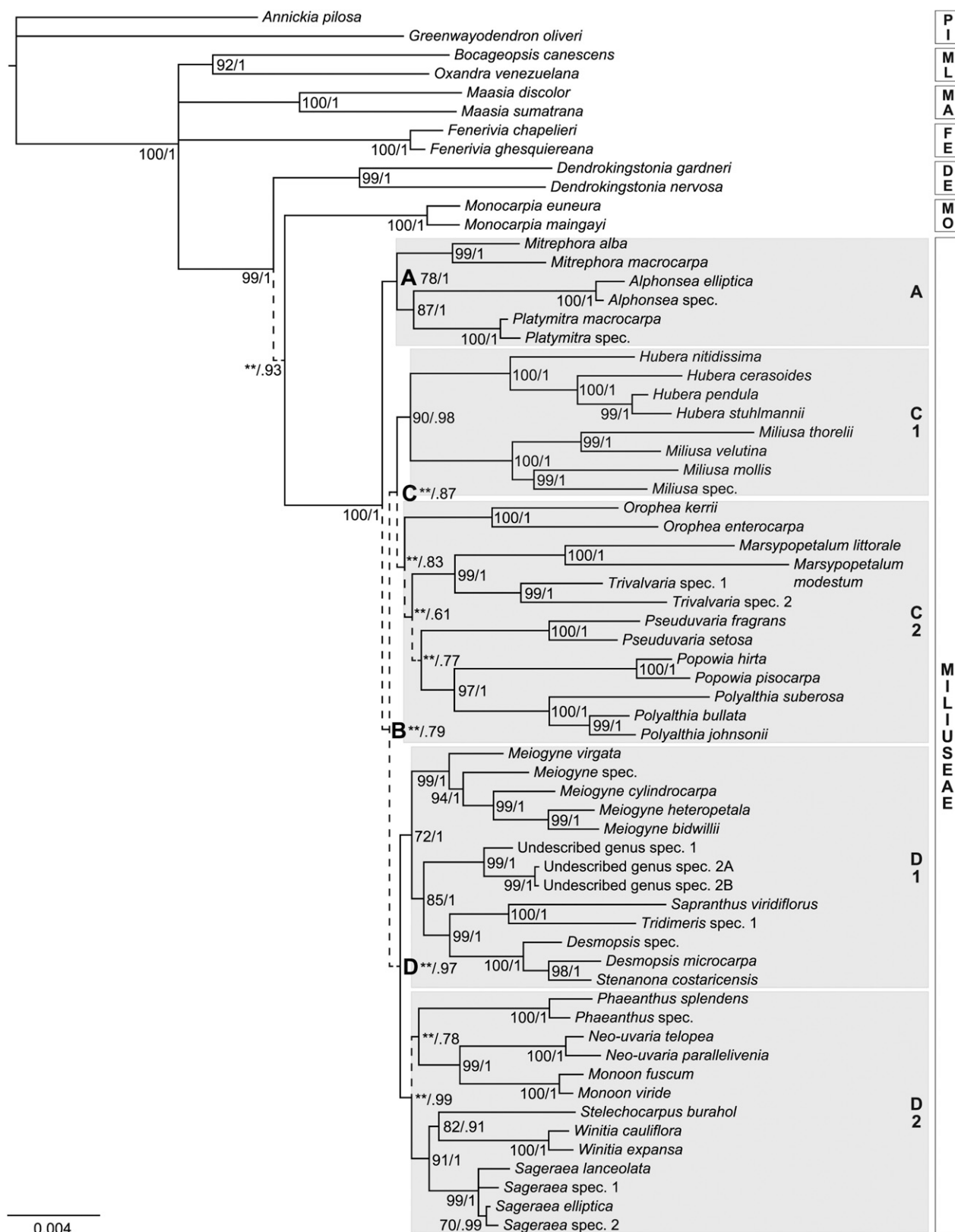
Fig. 1.   The 50% majority-rule consensus tree from Bayesian analysis of seven cpDNA markers. Scale bar unit: Substitutions per site; numbers at nodes indicate clade support: SR (symmetric resampling values of corresponding clades from the parsimony analysis)/PP (posterior probabilities); ** indicates SR < 50%; dashed lines indicate branches leading to nodes that are not present in the strict consensus tree from the parsimony analysis; PI, Piptostigmateae (= outgroups); ML = Malmeeae; MA = Maasieae; FE = Fenerivieae; DE = Dendrokingstonieae; MO = Monocarpieae.
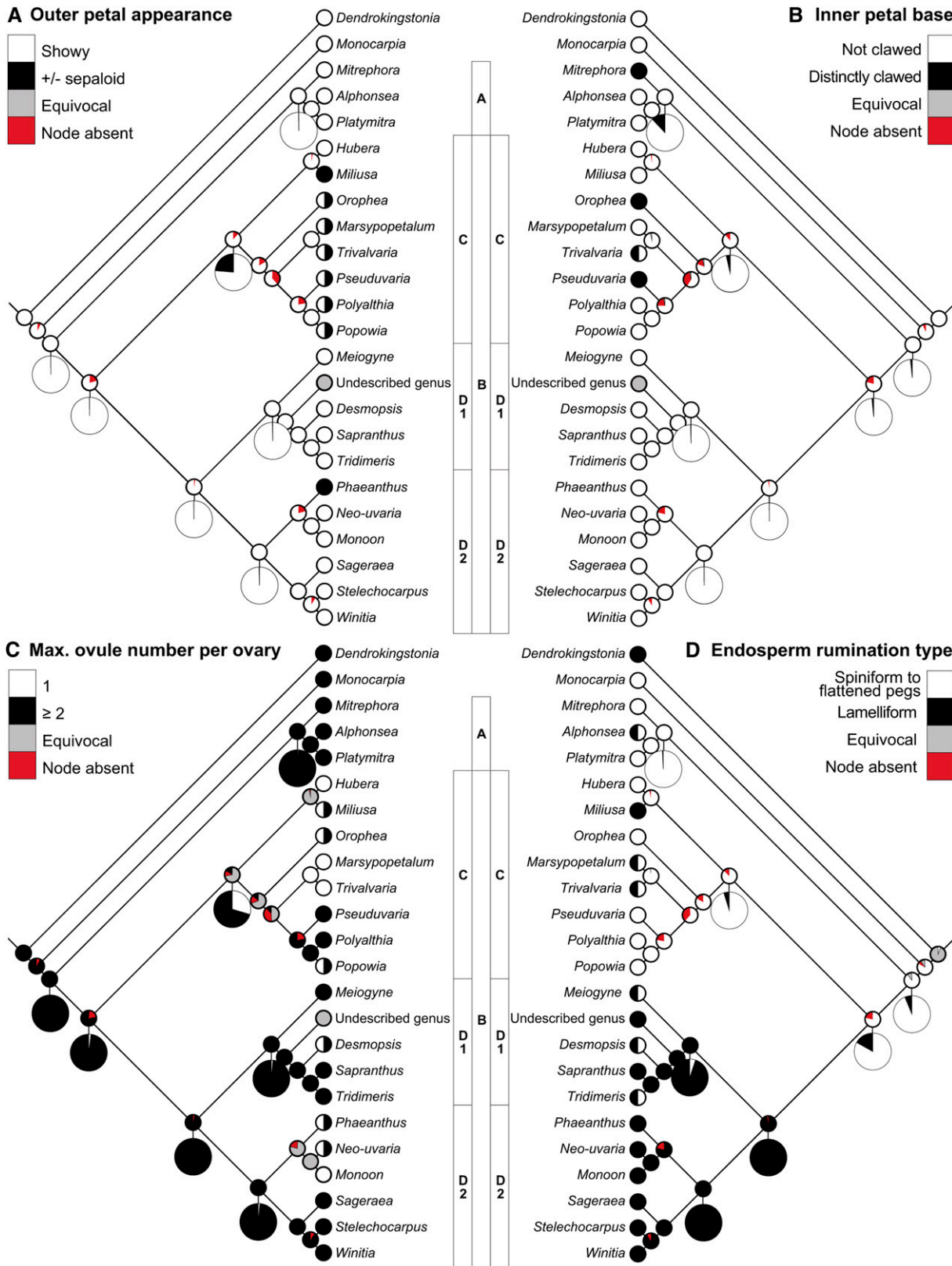
Fig. 2.   Bayesian and parsimony ancestral character-state reconstructions across 20 004 post-burn-in trees shown on pruned 50% majority-rule consensus tree of the Bayesian phylogenetic reconstructions (for details, see Materials and Methods). Parsimony analyses: Smaller pie charts at internal nodes illustrate the number and proportion of unequivocal state reconstructions, equivocal state reconstructions (gray), and the proportion of node absence in the input trees (red). Bayesian RJ-MCMC analyses: Larger pie charts at selected nodes indicate posterior probabilities for states at the node.
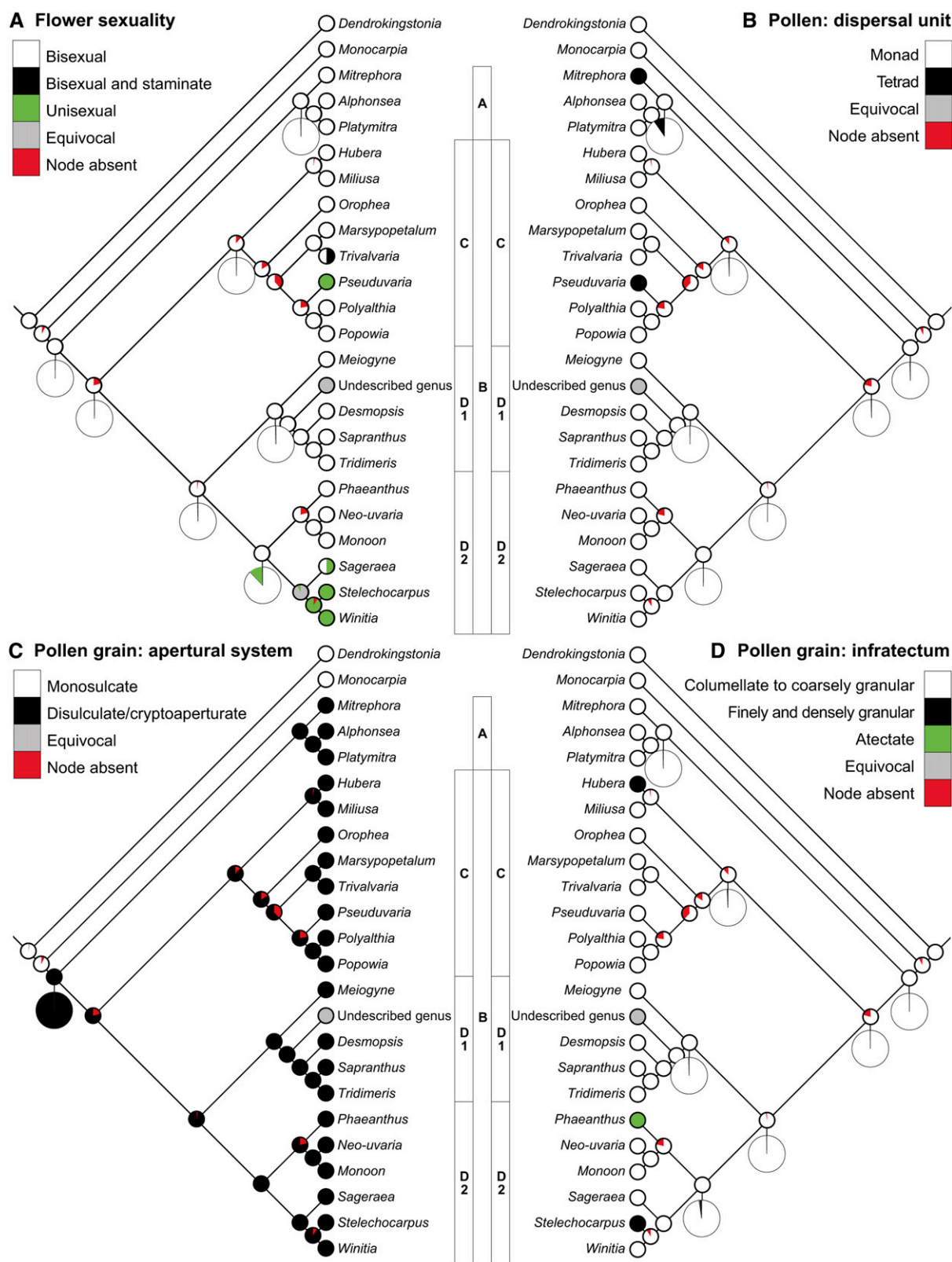
Fig. 3. Bayesian and parsimony ancestral character-state reconstructions across 20 004 post-burn-in trees shown on pruned 50% majority-rule consensus tree of the Bayesian phylogenetic reconstructions (for details, see Materials and Methods). Parsimony analyses: Smaller pie charts at internal nodes illustrate the number and proportion of unequivocal state reconstructions, equivocal state reconstructions (gray), and the proportion of node absence in the input trees (red). Bayesian RJ-MCMC analyses: Larger pie charts at selected nodes indicate posterior probabilities for states at the node.

