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Chapter 2

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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Abstract

Premise of study: Tribe Miliuseae (ca. 25 genera and ca. 510 species) include a substantial part of the species and generic diversity in the pantropical flowering plant family Annonaceae (ca. 108 genera and ca. 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 (ca. 89 % of generic diversity in Miliuseae) and plastid DNA sequence data (ca. 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: Dendrokingstoniae, 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, morphology, Miliuseae, phylogenetic analyses, palynology
Introduction

The flowering plant family Annonaceae comprises ca. 108 genera and ca. 2400 species of trees, shrubs and woody lianas (Rainer & Chatrou 2006, Chatrou et al. 2012) predominantly inhabiting 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. Sauquet et al. 2003, Keßler 1993).

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 subfamilial and tribal level. The family is now classified into four subfamilies, i.e. (1) Anaxagoreoideae, (2) Ambavioideae, (3) Annonoideae, and (4) Malmeoideae. The latter two subfamilies together constitute a large clade containing more than 95% of species in the family (Rainer & 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 & Le Thomas 2012, Koek-Noorman & Westra 2012), historical biogeography of the family (Couvreur et al. 2011), and patterns and timing of diversification (Erkens et al. 2012, Pirie & 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., Phoencanthus 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 ca. 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 (ca. 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. 2012b). 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 & Le Thomas 2012), the most obvious of which is apertures. Miliuseae pollen has been considered as being 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.* in press) 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 (ca. 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 (ca. 7 kb) and covering ca. 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 *Greenwayodenron* 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 eight 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, as (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 elaborated on below (see Reconstructions of ancestral character states section).

**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 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 $\geq 85\%$, 70–84 %, and $\leq 69\%$ was considered strongly, moderately, and weakly supported, respectively.

Bayesian MCMC (Yang & Rannala 1997) phylogenetic analysis was performed in MrBayes version 3.1.2 (Ronquist & 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, ycf1), and the Hasegawa, Kishino and 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 ten 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 v1.5 (Rambaut & 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 trees 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 & Le Thomas 1996, Mols et al. 2004a), including six macro-morphological 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 in size to inner petals]. (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 & Le Thomas 1996, Mols et al. 2004a), but none of the genera in Miliuseae invariably
exhibiting two-ovuled ovaries prompted us to differentiate only uniovulate and multi-ovuled 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 disulcate.

(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 as 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 & 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.: pers. obs. T. Chaowasku). The intermediate form was treated as an additional character state in Doyle & 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 & Keßler 2000a: Phaeanthus Hook.f. & Thomson, Schatz & 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 & Le Thomas (1996) and Mols et al. (2004a) included this character in their analyses and found some phylogenetic signal. We re-investigated this character, however, and found that character state determination is subjective, e.g. Van Setten & Koek-Noorman (1992) described the
endosperm texture of *Neo-uvaria* 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 & Maddison 2010), respectively. The outgroups (accessions of Piptostigmaeae) 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.* in press), 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 & 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 five million iterations were run, with sampling every 100th iteration, and discarding a burn-in of 500,000 iterations. In order 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 v1.5 (Rambaut & 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, infratectum, and basal layer, following Le Thomas (1980), is used.
Results

Phylogenetic analyses (Fig. 1)

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

<table>
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<th>DNA region</th>
<th>No. of included characters</th>
<th>No. of accessions (of all 65 accessions included) lacking sequence data (%)</th>
<th>No. of variable characters (%)</th>
<th>No. of parsimony-informative characters (%)</th>
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<td>124 (9.0)</td>
<td>65 (4.7)</td>
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<td>- trnL intron + trnL-F spacer</td>
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<td>0</td>
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<td>101 (10.9)</td>
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<td>828</td>
<td>0</td>
<td>184 (22.2)</td>
<td>84 (10.1)</td>
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<td>436 (21.4)</td>
<td>247 (12.2)</td>
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<tr>
<td>- psbA-trnH spacer</td>
<td>431</td>
<td>0</td>
<td>137 (31.8)</td>
<td>94 (21.8)</td>
</tr>
<tr>
<td>- ycf1 exon</td>
<td>1429</td>
<td>8 (12.3)</td>
<td>358 (25.1)</td>
<td>177 (12.4)</td>
</tr>
<tr>
<td>- Combined data</td>
<td>7027</td>
<td>NA</td>
<td>1440 (20.5)</td>
<td>768 (10.9)</td>
</tr>
</tbody>
</table>

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*, *Neuo-uvaria*, *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 (Figs. 2, 3; Appendices 5, 6)

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 greater than 1200, indicating adequate posterior sampling. Results of the parsimony and Bayesian ancestral character-state reconstructions were largely congruent and are illustrated in Figs. 2, 3 (see Appendices 5 and 6 for precise values of all characters reconstructed).