Fig. 4. Representatives of genera in the Miliuseae, showing different modifications of petals. (A–I) Both whorls ± equal and ± similar in shape. (J) Outer whorl ± similar in size to the sepals but the inner whorl much larger. (K, L) Inner petals distinctly clawed toward base. (A) *Alphonsea* sp. (B) *Platymitra macrocarpa*. (C) *Sapranthus campechianus*. (D) *Tridimeris* sp. 1. (E) *Desmopsis* sp. (F) *Stenanona costaricensis*. (G) *Neo-uvaria telopea*. (H) *Monoon* sp. (I) *Hubera jenkinsii*. (J) *Miliusa parviflora*. (K) *Mitrephora vulpina*. (L) *Orophea* sp. [Photographs: (A, B, G, K, L) Simon Gardner; (C) German Carnevali Fernández-Concha; (D–F) Paul Maas; (H) Aree Kala; (I) Kithisak Aongyong; (J) Tanawat Chaowasku.]

Inner petal base (character 2; Fig. 2B)—The derived state of distinctly clawed inner petals (Fig. 4K, L) is inferred to have evolved from the ancestral state of nonclawed inner petals (Fig. 4A–J) multiple times: in *Mitrephora* (clade A); and in *Orophea* and *Pseuduvaria*, and somewhere in *Trivalvaria* (clade C2).

Maximum ovule number per ovary (character 3; Fig. 2C)—Multiovuled ovaries (with ≥2 to ~18 ovules) are the ancestral character state of Miliuseae. Multiple shifts to the derived state of uniovulate ovaries can be inferred. One shift occurred somewhere in *Desmopsis* including *Stenanona* (clade D1). Uniovulate ovaries also occur in clade D2: in *Monoon*, most species of *Neo-uvaria*, and particular species of *Phaeanthus*, as well as clade C: in *Hubera*, the *Marsypopetalum–Trivalvaria* subclade, particular species of *Miliusa*, *Orophea* and *Popowia*; but reconstructions at the crown nodes of clades C and D2 are ambiguous.

Endosperm rumination type (character 4; Fig. 2D)—Spiniform to flattened peg-like endosperm ruminations are the most likely ancestral character state of Miliuseae. The derived state of lamelliform ruminations are inferred to have evolved multiple times: in *Miliusa* (clade C1), the major clade D, somewhere in *Alphonsea* (clade A), and somewhere in each of two genera in clade C2: *Marsypopetalum* and *Trivalvaria*. Reversals to spiniform/flattened peg are inferred to have occurred independently somewhere in each of three genera of clade D1: *Meiogyne*, *Tridimeris*, and *Desmopsis* incl. *Stenanona*.

Flower sexuality (character 5; Fig. 3A)—At least two shifts from the ancestral state of bisexual to the derived state of unisexual flowers are inferred. These shifts occurred in *Pseuduvaria* (clade C2) and somewhere in the *Sageraea–Winitia–Stelechocarpus* clade (subclade of clade D2). The ancestral reconstructions at the crown node of the latter clade, however, are equivocal. Shift(s) from bisexual to the derived state of bisexual and staminate flowers (andromonoecy: T. Chaowasku, personal observation) occurred somewhere in *Trivalvaria* (clade C2).

Inflorescence position (character 6)—The reconstructions of this character are highly ambiguous at all nodes of interest (nodes Miliuseae, A, B, C, D, D1, and D2; see Appendices 5 and 6).

Pollen dispersal unit (character 7; Fig. 3B)—The derived state of tetrad pollen is inferred to have evolved from the ancestral state of monad pollen twice: in *Mitrephora* (clade A) and *Pseuduvaria* (clade C2).

Pollen apertural system (character 8; Fig. 3C)—The derived state of cryptoaperturate/disulculate pollen grains (Fig. 5A–F), synapomorphic for Miliuseae, are inferred to have evolved from monosulcate pollen grains.

Pollen infratectum type (character 9; Fig. 3D)—A columellate to coarsely granular infratectum (Fig. 6A, B) is the ancestral character state of Miliuseae. The derived state of an atectate exine (i.e., exine not to very little differentiated into tectum, infratectum, and basal layer; Fig. 6D), is inferred to have evolved once in *Phaeanthus* (clade D2), while a finely and densely granular infratectum (Fig. 6C) is inferred to have evolved twice: in *Hubera* (clade C1) and *Stelechocarpus* (clade D2).

DISCUSSION

The deeper relationships within tribe Miliuseae have remained largely unresolved in previous molecular phylogenetic analyses (e.g., Couvreur et al., 2011; Xue et al., 2011, 2012; Chaowasku et al., 2012a; Chatrou et al., 2012; Thomas et al., 2012). In the present analyses, including a much expanded sampling of taxa and DNA regions in comparison to previous analyses, parts of the backbone of the Miliuseae still remain poorly supported, but the phylogeny corroborates previously indicated relationships and provides various new insights into the intertribal and infratribal relationships of this most recalcitrant clade. These relationships as well as diagnostic traits of the identified major clades and subclades within Miliuseae are discussed below.

Relationships among Miliuseae and related tribes—The phylogenetic relationships of tribe Dendrokingstonieae are here analyzed for the first time in a molecular phylogenetic framework. The Dendrokingstonieae, Monocarpieae, and Miliuseae form a strongly supported clade. Both Dendrokingstonieae and Monocarpieae are monogeneric and have a relatively narrow distribution in southern Thailand and western Malesia (Peninsular Malaysia, Sumatra, and, for Monocarpieae, Borneo; Mols and Keßler, 2000b; Chaowasku et al., 2012b). *Dendrokingstonia* and *Monocarpia* share a combination of features that are rarely found elsewhere in the family, such as remarkably enlarged peltate stigmas, highly reduced numbers of carpels per flower, huge and thick-walled monocarps, and percurrent tertiary venation of the leaves (Chaowasku et al., 2012b). Dendrokingstonieae are recovered as sister to a clade composed of Monocarpieae and Miliuseae, but the sister-group relationship of the latter two tribes received only weak to moderate support, implying that the sister group of Miliuseae could change if more molecular data became available.

The clade consisting of Dendrokingstonieae, Monocarpieae, and Miliuseae shows unresolved relationships with two monogeneric tribes, Fenerivieae and Maasieae, and tribe Malmeeae. Additional data are clearly required to resolve this part of the backbone of subfamily Malmeoideae. We applied a similar approach to character and taxon sampling as Pirie et al. (2006), carefully selecting a limited number of species to represent larger clades and focusing on the sampling of sequence data. Our sampling did not focus on species in Malmeoideae outside of the Miliuseae, in contrast to Pirie et al. (2006). Their analyses provided the most robust hypothesis for phylogenetic relationships of non-Miliuseae lineages of Malmeoideae thus far and inferred a sister group relationship of Miliuseae and Malmeeae, although with poor support (PP 0.84). However, Fenerivieae were absent from their analyses. Expansion of our characters, as well as taxon-sampling efforts to cover the entire Malmeoideae (i.e., adding more generic representatives for Piptostigmateae and Malmeeae and using species from other subfamilies as outgroups), might conclusively resolve phylogenetic relationships among all tribes and shed further light on character evolution.

Major clades and intergeneric relationships within Miliuseae—Several major clades can be identified in the Miliuseae on the basis of our molecular phylogenetic analyses:

Clade A.—This moderately to strongly supported clade includes *Mitrephora*, *Alphonsea*, and *Platymitra*. All three genera share several diagnostic features, such as an ovary with several to many ovules laterally attached in two rows (symplesiomorphy) and inner petals that do not fully separate from each other at anthesis (Keßler, 1988b; 1996; Weerasooriya and Saunders, 2010; Fig. 4A, B, K).
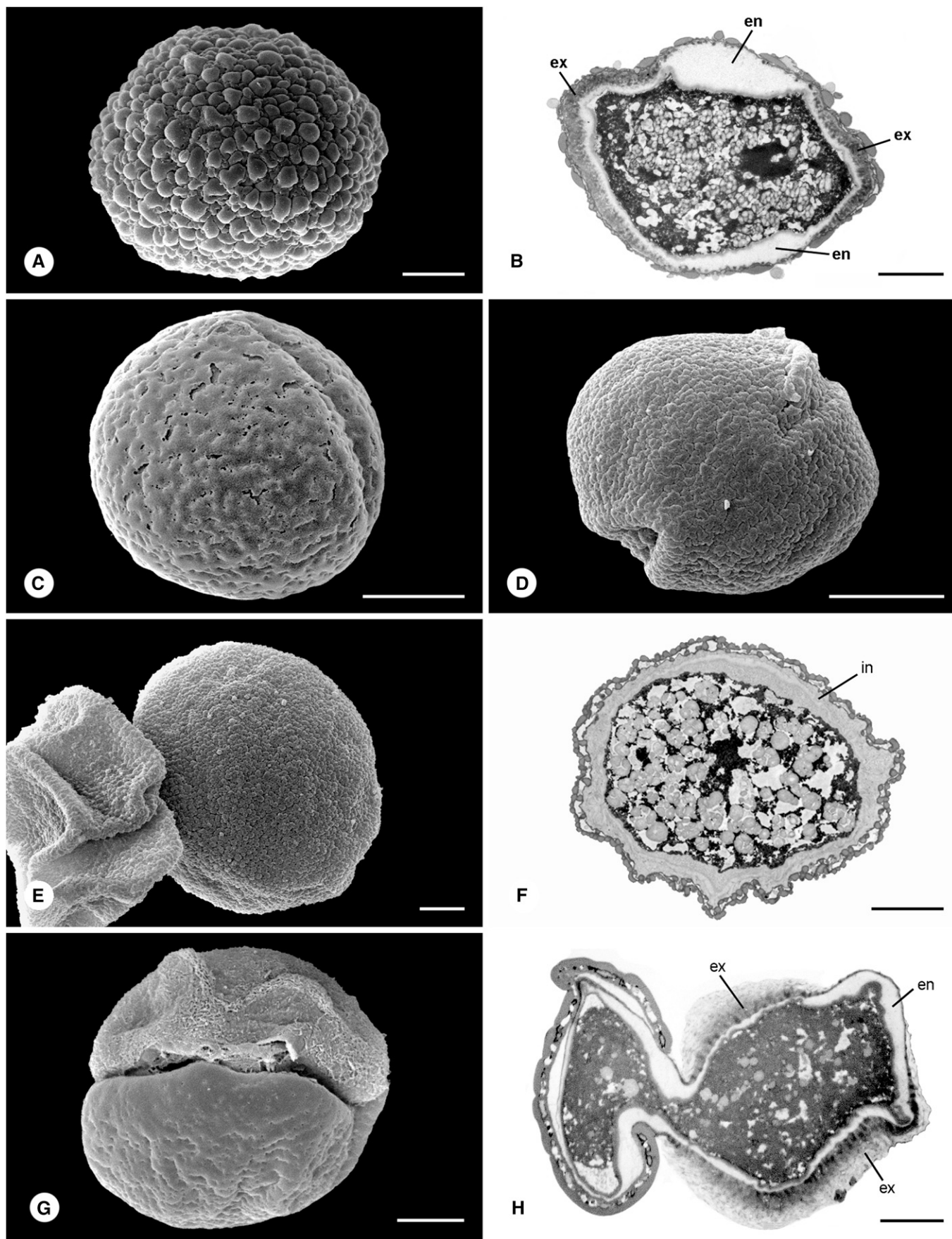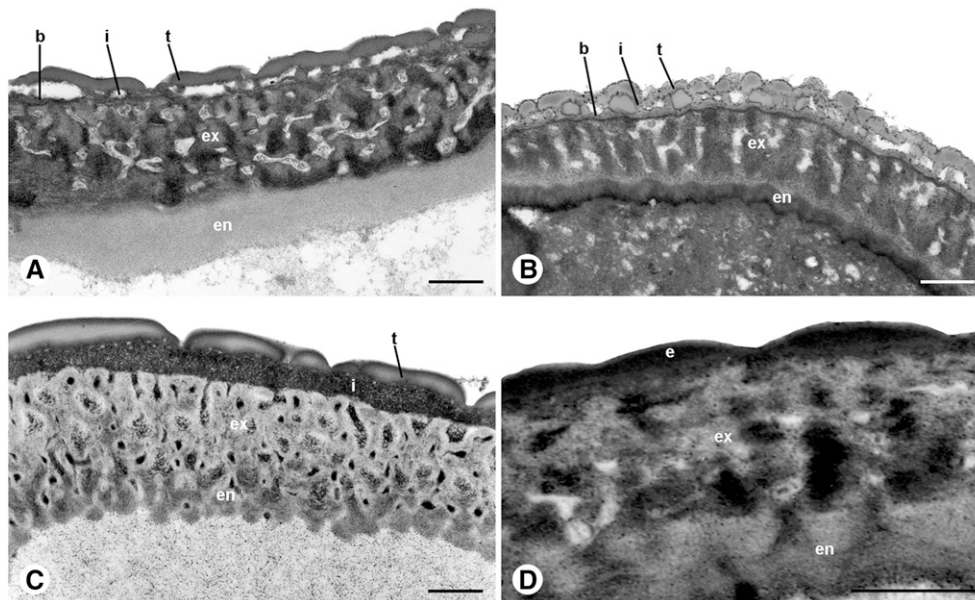
Fig. 6.   Pollen infratectum types in the Miliuseae (TEM). (A, B) Infratectum columellate to coarsely granular. (C) Infratectum finely and densely granular. (D) Exine not (to very weakly) differentiated into tectum, infratectum, and basal layer. (A) *Alphonsea boniana* (*van Beusekom & Smitinand 2116*). (B) *Platymitra macrocarpa* (*Gardner et al. 1648*). (C) *Hubera cerasoides* (*Larsen et al. 33731*). (D) *Phaeanthus splendens* (*S 15364*). Scale bars: 1 μm; b = basal layer; e = exine; en = endintine; ex = exintine; i = infratectum; t = tectum.

Mitrephora is recovered as sister to the latter two genera and differs from them in having distinctly clawed inner petals (Fig. 4K) and pollen that is shed in tetrads (Weerasooriya and Saunders, 2010). *Mitrephora* shows "uvarioid" stamens (Weerasooriya and Saunders, 2010), while *Alphonsea* (Keßler, 1996) and *Platymitra* (Keßler, 1988b) show diagnostically important "miliusoid" stamens.

Clade C.—This poorly supported clade contains nine genera (i.e., *Hubera, Miliusa, Orophea, Marsypopetalum, Trivalvaria, Pseuduvaria, Popowia,* and *Polyalthia*), whose phylogenetic relationships remain mostly uncertain. It is divided into two major subclades (clades C1 and C2), both of which are macromorphologically heterogeneous.

Clade C1.—This strongly supported clade comprises the genera *Hubera* and *Miliusa*. *Hubera*, which has recently been separated from the formerly highly polyphyletic genus *Polyalthia* (see Xue et al., 2011, 2012; Chaowasku et al., 2012a), is distributed from Fiji, New Caledonia, Australia, and Southeast Asian islands through mainland Asia to Madagascar and eastern Africa. This is the widest geographic distribution of any genus in the subfamily Malmeoideae (Chaowasku et al., 2012a). It is the only genus in the Miliuseae that is distributed in Madagascar and Africa. Morphological

synapomorphies for *Miliusa* and *Hubera* have not yet been identified. Rather, several conspicuous differences between them can be identified in stamen and petal morphology (Fig. 4I, J) and in endosperm rumination type (see Mols and Keßler, 2003; Chaowasku and Keßler, 2006; Chaowasku et al., 2012a).

Clade C2.—This weakly supported clade consists of six genera (*Orophea, Marsypopetalum, Trivalvaria, Pseuduvaria, Popowia,* and *Polyalthia*). Although the backbone phylogeny of this clade is not well supported, two strongly supported generic sister relationships can be differentiated as follows.

Marsypopetalum–Trivalvaria—The monophyly of *Trivalvaria*, as well as its sister-group relationship with *Marsypopetalum*, is confirmed with strong support. Both *Trivalvaria* and *Marsypopetalum* share some diagnostic features, such as the small arborescent growth from (rarely >5 m in height), considerably thickened leaves, a single ovule per ovary (likely synapomorphic), and more-or-less ellipsoid-cylindrical seeds (van Heusden, 1997b; Xue et al., 2011; T. Chaowasku, personal observation). For a discussion on the morphology and differentiation of *Marsypopetalum* and *Trivalvaria*, see Xue et al. (2011). Preliminary studies on the macromorphology of *Marsypopetalum*

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Fig. 5.   Overview of pollen apertural systems observed in tribe Miliuseae and closely related tribes: scanning (SEM) and transmission electron micrographs (TEM). (A–F) Miliuseae (cryptoaperturate/disulcate apertural system). (G, H) Dendrokingstonieae (monosulcate apertural system). (A) *Miliusa macropoda* (*Kostermans 13973*): pollen grain without externally visible aperture(s) (SEM). (B) *Miliusa horsfieldii* (*How 71794*): cross section of pollen grain showing thick tubular exintine (left and right) and two germination zones (top and bottom) characterized by thick homogeneous endintine and reduced exintine (TEM). (C) *Orophea kerrii* (*Chalermglin 440416-1*): pollen grain showing one (another one at the opposite side likely to be also present) slightly depressed exine area indicating an intinous germination zone (SEM). (D) *Orophea polycarpa* (*Chaowasku 21*): pollen grain showing two depressed exine areas indicating two intinous germination zones (SEM). (E, F) *Monoon paradoxum* (*Ambriansyah & Arifin B 1520*): (E) pollen grain without aperture(s) (SEM), (F) cross section of pollen grain showing continuous exine and intine without recognizable germination zone(s) (TEM). (G, H) *Dendrokingstonia gardneri*: (G) (*Kerr 19102*) pollen grain showing psilate/perforate exine (below) and bulging intine (above) (SEM), (H) (*FRI 32134*) cross section of pollen grain showing exine (left) and intine (right) strongly bulging outward (TEM). Scale bars = 5 μm (A, B, D–F, H); 10 μm (C, G); en = endintine; ex = exintine; in = intine.

and *Trivalvaria* indicate that there are undescribed species of both genera (see Appendix 2), necessitating thorough taxonomic, as well as molecular phylogenetic, investigations.

Popowia–Polyalthia—The sister-group relationship of *Popowia* and *Polyalthia* is strongly supported. Species of the two genera usually show a characteristic asymmetrical leaf base (Chaowasku et al., 2012a; Xue et al., 2012; T. Chaowasku, personal observation). For a detailed discussion of the two genera, see Xue et al. (2012).

Clade D.—This large clade is strongly supported in the Bayesian analyses, but only weakly supported in the parsimony analyses. Twelve genera (*Meiogyne*, *Sapranthus*, *Tridimeris*, *Desmopsis*, *Stenanona*, *Phaeanthus*, *Neo-uvaria*, *Monoon*, *Stelechocarpus*, *Winitia*, *Sageraea*, and an undescribed genus) belong to this clade. Two subclades within clade D can be identified: clades D1 and D2.

Clade D1.—The relationships of *Meiogyne* remained obscure in previous molecular phylogenetic analyses. The present analyses provide support for a sister-group relationship of *Meiogyne* with a clade comprising the only Neotropical genera in the predominantly Asian Miliuseae (the genera *Sapranthus*, *Tridimeris*, *Desmopsis*, and *Stenanona*), as well as samples of an undescribed Asian genus. The only known material of this undescribed genus are three accessions from Thailand including two sterile specimens (*Chaowasku 108*, *Nakorn-Thiemchan NTC 16*), both collected from a mountainous area of Chiang Mai province, and one fruiting specimen (*Chaowasku 111*) collected from a cultivated plant in Thailand showing a multiseeded subglobose monocarp (only a single detached monocarp is available). Endosperm ruminations of this genus are apparently four-parted lamelliform (T. Chaowasku, personal observation), which is also consistently found in the Neotropical genus *Sapranthus* (van Setten and Koek-Noorman, 1992). However, the flowers are currently unknown, and additional material is needed for a complete formal description of this genus.

The accession of *Meiogyne cylindrocarpa* (Burck) Heusden included in the present study was collected from the Mariana Islands (Appendix 1). This species was formerly known as *Guamia mariannae* (Saff.) Merr., which is the only species of the genus *Guamia* Merr. *Guamia* was morphologically synonymized with *Meiogyne* by van Heusden (1994), and results of the current study corroborate her synonymization.

The Neotropical genera *Sapranthus*, *Tridimeris*, *Desmopsis*, and *Stenanona* form a strongly supported clade. Divergence-time estimates indicate that the split between this clade and the *Meiogyne* clade occurred in the Oligocene or early Miocene (Thomas et al., 2012). Given that the Neotropical clade is nested among Asian taxa in the predominantly Asian Miliuseae, a dispersal event from Asia to America can be hypothesized. The genera of this Neotropical clade are macromorphologically quite similar to *Meiogyne* because of a suite of character states that are symplesiomorphic for the wider clade (D1), such as petals that are more or less similar in shape and size in both whorls (Fig. 4C–F and see Appendix 4: characters 1 and 2) and multiovuled ovaries (Appendix 4: character 3). However, deviations occur in *Meiogyne bidwillii* (ined.; = *Fitzalania bidwillii* [Benth.] Jessup, Kessler & Mols), which shows sepaloid outer petals (Jessup, 2007: under *Fitzalania* F.Muell.), and *Stenanona monticola* Maas & G.E.Schatz, which has uniovulate ovaries (Schatz and Maas, 2010). It is worthwhile to note that the maximum number of ovules per ovary is unknown in the undescribed Asian genus, but one accession (*Chaowasku 111*)

that exhibits a monocarp with multiple seeds implies that multiple ovules per ovary are present.

The genera *Sapranthus*, *Desmopsis*, and *Stenanona* can be differentiated from *Meiogyne* and *Tridimeris* by their terminal inflorescences (Appendix 4: character 6). The genus *Tridimeris* exhibits a peculiar floral morphology. It invariably possesses a dimerous perianth (i.e., a flower with two sepals, two outer petals, and two inner petals; Fig. 4D) instead of a trimerous one, which is the basic perianth structure in Annonaceae. Further, *Tridimeris* exhibits a highly reduced number of carpels per flower (van Heusden, 1992), which is a rare feature among Miliuseae genera. *Tridimeris* is sister to *Sapranthus* with strong support, but morphological synapomorphies uniting the two genera have not been identified yet.

The phylogenetic analyses indicate that *Stenanona* is nested within *Desmopsis* with strong support. The two genera do not clearly differ from each other in fruit morphology. With regard to flower morphology, the petals of *Stenanona* (Fig. 4F) are usually longer and narrower than in *Desmopsis* (Fig. 4E), and usually red-colored (while usually yellow-cream in *Desmopsis*), but intermediate forms exist (Schatz and Maas, 2010).

Clade D2.—This clade consists of the genera *Phaeanthus*, *Neo-uvaria*, *Monoon*, *Stelechocarpus*, *Winitia*, and *Sageraea*. It received only weak support in the parsimony analysis but was strongly supported in the Bayesian analysis. All genera in this clade exhibit exclusively lamelliform endosperm ruminations (Appendix 4: character 4). Within clade D2, a sister-group relationship of the genera *Monoon* and *Neo-uvaria* is strongly supported. Both genera share a number of traits, such as axillary inflorescences and a single ovule per ovary (with the exception of *N. telopea* Chaowasku, which sometimes possesses two ovules per ovary; Chaowasku et al., 2011a). The most recently described genus in Miliuseae is the monotypic genus *Wangia*, which also belongs to clade D2, but its position among this clade was rather obscure (Guo et al., 2014); unfortunately, we were not able to include it in the present study. *Wangia* also exhibits the four-parted lamelliform ruminations of the endosperm (Guo et al., 2014), which apparently support its placement in clade D.

The genera *Stelechocarpus*, *Winitia*, and *Sageraea* were recovered as a strongly supported clade possessing diagnostic multiovuled ovaries and (rather) thick leaves (van Heusden, 1995, 1997a; Chaowasku et al., 2013b). Furthermore, *Stelechocarpus*, *Winitia*, and some species of *Sageraea* are monoecious (van Heusden 1995, 1997a; Chaowasku et al., 2013b).

Stelechocarpus and *Winitia* are sister groups, although only with moderate support. *Stelechocarpus* differs considerably from *Winitia* in several macromorphological and palynological characters, particularly the stigma morphology, the distribution of male and female flowers in the same individual (mixed in *Winitia* with both male and female flowers borne on the trunk vs. separated in *Stelechocarpus* with male flowers ramiflorous and female flowers cauliflorous), and the pollen infratectum. These differences were the main basis for the recent separation of *Winitia* from *Stelechocarpus* (Chaowasku et al., 2013b).

Character evolution within Miliuseae—*Macromorphological characters.*—The ancestral character-state reconstructions of the first five selected macromorphological characters reveal a considerable degree of homoplasy. Most derived character states are diagnostically important at the generic level—for example, the synapomorphic sepaloid outer petals of *Miliusa* (Fig. 4J) and of *Phaeanthus*; the synapomorphic distinctly clawed

inner petals of *Mitrephora* (Fig. 4K), of *Orophea* (Fig. 4L), and of *Pseuduvaria* (occurring in most species); the uniovulate ovaries of *Hubera*, of the clade consisting of *Marsypopetalum* and *Trivalvaria*, of *Neo-uvaria* (usually), and of *Monoon*; and the unisexual flowers of *Pseuduvaria* (occurring in most species) and of the *Stelechocarpus–Winitia* clade.

Although the ancestral reconstructions of the inflorescence position are mainly equivocal (see Appendices 5 and 6), the distribution of the character states has taxonomic value for identifying Miliuseae genera: *Platymitra*, *Hubera*, *Meiogyne*, *Tridimeris*, *Monoon*, *Neo-uvaria*, *Stelechocarpus*, and *Sageraea* have axillary inflorescences, while *Mitrephora*, *Alphonsea*, *Trivalvaria*, *Popowia*, *Sapranthus*, *Desmopsis* incl. *Stenanona*, and *Phaeanthus* show terminal inflorescences.

Endosperm ruminations in Annonaceae can be divided into two main types, lamelliform and spiniform, which are of considerable systematic importance (van Setten and Koek-Noorman, 1992). Spiniform ruminations have been inferred to be ancestral for the crown group of subfamily Malmeoideae, but within the subfamily there is considerable variation (Pirie and Doyle, 2012). The present analyses highlight the fact that transitions between spiniform and lamelliform ruminations have been frequent in the evolution of Miliuseae. The ancestral character-state reconstructions indicate that (1) spiniform ruminations are plesiomorphic for the Miliuseae; (2) lamelliform ruminations are synapomorphic for a major clade (clade D) in the tribe, with some independent reversals to spiniform ruminations; and (3) several additional independent shifts from spiniform to lamelliform ruminations have occurred in the tribe (e.g., as a synapomorphy of *Miliusa*).

Shifts from multiovuled to uniovulate ovaries within Miliuseae may sometimes be correlated with shifts in dispersal agents. Multiovuled ovaries usually result in relatively large multiseeded monocarps, which tend to be dispersed by larger animals such as primates, whereas uniovulate ovaries generally develop into relatively small single-seeded monocarps that tend to be dispersed by smaller animals, mainly birds (Su and Saunders, 2006). The single-seeded monocarps of *Hubera*, *Marsypopetalum*, and *Trivalvaria* show a relatively small size and bright red and fleshy pericarps (*Hubera*: Chaowasku et al., 2012a; *Marsypopetalum* and *Trivalvaria*: Xue et al., 2011), indicative of bird dispersal (van der Pijl, 1969). Single-seeded monocarps of certain species of *Neo-uvaria* (e.g., *N. foetida* [Maingay ex Hook.f. & Thomson] Airy Shaw and *N. telopea*), however, are unlikely to be bird-dispersed, because they are relatively large (6.5–7.0 × 5.2–5.5 cm) and have brown, hairy, and fetid pericarps (Chaowasku et al., 2011a).

Palynological characters—Similar to the macromorphological characters discussed above, the ancestral character-state reconstructions of the pollen dispersal unit and pollen infratectum type (Fig. 6) indicate some levels of homoplasy. Nevertheless, derived states of these characters are important for generic circumscription: tetrad pollen is a synapomorphy of *Mitrephora* and of *Pseuduvaria* (occurring in most species); a finely and densely granular infratectum (Fig. 6C) is a synapomorphy of *Hubera* and an autapomorphy of *Stelechocarpus burahol* (Blume) Hook.f. & Thomson, the only species of the genus, whereas an atectate exine (Fig. 6D) is a synapomorphy of *Phaeanthus* (the exine of *Phaeanthus* was previously considered to be similar to that of *Mezzettia*, i.e., only differentiated into basal and upper layers; Chaowasku et al., 2008).

The distribution of major pollen apertural types in the Malmeoideae corroborates the molecular phylogenetic results. The basal grade of the Malmeoideae, including the monogeneric tribes Dendrokingstonieae and Monocarpieae, possesses monosulcate pollen (Fig. 5G, H; Le Thomas, 1980; Waha, 1985; Waha and Hesse, 1988; Schatz and Le Thomas, 1990; Couvreur et al., 2009; Chaowasku et al., 2012b), while the tribe Miliuseae shows synapomorphic cryptoaperturate or disulculate pollen (Fig. 5A–F). Using light microscopy (LM) and SEM, pollen grains of the Miliuseae are generally observed as subglobose objects lacking any apertures (Fig. 5A). However, using TEM, intine features (i.e., thickenings of the endintine and reductions of the exintine below the exine) indicate apertural conditions (germination zones; Fig. 5B; Waha and Hesse, 1988; Waha and Morawetz, 1988; Chaowasku et al., 2008). "Cryptoaperturate" is the term describing pollen that exhibits such features. Sometimes, the deviating intine parts are recognizable by a (slightly) sunken overlying exine (disulculate; Fig. 5C, D). Disulculate pollen may merely represent partially collapsed cryptoaperturate pollen; further examination is indispensable to understand the relation of cryptoaperturate and disulculate pollen. Doyle and Le Thomas (2012) argued that TEM studies indicate that the exine above the intinous germination zones of cryptoaperturate pollen can be thinner than the remaining exine; thus, these thin exine areas deserved recognition as apertures, and pollen grains showing this feature should also be considered "disulculate." We found that most cryptoaperturate pollen grains do not exhibit any thin exine areas, and that if they are present, they can occur anywhere around the grains. Therefore, thin exine areas are not a conclusive indicator of apertures, and the term "disulculate" should be restricted to pollen grains with two sunken exine areas.

The pollen grains of *Mitrephora* (T. Chaowasku, personal observation); *Neo-uvaria* (Chaowasku et al., 2011a); *Pseuduvaria* (Su and Saunders, 2003); *Sageraea*, *Stelechocarpus* and *Winitia* (T. Chaowasku, personal observation); and certain species of *Monoon* (Fig. 5F; Waha and Hesse, 1988; Chaowasku et al., 2011a; T. Chaowasku, personal observation) do not show germination zones. Further studies (i.e., more attempts at sectioning, more pollen samples of various developmental stages) need to be undertaken to investigate whether these pollen grains are inaperturate (omniaperturate), as observed in most members of the Annonoideae (Doyle and Le Thomas, 2012).

In Annonaceae, disulculate pollen, apart from the Miliuseae, has been reported from only one other genus, *Afroguatteria* Boutique, in tribe Uvarieae of subfamily Annonoideae (Le Thomas and Thanikaimoni, 1987; Doyle and Le Thomas, 2012). However, TEM studies investigating whether there are enlargements or reductions of the intine sublayers characteristic for disulculate pollen (Waha and Hesse, 1988; Waha and Morawetz, 1988) are required for confirmation.

LITERATURE CITED

AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

CHAOWASKU, T. 2013. *Miliusa codonantha* (Annonaceae), a new species from the Indian eastern Himalaya, with a new combination, *M. dioeca*. Willdenowia 43: 101–105.

CHAOWASKU, T., D. M. JOHNSON, R. W. J. M. VAN DER HAM, AND L. W. CHATROU. 2012a. Characterization of *Hubera* (Annonaceae), a new genus segregated from *Polyalthia* and allied to *Miliusa*. Phytotaxa 69: 33–56.

CHAOWASKU, T., AND P. J. A. KEßLER. 2006. *Miliusa lanceolata* (Annonaceae), a new species from Papua New Guinea. *Blumea* 51: 553–557.

CHAOWASKU, T., AND P. J. A. KEßLER. 2013. Seven new species of *Miliusa* (Annonaceae) from Thailand. *Nordic Journal of Botany* 31: 680–699.