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*).

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 non-clawed inner petals (Fig. 4A–J) multiple times: in *Mitrephora* (clade A); and in *Orophea*, *Pseuduvaria* and somewhere in *Trivalvaria* (clade C2).
Phylogeny of and character evolution within Miliuseae

**a) Outer petal appearance**
- Showy
- +/- sepaloid
- Equivocal
- Node absent

**b) Inner petal base**
- Not clawed
- Distinctly clawed
- Equivocal
- Node absent

**c) Max. ovule number per ovary**
- 1
- ≥ 2
- Equivocal
- Node absent

**d) Endosperm rumination type**
- Spiniform to flattened pegs
- Lamelliform
- Equivocal
- Node absent

**FIGURE 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 (see Materials and methods for details). Parsimony analyses: Smaller pie charts at internal nodes illustrate the number and proportion of unequivocal state reconstructions, equivocal state reconstructions (grey), 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.
The Miliuseae revisited – Chapter 2

a) Flower sexuality

- Monod
- Tetrad
- Equivocal
- Node absent

b) Pollen: dispersal unit

- Dendrokingstonia
- Monocarpia
- Mitrephora
- Alphonsea
- Platymitra
- Hubera
- Milusa
- Orphoea
- Marsyopetalum
- Trivaria
- Pseuduvaria
- Polyalthia
- Popowia
- Meiogyne
- Undescribed genus
- Desmopsis
- Sapranthus
- Tridimeris
- Phaeanthus
- Neo-uvaria
- Monoon
- Sageraea
- Stelechocarpus
- Winitia

FIGURE 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 (see Materials and methods for details). Parsimony analyses: Smaller pie charts at internal nodes illustrate the number and proportion of unequivocal state reconstructions, equivocal state reconstructions (grey), 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.
Maximum ovule number per ovary (character 3; Fig. 2c)

Multi-ovuled ovaries (with ≥ 2 to c. 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 *Neouvaria*, 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: pers. obs. T. Chaowasku) 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, synapomorphic for Miliuseae (Fig. 5A–F), 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, Chaowasku et al. 2012a, Chatrou et al. 2012, Thomas et al. 2012, Xue et al. 2011, 2012). In the current 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 inter- 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 & 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), viz. the careful selection of a limited number of species to represent larger clades, and a focus on the sampling of sequence data. Our sampling did not focus on species of 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 so 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 character, 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 based on the 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 & Saunders, 2010; Fig. 4A, B, K). *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 & Saunders, 2010). *Mitrephora* shows ‘uvarioid’ stamens (Weerasooriya & 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, Southeast Asian Islands through mainland Asia to Madagascar and eastern Africa. This is the widest geographical 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 endosperm rumination type (see Mols & Keßler 2003, Chaowasku & 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 exceeding 5 m in height), considerably thickened leaves, a single ovule per ovary (likely synapomorphic), and more or less ellipsoid-cylindrical
FIGURE 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...continued on page 27
seeds (Van Heusden 1997b, Xue et al. 2011, pers. obs. T. Chaowasku). For a discussion on the morphology and differentiation of *Marsypopetalum* and *Trivalvaria*, see Xue et al. (2011). Preliminary studies on the macromorphology of *Marsypopetalum* 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, pers. obs. T. Chaowasku). 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 current 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 multi-seeded subglobose monocarp (only a single detached monocarp is available). Endosperm ruminations of this genus are apparently four-parted lamelliform (pers. obs. T. Chaowasku), which is also consistently found in the Neotropical genus *Sapranthus* (Van Setten & 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 this 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. *Guamía* was morphologically synonymized with *Meiogyne* by Van Heusden (1994) and results of the present 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 which 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 multi-ovuled ovaries (Appendix 4: character 3). However, deviations occur in Meiogyne bidwillii (Benth.) D.C.Thomas, Chaowasku & R.M.K. Saunders, which shows sepaloid outer petals (Jessup 2007: under Fitzalania F.Muell.), and Stenanona monticola Maas & G.E.Schatz, which has uniovulate ovaries (Schatz & 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) exhibiting 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 do exist (Schatz & Maas 2010).