CHAOWASKU, T., P. J. A. KEßLER, AND L. W. CHATROU. 2013a. Phylogeny of *Miliusa* (Magnoliales: Annonaceae: Malmeoideae: Miliuseae), with descriptions of two new species from Malesia. *European Journal of Taxonomy* 54: 1–21.

CHAOWASKU, T., P. J. A. KEßLER, S. PUNNADEE, AND R. W. J. M. VAN DER HAM. 2011a. Taxonomic novelties and pollen morphological study in the genus *Neo-uvaria* (Annonaceae). *Phytotaxa* 32: 27–42.

CHAOWASKU, T., P. J. A. KEßLER, AND R. W. J. M. VAN DER HAM. 2012b. A taxonomic revision and pollen morphology of the genus *Dendrokingstonia* (Annonaceae). *Botanical Journal of the Linnean Society* 168: 76–90.

CHAOWASKU, T., J. B. MOLS, AND R. W. J. M. VAN DER HAM. 2008. Pollen morphology of *Miliusa* and relatives (Annonaceae). *Grana* 47: 175–184.

CHAOWASKU, T., R. W. J. M. VAN DER HAM, AND L. W. CHATROU. 2013b. Integrative systematics supports the establishment of *Winitia*, a new genus of Annonaceae (Malmeoideae, Miliuseae) allied to *Stelechocarpus* and *Sageraea*. *Systematics and Biodiversity* 11: 195–207.

CHAOWASKU, T., G. ZIJLSTRA, AND L. W. CHATROU. 2011b. (2029) Proposal to conserve the name *Meiogyne* against *Fitzalania* (Annonaceae). *Taxon* 60: 1522–1523.

CHATROU, L. W., M. D. PIRIE, R. H. J. ERKENS, T. L. P. COUVREUR, K. M. NEUBIG, J. R. ABBOTT, J. B. MOLS, ET AL. 2012. A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society* 169: 5–40.

COUVREUR, T. L. P., M. D. PIRIE, L. W. CHATROU, R. M. K. SAUNDERS, Y. C. F. SU, J. E. RICHARDSON, AND R. H. J. ERKENS. 2011. Early evolutionary history of the flowering plant family Annonaceae: Steady diversification and boreotropical geodispersal. *Journal of Biogeography* 38: 664–680.

COUVREUR, T. L. P., J. E. RICHARDSON, M. S. M. SOSEF, R. H. J. ERKENS, AND L. W. CHATROU. 2008. Evolution of syncarpy and other morphological characters in African Annonaceae: A posterior mapping approach. *Molecular Phylogenetics and Evolution* 47: 302–318.

COUVREUR, T. L. P., R. W. J. M. VAN DER HAM, Y. M. MBELE, F. M. MBAGO, AND D. M. JOHNSON. 2009. Molecular and morphological characterization of a new monotypic genus of Annonaceae, *Mwasumbia*, from Tanzania. *Systematic Botany* 34: 266–276.

DOYLE, J. A., AND A. LE THOMAS. 1996. Phylogenetic analysis and character evolution in Annonaceae. *Bulletin du Muséum National d'Histoire Naturelle, section B. Adansonia, série 4* 18: 279–334.

DOYLE, J. A., AND A. LE THOMAS. 2012. Evolution and phylogenetic significance of pollen in Annonaceae. *Botanical Journal of the Linnean Society* 169: 190–221.

ERKENS, R. H. J., L. W. CHATROU, AND T. L. P. COUVREUR. 2012. Radiations and key innovations in an early branching angiosperm lineage (Annonaceae; Magnoliales). *Botanical Journal of the Linnean Society* 169: 117–134.

GOLOBOFF, P. A., J. S. FARRIS, M. KÄLLERSJÖ, B. OXELMAN, M. J. RAMIREZ, AND C. A. SZUMIK. 2003. Improvements to resampling measures of group support. *Cladistics* 19: 324–332.

GOLOBOFF, P. A., J. S. FARRIS, AND K. C. NIXON. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.

GUO, X., J. WANG, B. XUE, D. C. THOMAS, Y. C. F. SU, Y.-H. TAN, AND R. M. K. SAUNDERS. 2014. Reassessing the taxonomic status of two enigmatic *Desmos* species (Annonaceae): Morphological and molecular phylogenetic support for a new genus, *Wangia*. *Journal of Systematics and Evolution* 52: 1–15.

HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160–174.

JESSUP, L. W. 2007. Annonaceae. *In* A. J. G. Wilson [ed.], Flora of Australia, vol. 2, 18–57 + 447–450. Australian Biological Resources Study/CSIRO Publishing, Canberra, Australia.

KEßLER, P. J. A. 1988a. Revision der Gattung *Orophea* Blume (Annonaceae). *Blumea* 33: 1–80.

KEßLER, P. J. A. 1988b. Studies on the tribe Saccopetaleae (Annonaceae) I. Revision of the genus *Platymitra* Boerlage. *Blumea* 33: 471–476.

KEßLER, P. J. A. 1993. Annonaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 93–129. Springer, Berlin, Germany.

KEßLER, P. J. A. 1996. Studies on the tribe Saccopetaleae (Annonaceae) IV. Revision of the genus *Alphonsea* Hook.f. & Thomson. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 118: 81–112.

KOEK-NOORMAN, J., AND L. Y. T. WESTRA. 2012. Macrophotographic wood atlas of Annonaceae. *Botanical Journal of the Linnean Society* 169: 135–189.

LE THOMAS, A. 1980. Ultrastructural characters of the pollen grains of African Annonaceae and their significance for the phylogeny of primitive angiosperms (first part). *Pollen et Spores* 22: 267–342.

LE THOMAS, A., AND G. THANIKAIMONI. 1987. Variation de l'aperture des Annonacées: Tendances palynologiques nouvelles. *Mémoires et Travaux de l'École Pratique des Hautes Études. Institut de Montpellier* 17: 333–353.

MADDISON, W. P., AND D. R. MADDISON. 2010. Mesquite: A modular system for evolutionary analysis, version 2.74. http://mesquiteproject.org/mesquite/mesquite.html

MARSHALL, D. C. 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: Lost in the land of long trees. *Systematic Biology* 59: 108–117.

MOLS, J. B., D. L. V. CO, B. GRAVENDEEL, L. W. CHATROU, M. D. PIRIE, R. W. J. M. VAN DER HAM, E. J. VAN MARLE, AND P. J. A. KEßLER. 2004a. Morphological character evolution in the miliusoid clade (Annonaceae). *In* J. B. Mols [ed.], From Miliusa to Miliuseae to Miliusoid: Identifying clades in Asian Annonaceae, 37–75. Ph.D. thesis, Leiden University, The Netherlands.

MOLS, J. B., B. GRAVENDEEL, L. W. CHATROU, M. D. PIRIE, P. C. BYGRAVE, M. W. CHASE, AND P. J. A. KEßLER. 2004b. Identifying clades in Asian Annonaceae: Monophyletic genera in the polyphyletic Miliuseae. *American Journal of Botany* 91: 590–600.

MOLS, J. B., AND P. J. A. KEßLER. 2000a. Revision of the genus *Phaeanthus* (Annonaceae). *Blumea* 45: 205–233.

MOLS, J. B., AND P. J. A. KEßLER. 2000b. The genus *Monocarpia* (Annonaceae) in Borneo including a new species *Monocarpia borneensis*. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 122: 233–240.

MOLS, J. B., AND P. J. A. KEßLER. 2003. The genus *Miliusa* (Annonaceae) in the Austro-Malesian area. *Blumea* 48: 421–462.

MOLS, J. B., P. J. A. KEßLER, S. H. ROGSTAD, AND R. M. K. SAUNDERS. 2008. Reassignment of six *Polyalthia* species to the new genus *Maasia* (Annonaceae): Molecular and morphological congruence. *Systematic Botany* 33: 490–494.

NYLANDER, J. A. A., J. C. WILGENBUSCH, D. L. WARREN, AND D. L. SWOFFORD. 2008. AWTY (Are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics (Oxford, England)* 24: 581–583.

PAGEL, M., AND A. MEADE. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *American Naturalist* 167: 808–825.

PAGEL, M., A. MEADE, AND D. BARKER. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53: 673–684.

PIRIE, M. D., L. W. CHATROU, J. B. MOLS, R. H. J. ERKENS, AND J. OOSTERHOF. 2006. 'Andean-centred' genera in the short-branch clade of Annonaceae: Testing biogeographical hypotheses using phylogeny reconstruction and molecular dating. *Journal of Biogeography* 33: 31–46.

PIRIE, M. D., AND J. A. DOYLE. 2012. Dating clades with fossils and molecules: The case of Annonaceae. *Botanical Journal of the Linnean Society* 169: 84–116.

PUNT, W., P. P. HOEN, S. BLACKMORE, S. NILSSON, AND A. LE THOMAS. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143: 1–81.

RAINER, H., AND L. W. CHATROU. 2006. AnnonBase: World species list of Annonaceae, version 1.1. http://www.sp2000.org and http://www.annonaceae.org.

RAMBAUT, A., AND A. DRUMMOND. 2009. Tracer, version 1.5. http://tree.bio.ed.ac.uk/software/tracer.

RICHARDSON, J. E., L. W. CHATROU, J. B. MOLS, R. H. J. ERKENS, AND M. D. PIRIE. 2004. Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Philosophical Transactions of the Royal Society of London, Series B* 359: 1495–1508.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* 19: 1572–1574.

SAFFORD, W. E. 1916. *Desmopsis*, a new genus of Annonaceae. *Bulletin of the Torrey Botanical Club* 43: 183–193.

SAUNDERS, R. M. K. 2010. Floral evolution in the Annonaceae: Hypotheses of homeotic mutations and functional convergence. *Biological Reviews of the Cambridge Philosophical Society* 85: 571–591.

SAUNDERS, R. M. K. 2012. The diversity and evolution of pollination systems in Annonaceae. *Botanical Journal of the Linnean Society* 169: 222–244.

SAUNDERS, R. M. K., Y. C. F. SU, AND P. CHALERMGLIN. 2004. *Craibella phuyensis* (Annonaceae): A new genus and species from Thailand. *Systematic Botany* 29: 42–49.

SAUNDERS, R. M. K., Y. C. F. SU, AND B. XUE. 2011. Phylogenetic affinities of *Polyalthia* species (Annonaceae) with columellar-sulcate pollen: Enlarging the Madagascan endemic genus *Fenerivia*. *Taxon* 60: 1407–1416.

SAUQUET, H., J. A. DOYLE, T. SCHARASCHKIN, T. BORSCH, K. W. HILU, L. W. CHATROU, AND A. LE THOMAS. 2003. Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: Implications for character evolution. *Botanical Journal of the Linnean Society* 142: 125–186.

SCHATZ, G. E. 1998. New species of *Sapranthus* B. C. Seemann and *Unonopsis* R. E. Fries (Annonaceae) from Mesoamerica. *Novon* 8: 436–440.

SCHATZ, G. E., AND A. LE THOMAS. 1990. The genus *Polyalthia* Blume (Annonaceae) in Madagascar. *Bulletin du Muséum National d'Histoire Naturelle, section B. Adansonia, série 4* 12: 113–130.

SCHATZ, G. E., AND P. J. M. MAAS. 2010. Synoptic revision of *Stenanona* (Annonaceae). *Blumea* 55: 205–223.

SEELANAN, T., A. SCHNABEL, AND J. F. WENDEL. 1997. Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany* 22: 259–290.

SIMMONS, M. P. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* 31: 874–879.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

SU, Y. C. F., T. CHAOWASKU, AND R. M. K. SAUNDERS. 2010. An extended phylogeny of *Pseuduvaria* (Annonaceae) with descriptions of three new species and a reassessment of the generic status of *Oreomitra*. *Systematic Botany* 35: 30–39.

SU, Y. C. F., AND R. M. K. SAUNDERS. 2003. Pollen structure, tetrad cohesion and pollen-connecting threads in *Pseuduvaria* (Annonaceae). *Botanical Journal of the Linnean Society* 143: 69–78.

SU, Y. C. F., AND R. M. K. SAUNDERS. 2006. Monograph of *Pseuduvaria* (Annonaceae). *Systematic Botany Monographs* 79: 1–204.

SU, Y. C. F., G. J. D. SMITH, AND R. M. K. SAUNDERS. 2008. Phylogeny of the basal angiosperm genus *Pseuduvaria* (Annonaceae) inferred from five chloroplast DNA regions, with interpretation of morphological character evolution. *Molecular Phylogenetics and Evolution* 48: 188–206.

TAVARÉ, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.

THIERS, B. 2008 [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium, New York, USA. http://sweetgum.nybg.org/ih/.

THOMAS, D. C., S. SURVESWARAN, B. XUE, G. SANKOWSKY, J. B. MOLS, P. J. A. KEßLER, AND R. M. K. SAUNDERS. 2012. Molecular phylogenetics and historical biogeography of the *Meiogyne-Fitzalania* clade (Annonaceae): Generic paraphyly and late Miocene-Pliocene diversification in Australasia and the Pacific. *Taxon* 61: 559–575.

VAN DER HAM, R. W. J. M. 1990. Nephelieae pollen (Sapindaceae): Form, function, and evolution. *Rijksherbarium/Hortus Botanicus. Leiden Botanical Series* 13: 1–255.

VAN DER PIJL, L. 1969. Principles of dispersal in higher plants. Springer-Verlag, New York, New York, USA.

VAN HEUSDEN, E. C. H. 1992. Flowers of Annonaceae: Morphology classification and evolution. *Blumea* 7 (supplement): 1–218.

VAN HEUSDEN, E. C. H. 1994. Revision of *Meiogyne* (Annonaceae). *Blumea* 38: 487–511.

VAN HEUSDEN, E. C. H. 1995. Revision of the southeast Asian genus *Stelechocarpus* (Annonaceae). *Blumea* 40: 429–438.

VAN HEUSDEN, E. C. H. 1997a. Revision of the southeast Asian genus *Sageraea* (Annonaceae). *Nordic Journal of Botany* 17: 39–54.

VAN HEUSDEN, E. C. H. 1997b. Revision of the southeast Asian genus *Trivalvaria* (Annonaceae). *Nordic Journal of Botany* 17: 169–180.

VAN SETTEN, A. K., AND J. KOEK-NOORMAN. 1992. Fruits and seeds of Annonaceae: Morphology and its significance for classification and identification. *Bibliotheca Botanica* 142: 1–101.

WAHA, M. 1985. Ultrastruktur und systematische Bedeutung des Pollens bei *Bocageopsis, Ephedranthus, Malmea* und *Unonopsis* (Annonaceae). *Plant Systematics and Evolution* 150: 165–177.

WAHA, M., AND M. HESSE. 1988. Aperture types within *Sapranthus* and *Polyalthia* (Annonaceae). *Plant Systematics and Evolution* 161: 135–146.

WAHA, M., AND W. MORAWETZ. 1988. Pollen evolution and systematics in Annonaceae with special reference to the disulcate Australian endemic genera. *Plant Systematics and Evolution* 161: 1–12.

WEERASOORIYA, A. D., AND R. M. K. SAUNDERS. 2010. Monograph of *Mitrephora* (Annonaceae). *Systematic Botany Monographs* 90: 1–167.

WIENS, J. J. 1998. Combining data sets with different phylogenetic histories. *Systematic Biology* 47: 568–581.

XUE, B., Y. C. F. SU, J. B. MOLS, P. J. A. KEßLER, AND R. M. K. SAUNDERS. 2011. Further fragmentation of the polyphyletic genus *Polyalthia* (Annonaceae): Molecular phylogenetic support for a broader delimitation of *Marsypopetalum*. *Systematics and Biodiversity* 9: 17–26.

XUE, B., Y. C. F. SU, D. C. THOMAS, AND R. M. K. SAUNDERS. 2012. Pruning the polyphyletic genus *Polyalthia* (Annonaceae) and resurrecting the genus *Monoon*. *Taxon* 61: 1021–1039.

XUE, B., D. C. THOMAS, T. CHAOWASKU, D. M. JOHNSON, AND R. M. K. SAUNDERS. 2014. Molecular phylogenetic support for the taxonomic merger of *Fitzalania* and *Meiogyne* (Annonaceae): New nomenclatural combinations under the conserved name *Meiogyne*. *Systematic Botany*: in press.

YANG, Z., AND B. RANNALA. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. *Molecular Biology and Evolution* 14: 717–724.

APPENDIX 1. Voucher specimens for molecular phylogenetic analyses; specimens are deposited in CNS, K, L, MO, SAN, TISTR (Bangkok), U, WAG, and WU herbaria (acronyms follow Index Herbariorum [Thiers, 2008], except for TISTR [Thailand Institute of Scientific and Technological Research]). * = sequences newly generated for this study.

Taxon; GenBank accessions: rbcL; trnLF; matK; ndhF; psbA-trnH; ycf1; *Voucher specimen*; Collection location; Herbarium acronym. Long dash (—) = sequence not available.

Alphonsea elliptica Hook.f. & Thomson; AY318966; AY319078; AY518807; JQ690401; JQ690402; JQ690403; *van Balgooy 5141*; Bogor Bot. Gard.; L. *A.* sp.;—; AY319082; AY518808; JQ690404; JQ690405; JQ690406; *Keßler PK 3186*; Thailand; TISTR, Bangkok.

Annickia pilosa (Exell) Setten & Maas; AY743450; AY743469; AY743488; AY841402; AY841444;—; *Sosef 1803*; Gabon; WAG.

Bocageopsis canescens (Spruce ex Benth.) R.E.Fr.; JQ690407; JQ690408; JQ690409; JQ690410; JQ690411; JQ690412; *Maas et al. 9243*; Brazil; U.

Dendrokingstonia gardneri Chaowasku; *KJ418381; *KJ418406; *KJ418391; *KJ418385; *KJ418399; *KJ418378; *Gardner & Sidisunthorn ST 2214*; Thailand; L. *D. nervosa* Rauschert; *KJ418382; *KJ418407; *KJ418392; *KJ418386; *KJ418400;—; *Rogstad 961*; Peninsular Malaysia; L.

Desmopsis microcarpa R.E.Fr.; AY319059; AY319173; AY518804; JX544771; AY841461; JX544758; *Chatrou et al. 85*; Costa Rica; U. *D.* sp.;—; AY841701; KC857552; KC857553; KC857554; KC857555; *Rainer 1593*; Mexico; WU.

Fenerivia chapelieri (Baill.) R.M.K.Saunders; *KJ418383; *KJ418403; *KJ418404; *KJ418393; *KJ418387; *KJ418397;—; *Rabevohitra et al. 4439*; Madagascar; MO. *F. ghesquiereana* (Cavaco & Keraudren) R.M.K.Saunders; *KJ418384; *KJ418405; *KJ418394; *KJ418388; *KJ418398;—; *Schatz et al. 3611*; Madagascar; MO.

Greewayodendron oliveri (Engl.) Verdc.; AY743451; AY743470; AY743489; AY841408; AY841465;—; *Jongkind et al. 1795*; Ghana; WAG.

Hubera cerasoides (Roxb.) Chaowasku; AY319017; AY319131; AY518854; JQ889985; JQ889980; JQ889975; *Chalermglin 440214-4*; Thailand; L. *H. nitidissima* (Dunal) Chaowasku;—; JQ889988; JQ889989; JQ889986; JQ889981; JQ889976; *Ford AF 4967*; Australia; L. *H. pendula* (Capuron ex G.E.Schatz & Le Thomas) Chaowasku;—; AY319144; AY518852; JQ889987; JQ889982; JQ889977; *Rabevohitra 2386*; Madagascar; WAG. *H. stuhlmannii* (Engl.) Chaowasku;—; AY319149; AY518853; JX544882; JX544862; JX544852; *Luke & Robertson 1424*; Kenya; K.

Maasia discolor (Diels) Mols, Kessler & Rogstad; AY319021; AY319135; AY518872; AY841416; AY841500;—; *Takeuchi & Ama 16394*; Papua New Guinea; L. *M. sumatrana* (Miq.) Mols, Kessler & Rogstad; AY319039; AY319153; AY518873; AY841418; AY841503;—; *SAN 143918*; Borneo; SAN.

Marsypopetalum littorale (Blume) B.Xue & R.M.K.Saunders; AY319026; AY319140; AY518835; JX544827; JX544804; JX544813; *Rastini 153*; Bogor Bot. Gard.; L. *M. modestum* (Pierre) B.Xue & R.M.K.Saunders; AY318980; AY319092; AY518834; KC857561; KC857562; KC857563; *Keßler PK 3192*; Thailand; L.

Meiogyne bidwillii ined. = *Fitzalania bidwillii* (Benth.) Jessup, Kessler & Mols;—; *KJ418408; *KJ418396; *KJ418390; *KJ418401; *KJ418380; *Randall 624*; Australia; L. *M. cylindrocarpa* (Burck) Heusden;—; *KJ418409; *KJ418395; *KJ418389; *KJ418402; *KJ418379; *Marler s.n.*; Tinian Island; L. *M. heteropetala* ined. = *Fitzalania heteropetala* (F.Muell.) F. Muell.;—; KC857556; KC857557; KC857558; KC857559; KC857560; *Kemp TH 7267*; Australia; L. *M. virgata* (Blume) Miq.; AY318982; AY319094; AY518798; JX544769; JX544784; JX544756; *Keßler PK 2751*; Borneo; L. *M.* sp.; KC857564; KC857565; KC857566; KC857567; KC857568; KC857569; *Gardner et al. ST 2014*; Thailand; L.

Miliusa mollis Pierre;—; AY319102; AY518851; JQ690503; JQ690504; JQ690505; *Keßler PK 3207*; Thailand; L. *M. thorelii* Finet & Gagnep.;—; AY319104; AY518846; JQ690519; JQ690520; JQ690521; *Keßler PK 3184*; Thailand; L. *M. velutina* (DC.) Hook.f. & Thomson; AY318993; AY319105; AY518847; JQ690536; JQ690537; JQ690538; *Pholsena & Koonkhunthod 2842*; L. *M.* sp.;—; JQ690526; JQ690527; JQ690528; JQ690529; JQ690530; *Nakorn-Thienchan NTC 7*; Thailand; L.

Mitrephora alba Ridl.; AY318994; AY319106; AY518855; JQ889983; JQ889978; JQ889973; *Chalermglin 440304-1*; Thailand; TISTR, Bangkok. *M. macrocarpa* (Miq.) Weeras. & R.M.K.Saunders;—; AY319107; AY518859; JQ889984; JQ889979; JQ889974; *Mols 8*; Bogor Bot. Gard.; L.

Monocarpia euneura Miq.; AY318998; AY319111; AY518865; AY841412; AY841477;—; *Slik 2931*; Borneo; L. *M. maingayi* (Hook.f. & Thomson) I.M.Turner; JQ690395; JQ690396; JQ690397; JQ690398; JQ690399; JQ690400; *Kaewruang 1*; Thailand; L.