Clade D2: This clade consists of the genera Phaenanthis, Neo-uvaria, Monoon, Steleichocarpus, 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 Steleichocarpus, Winitia, and Sageraea were recovered as a strongly supported clade possessing diagnostic multi-ovuled ovaries and (rather) thick leaves (Van Heusden 1995, 1997a, Chaowasku et al. 2013b). Furthermore, Steleichocarpus, Winitia, and some species of Sageraea are monoecious (Van Heusden 1995, 1997a, Chaowasku et al. 2013b).

Steleichocarpus 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 & 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 & 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 multi-ovuled to uniovulate ovaries within Miliuseae may sometimes be correlated with shifts in dispersal agents. Multi-ovuled ovaries usually result in relatively large multi-seeded 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 & 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 de 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, as 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 Mezzetta, 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 Dendrokingstoniaeae and Monocarpieae, possesses monosulcate pollen (Fig. 5G, H; Le Thomas 1980, Waha 1985, Waha & Hesse 1988, Schatz & Le Thomas 1990, Couvreur et al. 2009, Chaowasku et al. 2012b), while the tribe Miliuseae shows synapomorphic cryptoaperturate or disulcate 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 & Hesse 1988, Waha & Morawetz 1988, Chaowasku et al. 2008). Cryptoaperturate is the term describing the pollen exhibiting such features. Sometimes, the deviating intine parts are recognizable by a (slightly) sunken overlying exine (disulcate; Fig. 5C, D). Disulculate pollen may just represent partially collapsed cryptoaperturate pollen; further examination is indispensable to understand the relation of cryptoaperturate and disulculate pollen. Doyle & Le Thomas (2012) argued that TEM studies indicate that the exine above the intinuous 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 be also 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.

(Chaowasku 21): pollen grain showing two depressed exine areas indicating two intinuous 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 outwards (TEM). Scale bars: 5 µm (A), (B), (D)–(F), (H); 10 µm (C), (G); en, endintine; ex, exintine; in, intine.
FIGURE 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/disulculate 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 intinuous germination zone (SEM). (D) *Orophea polycarpa* ...continued on page 30
The pollen grains of *Mitrephora* (pers. obs. T. Chaowasku); *Neo-uvaria* (Chaowasku et al. 2011a); *Pseudeuvaria* (Su & Saunders 2003); *Sageraea*, *Stelechocarpus* and *Winitia* (pers. obs. T. Chaowasku); and certain species of *Monoon* (Fig. 5F; Waha & Hesse 1988, Chaowasku et al. 2011a, pers. obs. T. Chaowasku) 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 & 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 & Thanikaimoni 1987, Doyle & Le Thomas 2012). However, TEM studies investigating whether there are enlargements/reductions of the intine sublayers characteristic for disulculate pollen (Waha & Hesse 1988, Waha & Morawetz 1988) are required for confirmation.

**Appendices**

**APPENDIX 1.** Voucher specimens for molecular phylogenetic analyses. * = sequences newly generated for this study; --- = sequence not available.

**Taxon:** GenBank accessions: rbcL; trnLF; matK; ndhF; psbA-trnH; ycf1; **Voucher specimen**; Collection location; Herbarium acronym.
Phylogeny of and character evolution within Miliuseae

**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.

**Anickia pilosa** (Exell) Setten & Maas; Ay743450; Ay743469; Ay743488; Ay841402; Ay841444; --; Sofe 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; KJ418400; --; Rogstad 961; Peninsular Malaysia; L.

**Desmopsis microcarpa** R.E. Fr.; Ay319059; Ay319173; Ay518804; JX544771; Ay841461; JX544758; Chateau et al. 85; Costa Rica; U. D. sp.; --; Ay841701; KC857552; KC857553; KC857554; KC857555; Rainer 1593; Mexico; WU.