Monoon fuscum (King) B.Xue & R.M.K.Saunders; AY318973; AY319085; AY518787; JX544779; JX544792; JX544767; *Keßler PK 3222*; Thailand; L. *M. viride* (Craib) B.Xue & R.M.K.Saunders; AY319040; AY319154; AY518784; JX544780; JX544793; JX544768; *Chalermglin 440214-3*; Thailand; L.

Neo-uvaria telopea Chaowasku; JX544755; JX544783; JX544751; JX544778; JX544791; JX544766; *Chaowasku 77*; Thailand; L. *N. parallelivenia* (Boerl.) H.Okada & K.Ueda; AY319000; AY319113; AY518794; KC857570; KC857571; KC857572; *Keßler sub IV-H-73*; Bogor Bot. Gard.; L.

Orophea enterocarpa Maingay ex Hook.f. & Thomson; AY319006; AY319119; AY518815; JQ690416; JQ690417; JQ690418; *Chalermglin 440403*; Thailand; TISTR, Bangkok. *O. kerrii* Kessler; AY319008; AY319121; AY518818; JQ690419; JQ690420; JQ690421; *Chalermglin 440416-1*; Thailand; L.

Oxandra venezuelana R.E.Fr.; AY841645; AY841723; JQ690413; JQ690414; AY841495; JQ690415; *Chatrou et al. 120*; Costa Rica; U.

Phaeanthus splendens Miq.; JX544754; AY319126; AY518864; JX544777; JX544790; JX544765; *Keßler B 1564*; Borneo; L. *P.* sp.;—; KC857573; KC857574; KC857575; KC857576; KC857577; *Takeuchi 18407*; Sumatra; L.

Platymitra macrocarpa Boerl.; AY319013; AY319127; AY518812; JQ690422; JQ690423; JQ690424; *Okada 3457*; Bogor Bot. Gard.; L. *P.* sp.;—; JQ690425; JQ690426; JQ690427; JQ690428; JQ690429; *Chaowasku 100*; Thailand; L.

Polyalthia bullata King;—; JX544800; JX544825; JX544839; JX544809; JX544818; *Chaowasku 34*; Thailand; L. *P. johnsonii* (F.Muell.) B.Xue & R.M.K.Saunders;—; JX544801; JX544826; JX544840; JX544810; JX544819; *Ford AF 3625*; Australia; CNS. *P. suberosa* (Roxb.) Thwaites; AY238956; AY231289+AY238949; AY238965; AY841417; AY841502; JX544817; *Chatrou 480*; Utrecht Univ. Bot. Gard.; U.

Popowia hirta Miq.; AY319042; AY319156; AY518860; JX544830; JX544806; JX544816; *Keßler B 1628*; Borneo; L. *P. pisocarpa* (Blume) Endl.; AY319044; AY319158; AY518862; KC857578; KC857579; KC857580; *van Balgooy & van Setten 5683*; Bogor Bot. Gard.; L.

Pseuduvaria fragrans Y.C.F.Su, Chaowasku & R.M.K.Saunders; EU522341; EU522231; EU522286; JX544829; EU522176; JX544815; *Chaowasku 27*; Thailand; L. *P. setosa* (King) J.Sinclair;—; KC857581; KC857582; KC857583; KC857584; KC857585; *Chaowasku 66*; Thailand; L.

Sageraea elliptica (A.DC.) Hook.f. & Thomson;—; KC857586; KC857587; KC857588; KC857589; KC857590; *Chaowasku 45*; Thailand; L. *S. lanceolata* Miq.; AY319050; AY319164; AY518799; JX544774; JX544787; JX544762; *Ridsdale DV-M2-1692*; Borneo; L. *S.* sp. 1;—; KC857591; KC857592; KC857593; KC857594; KC857595; *Slik 3868*; Borneo; L. *S.* sp. 2;—; KC857596; KC857597; KC857598; KC857599; KC857600; *Gardner & Sidisunthorn ST 1006*; Thailand; L.

Sapranthus viridiflorus G.E.Schatz; AY319051; AY319165; AY743493; AY841422; AY841515; JX544760; *Chatrou et al. 55*; Costa Rica; U.

Stelechocarpus burahol (Blume) Hook.f. & Thomson; AY319053; AY319167; AY518803; JX544775; JX544788; JX544763; *Mols 13*; Bogor Bot. Gard.; L.

Stenanona costaricensis R.E.Fr.; AY319069; AY319183; AY518801; JX544772; AY841516; JX544759; *Chatrou et al. 67*; Costa Rica; U.

Tridimeris sp. 1; JX544753; JX544782; JX544750; JX544773; JX544786; JX544761; *Maas 8646*; Missouri Bot. Gard.; U.

Trivalvaria sp. 1; JX544822; JX544794; JX544824; JX544828; JX544805; JX544814; *Chaowasku 35*; Thailand; L. *T.* sp. 2;—; KC857601; KC857602; KC857603; KC857604; KC857605; *Chaowasku 56*; Thailand; L.

Winitia cauliflora (Scheff.) Chaowasku; AY319054; AY319168; AY518800; JX544776; JX544789; JX544764; *Unknown s.n.*; Bogor Bot. Gard.

(XV-A-196); L. *W. expansa* Chaowasku;—; KC857616; KC857617; KC857618; KC857619; KC857620; *Chaowasku 93*; Thailand; L.

Undescribed genus sp. 1;—; KC857611; KC857612; KC857613; KC857614; KC857615; *Chaowasku 111*; Thailand; L. **Undercribed genus** sp. 2A; JX544752; JX544781; JX544749; JX544770; JX544785; JX544757; *Chaowasku 108*; Thailand; L. **Undescribed genus** sp. 2B;—; KC857606; KC857607; KC857608; KC857609; KC857610; *Nakorn-Thiemchan NTC 16*; Thailand; L.

APPENDIX 2. Voucher specimens for macromorphological observations; specimens are deposited in L and U herbaria (acronyms follow Index Herbariorum: Thiers, 2008).

Taxon; *Voucher specimens*; Collection location; Herbarium acronym.

Marsypopetalum littorale (Blume) B.Xue & R.M.K.Saunders; *Chaowasku 80*; Hortus Botanicus Leiden; L. *M. modestum* (Pierre) B.Xue & R.M.K.Saunders; *Keßler PK 3192*; Thailand; L. *M. pallidum* (Blume) Backer; *Unknown 7706*; Bogor Bot. Gard.; L. *M.* sp. 1; *Chaowasku 90*; Thailand; L. *M.* sp. 2; *Chaowasku 102*; Thailand; L; *Sidisunthorn & Tippayasri ST 1416*; Thailand; L. *M.* sp. 3; *SAN 138345*; Borneo; L. *M.* sp. 4; *Kokawa & Hotta 704*; Borneo; L. *M.* sp. 5; *S 47179*; Borneo; L. *M.* sp. 6; *SAN 108682*; Borneo; L.

Meiogyne bidwillii ined. = *Fitzalania bidwillii* (Benth.) Jessup, Kessler & Mols; *Randall 624*; Australia; L. *M. cylindrocarpa* (Burck) Heusden; *Marler s.n.*; Tinian Island; L. *M. heteropetala* ined. = *Fitzalania heteropetala* (F.Muell.) F. Muell.; *Kemp TH 7267*; Australia; L. *M.* sp.; *Gardner et al. ST 2014*; Thailand; L.

Polyalthia bullata King; *Chaowasku 34*; Thailand; L. *P.* sp. 1; *Punnadee 1*; Thailand; L. *P.* sp. 2; *Chaowasku 50*; Thailand; L. *P.* sp. 3; *Chaowasku 47*; Thailand; L.

Popowia fusca King; *FRI 16340*; Peninsular Malaysia; L. *P. hirta* Miq.; *Ismail & Arifin BRF 1732*; Borneo; L. *P.* sp. 1; *Ambri & Arifin W632*;

Borneo; L. *P.* sp. 2; *van Balgooy 6801*; Moluccas; L. *P.* sp. 3; *LAE 78481*; Papua New Guinea; L. *P.* sp. 4: photographs available at http://www.nature-museum.net/%28S%28bhhb0445z22hbtbgh1ba3a55%29%29/Album/ShowAlbum.aspx?albumid=1cbe65df-1919-4d7b-a4f9-59251c278954&Username=pcssw

Pseuduvaria fragrans Y.C.F.Su, Chaowasku & R.M.K.Saunders; *Chaowasku 27*; Thailand; L. *P. setosa* (King) J.Sinclair; *Chaowasku 66*; Thailand; L.

Sageraea bracteolata R.Parker; *Gardner ST 2068*; Thailand; L. *S. elliptica* (A.DC.) Hook.f. & Thomson; *Chaowasku 45*; Thailand; L.

Sapranthus campechianus (Kunth) Standl.; *Cabrera 4965*; Mexico; U. *S. violaceus* (Dunal) Saff.; *van Rooden 822*; Guatemala; U.

Tridimeris sp. 1; *Maas 8646*; Missouri Bot. Gard.; U. *T.* sp. 2; *Calzada 1590*; Mexico; U.

Trivalvaria sp. 1; *Chaowasku 35*; Thailand; L. *T.* sp. 2; *Chaowasku 56*; Thailand; L. *T.* sp. 3; *Chaowasku 73*; Thailand; L. *T.* sp. 4; *Chaowasku 86*; Thailand; L.

Undescribed genus sp. 1; *Chaowasku 111*; Thailand; L.

APPENDIX 3. Voucher specimens for pollen morphological study; specimens are deposited in A, BO, K, L, and U herbaria (acronyms follow Index Herbariorum: Thiers, 2008). SEM = scanning electron microscopy; TEM = transmission electron microscopy.

Taxon; Technique applied; *Voucher specimen*; Collection location; Herbarium acronym.

Alphonsea boniana Finet & Gagnep.; TEM; van Beusekom & Smitinand 2116; Thailand; L.

Dendrokingstonia gardneri Chaowasku; TEM; *FRI 32134*; Peninsular Malaysia; L; SEM; *Kerr 19102*; Thailand; K.

Desmopsis verrucipes Chatrou, G.E.Schatz & N.Zamora; TEM; *Chatrou et al. 102*; Costa Rica; U.

Hubera cerasoides (Roxb.) Chaowasku; TEM; *Larsen et al. 33731*; Thailand; L.

Marsypopetalum littorale (Blume) B.Xue & R.M.K.Saunders; TEM; *Backer s.n.*; Java; L. *M.* sp. 1; TEM; *Chaowasku 90*; Thailand; L. *M.* sp. 2; TEM; *Chaowasku 102*; Thailand; L.

Miliusa horsfieldii (Benn.) Baill. ex Pierre; TEM; *How 71794*; China; A. *Miliusa macropoda* Miq.; SEM; *Kostermans 13973*; Borneo; BO.

Mitrephora keithii Ridl.; TEM; *Chin 931*; Peninsular Malaysia; L. *M. macrocarpa* (Miq.) Weeras. & R.M.K.Saunders; TEM; *Sutrisno 60*; Bogor Bot. Gard. (XI-B-XIX-209); L. *M. teysmannii* Scheff.; TEM; *Keßler PK 3226*; Thailand; L.

Monoon paradoxum (Becc.) B.Xue & R.M.K.Saunders; SEM, TEM; *Ambriansyah & Arifin B 1520*; Borneo; L. *M. viride* (Craib) B.Xue & R.M.K.Saunders; TEM; *Phengklai et al. 4244*; Thailand; L.

Orophea kerrii Kessler; SEM; Chalermglin 440416-1; Thailand; L. *O. polycarpa* A.DC.; SEM; Chaowasku 21; Thailand; L.

Phaeanthus ophthalmicus (Roxb. ex G.Don) J.Sinclair; TEM; *PPI 17972*; the Philippines; L. *P. splendens* Miq.; TEM; *S 15364*; Borneo; L.

Platymitra macrocarpa Boerl.; TEM; Gardner et al. ST 1648; Thailand; L.

Polyalthia sp. 1; TEM; *Punnadee 1*; Thailand; L. *P.* sp. 2; TEM; *Chaowasku 50*; Thailand; L.

Popowia odoardi Diels; TEM; *S 74222*; Peninsular Malaysia; L. *P. pisocarpa* (Blume) Endl.; TEM; *van Balgooy & van Setten 5683*; Bogor Bot. Gard.; L.

Sageraea elliptica (A.DC.) Hook.f. & Thomson; TEM; Chaowasku 45; Thailand; L. *S. lanceolata* Miq.; TEM; Ambriansyah & Arbainsyah AA 1673; Borneo; L.

Sapranthus viridiflorus G.E.Schatz; TEM; Maas et al. 7961; Costa Rica; U.

Stelechocarpus burahol (Blume) Hook.f. & Thomson; TEM; *Lörzing 11332*; Sumatra; L.

Stenanona tuberculata G.E.Schatz & Maas; TEM; *Maas et al. 8476*; Honduras; U.

Tridimeris sp. 1; TEM; *Maas 8646*; Missouri Bot. Gard.; U.

Trivalvaria sp. 2; TEM; Chaowasku 56; Thailand; L. *T.* sp. 3; TEM; *Chaowasku 73*; Thailand; L. *T.* sp. 4; TEM; *Chaowasku 86*; Thailand; L.

Winitia expansa Chaowasku; TEM; *Chaowasku 93*; Thailand; L.

APPENDIX 4. Selected characters and their states scored for reconstructing the ancestral character states. Char. = character.

Genus	Char. 1	Char. 2	Char. 3	Char. 4	Char. 5	Char. 6	Char. 7	Char. 8	Char. 9
Dendrokingstonia	0[a]	0[a]	1[a]	1[a,b]	0[a]	0[a]	0[a]	0[a]	0[a]
Monocarpia	0[c]	0[c]	1[c]	0[b]	0[c]	1[a,c]	0[a]	0[a]	0[a]
Mitrephora	0[d]	1[d]	1[d]	0[b]	0[d]	1[d]	1[d,e]	1[e]	0[e]
Alphonsea	0[f]	0[f]	1[f]	01[b]	0[f]	1[f]	0[g]	1[g]	0[g]
Platymitra	0[h]	0[h]	1[h]	0[b]	0[h]	0[h]	0[g]	1[g]	0[g]
Hubera	0[i]	0[i]	0[i]	0[i]	0[i]	0[i]	0[g,i]	1[g,i]	1[g,i]
Miliusa	1[j,k]	0[j,k]	01[j,k]	1[b]	0[j,ii,jj]	01[j]	0[g]	1[g]	0[g]
Orophea	01[l]	1[l]	01[l]	0[b]	0[l]	01[l,m]	0[g]	1[g]	0[g]
Marsypopetalum	01[e]	0[e]	0[n]	01[b,e]	0[n]	01[e,n]	0[e,n]	1[e]	0[e]
Trivalvaria	01[o]	01[o]	0[o]	01[b,e]	02[e,o]	1[o]	0[e,n]	1[e]	0[e]
Pseuduvaria	01[p,q]	1[p,q]	1[p,q]	0[b]	1[p,q]	0[p,q,kk]	1[q,r,kk]	1[r]	0[r]
Polyalthia	01[e,i]	0[e,m]	1[i,m,s]	0[i]	0[e]	01[m,s]	0[e,i]	1[e,t]	0[e,i]
Popowia	01[e]	0[e]	01[m,u]	0[b]	0[u]	1[e,m]	0[e]	1[e]	0[e]
Meiogyne	0[v,w,x]	0[v,w]	1[v,w]	01[b]	0[v,w]	0[v,w]	0[t]	1[t]	0[t]
Undescribed genus	?	?	?	1[e]	?	?	?	?	?
Desmopsis (incl. *Stenanona*)	0[y,z]	0[y,z]	01[y,z]	01[b]	0[y,z]	1[y,z]	0[e]	1[e]	0[e]
Sapranthus	0[e,aa]	0[e,aa]	1[m,aa]	1[b]	0[u]	1[e,aa]	0[e,bb]	1[e,bb]	0[e]
Tridimeris	0[e]	0[e]	1[e]	01[b,e]	0[u]	0[e]	0[e]	1[e]	0[e]
Phaeanthus	1[cc]	0[cc]	01[cc]	1[b]	0[cc]	1[cc]	0[e]	1[e]	2[e]
Neo-uvaria	0[dd]	0[dd]	01[dd]	1[b,dd]	0[dd]	0[dd]	0[dd]	1[dd]	0[dd]
Monoon	0[i,m]	0[i,m]	0[s]	1[b,s]	0[dd]	0[s]	0[i,dd]	1[e,dd]	0[e,dd]
Sageraea	0[ee]	0[e,ee]	1[ee]	1[b]	01[ee]	0[e,ee]	0[ff]	1[e]	0[ff]
Stelechocarpus	0[ff,gg]	0[ff,gg]	1[ff]	1[b,ff]	1[ff,gg]	0[ff]	0[ff]	1[ff]	1[ff]
Winitia	0[ff,gg]	0[ff,gg]	1[ff]	1[b,ff]	1[ff,gg]	?[ff,gg,hh]	0[ff]	1[ff]	0[ff]

[a] Chaowasku et al. (2012b); [b] van Setten and Koek-Noorman (1992); [c] Mols and Keßler (2000b); [d] Weerasooriya and Saunders (2010); [e] T. Chaowasku, personal observation; [f] Keßler (1996); [g] Chaowasku et al. (2008); [h] Keßler (1988b); [i] Chaowasku et al. (2012a); [j] Chaowasku and Keßler (2013); [k] Mols and Keßler (2003); [l] Keßler (1988a); [m] Mols et al. (2004a); [n] Xue et al. (2011); [o] van Heusden (1997b); [p] Su and Saunders (2006); [q] Su et al. (2010); [r] Su and Saunders (2003); [s] Xue et al. (2012); [t] Waha and Morawetz (1988); [u] van Heusden (1992); [v] Jessup (2007); [w] van Heusden (1994); [x] Thomas et al. (2012); [y] Schatz and Maas (2010); [z] Safford (1916); [aa] Schatz (1998); [bb] Waha and Hesse (1988); [cc] Mols and Keßler (2000a); [dd] Chaowasku et al. (2011a); [ee] van Heusden (1997a); [ff] Chaowasku et al. (2013b); [gg] van Heusden (1995); [hh] Inflorescence position undeterminable because of the occurrence of only cauliflory; [ii] Chaowasku (2013); [jj] Chaowasku et al. (2013a); [kk] Saunders et al. (2004).

APPENDIX 5. Results of parsimony ancestral character-state reconstructions at nodes of interest. Char. = character; node present = number of input trees with presence of nodes of interest; 0 = number of trees adopting state 0; 1 = number of trees adopting state 1; 2 = number of trees adopting state 2; eq. = number of trees with equivocal state reconstruction; NC = not calculated.

Node	Char. 1 (node present: 0/1/eq.)	Char. 2 (node present: 0/1/eq.)	Char. 3 (node present: 0/1/eq.)	Char. 4 (node present: 0/1/eq.)	Char. 5 (node present: 0/1/2/eq.)	Char. 6 (node present: 0/1/eq.)	Char. 7 (node present: 0/1/eq.)	Char. 8 (node present: 0/1/eq.)	Char. 9 (node present: 0/1/2/eq.)
Miliuseae	(20004: 20004/0/0)	(20004: 20004/0/0)	(20004: 0/20004/0)	(20004: 17993/1/2010)	(20004: 20004/0/0/0)	(20004: 207/1974/17823)	(20004: 20004/0/0)	(20004: 0/20004/0)	(20004: 20004/0/0/0)
A	(19997: 19997/0/0)	(19997: 19992/0/5)	(19997: 0/19997/0)	(19997: 19997/0/0)	(19997: 19997/0/0/0)	(19997: 10/1986/18001)	(19997: 19995/0/2)	NC	(19997: 19997/0/0/0)
B	(15884: 15883/0/1)	(15884: 15884/0/0)	(15884: 0/15607/277)	(15884: 15720/0/164)	(15884: 15884/0/0/0)	(15884: 585/1564/13735)	(15884: 15884/0/0)	NC	(15884: 15884/0/0/0)
C	(17686: 17685/0/1)	(17686: 17677/0/9)	(17686: 0/3313/14373)	(17686: 17509/1/176)	(17686: 17686/0/0/0)	(17686: 698/1494/15494)	(17686: 17686/0/0)	NC	(17686: 17686/0/0/0)
D	(19523: 19523/0/0)	(19523: 19523/0/0)	(19523: 0/19523/0)	(19523: 0/19518/5)	(19523: 19523/0/0/0)	(19523: 1075/1598/16850)	(19523: 19523/0/0)	NC	(19523: 19523/0/0/0)
D1	(19986: 19986/0/0)	(19986: 19986/0/0)	(19986: 0/19986/0)	(19986: 0/19649/337)	(19986: 19986/0/0/0)	(19986: 1208/1631/17147)	(19986: 19986/0/0)	NC	(19986: 19986/0/0/0)
D2	(19859: 19858/0/1)	(19859: 19859/0/0)	(19859: 0/19784/75)	(19859: 0/19859/0)	(19859: 19859/0/0/0)	(19859: 1162/1639/17058)	(19859: 19859/0/0)	NC	(19859: 19859/0/0/0)

APPENDIX 6. Results of Bayesian ancestral character-state reconstructions at nodes of interest. Char. = character; pp = posterior probabilities; 0 = state 0; 1 = state 1; 2 = state 2; NC = not calculated.

Node	Char. 1 (pp 0/1)	Char. 2 (pp 0/1)	Char. 3 (pp 0/1)	Char. 4 (pp 0/1)	Char. 5 (pp 0/1/2)	Char. 6 pp (0/1)	Char. 7 (pp 0/1)	Char. 8 (pp 0/1)	Char. 9 (pp 0/1/2)
Miliuseae	0.999/0.001	0.983/0.017	0.003/0.997	0.938/0.062	0.999/0.001/0	0.824/0.176	0.996/0.004	0/1	0.999/0.001/0
A	0.998/0.002	0.879/0.121	0.006/0.994	0.992/0.008	0.997/0.002/0.001	0.554/0.446	0.903/0.097	NC	0.997/0.002/0.001
B	0.994/0.006	0.984/0.016	0.019/0.981	0.832/0.168	0.999/0.001/0	0.855/0.145	0.996/0.004	NC	0.999/0.001/0
C	0.763/0.237	0.964/0.036	0.296/0.704	0.952/0.048	0.997/0.002/0.001	0.634/0.366	0.995/0.005	NC	0.994/0.004/0.002
D	0.999/0.001	0.999/0.001	0.005/0.995	0.004/0.996	0.996/0.004/0	0.883/0.117	1/0	NC	0.998/0.001/0.001
D1	0.997/0.003	0.995/0.005	0.013/0.987	0.05/0.95	0.996/0.002/0.002	0.703/0.297	0.998/0.002	NC	0.996/0.002/0.002
D2	0.995/0.005	0.999/0.001	0.052/0.948	0.003/0.997	0.878/0.12/0.002	0.877/0.123	1/0	NC	0.977/0.01/0.013