**Fenerivia chapeleri** (Baill.) R.M.K.Saunders; KJ418383; KJ418403; KJ418393; KJ418387; KJ418397; --; Rabevohitra et al. 4439; Madagascar; F. ghesquiereana (Caravo & 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; JQ889975; Chalermglin 440214-4; Thailand; L. H. nititissima (Dunal) Chaowasku; --; JQ889988; JQ889986; JQ889986; JQ889981; JQ889987; Ford AF 4967; Australia; L. H. pendula (Capuron ex G.E.Schatz & Le Thomas) Chaowasku; --; Ay319144; Ay818852; JQ889987; JQ889982; Rabevohitra 2386; Madagascar; WAG.

**H. stuhlmannii** --; Chaowasku 77; Thailand; L.

**Maasia discolor** (Diels) Mols, Kessler & Rogstad; Ay319021; Ay319135; Ay518847; Ay841410; --; Takeuchi & Ama 16395; 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; JX544840; 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.

**Meioigyne bidwillii** (Benth.) D.C.Thomas, Chaowasku & R.M.K.Saunders; --; KJ418408; KJ418396; KJ418390; KJ418401; KJ418380; Randall 624; Australia; L. M. cylindrocarpa (Burck) Heusden; --; KJ418409; KJ418395; KJ418389; KJ418402; KJ18379; Marler s.n.; Tinian Island; L. M. heteropetala (F.Muell.) D.C.Thomas, Chaowasku & R.M.K.Saunders; --; KC857556; KC857557; KC857558; KC857559; KC857560; Kemp Th 7267; Australia; L. M. virgata (Blume) Miq.; Ay318982; Ay319194; Ay518798; JX544769; JX544765; JX544784; JX544758; Keßler PK 2751; Borneo; L. M. sp.; JX544764; JX544765; JX544766; JX544767; Gardner et al. ST 2014; Thailand; L.

**Miliusa mollis** Pierre; --; Ay319102; Ay518851; JQ690503; JQ690504; JQ690505; Keßler PK 3207; Thailand; L. M. thorelli Finet & Gagnep.; --; Ay319104; Ay518846; JQ690519; JQ690520; JQ690521; Keßler PK 3184; Thailand; L. M. velutina (DC.) Hook.f. & Thomson; Ay318933; Ay319105; Ay518847; JQ690536; JQ690537; JQ690538; Pholsena & koonkhunthod 2842; L. M. sp.; --; JQ690526; JQ690527; JQ690529; JQ690530; Nakorn-Thiemchan NTC 7; Thailand; L.

**Mitrephora alba** Ridl.; Ay318994; Ay319106; JQ690503; JQ889978; JQ889973; Chalermglin 440204-1; Thailand; TISTR, Bangkok. M. macrocarpa (Miq.) Weeras. & R.M.K.Saunders; --; Ay319107; Ay518859; JQ889984; JQ889987; JQ889973; Mols 8; Bogor Bot. Gard.; L.

**Monocarpia euneura** (Miq.) Weeras.; 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.

**Mononcus 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; JX544791; JX544766; Chalermglin 77; Thailand; L. N. parallelevinina (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;
APPENDIX 2. Voucher specimens for macromorphological observations.

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. **S. lanceolata** (Roxb.) Thwaites; AY319050; AY319164; AY518799; JX544774; JX544778; KC857601; KC857602; KC857603; KC857604; KC857605; Chaowasku 56; Thailand; L. **S. sp. 1**; Thailand; L. **S. sp. 2**; Thailand; L. **S. sp. 3**; Thailand; L. **S. sp. 4**; Thailand; L. **Trivalvaria** (King) J.Sinclair; ---; KC857581; KC857582; KC857583; KC857584; KC857585; Chaowasku 66; Thailand; L. **Winitia cauliflora** (Scheff.) Chaowasku; AY319008; AY319121; AY518799; JX544774; JX544778; KC857601; KC857602; KC857603; KC857604; KC857605; Chaowasku 56; Thailand; L. **W. expansa** (Blume) Backer; Unknown 7706; Bogor Bot. Gard.; L. **W. sp. 1**; Thailand; L. **W. sp. 2**; Thailand; L. **W. sp. 3**; Thailand; L. **W. sp. 4**; Thailand; L. **W. sp. 5**; Thailand; L.
**Phylogeny of and character evolution within Miliuseae**

*Popowia fusca* King; FRI 16340; Peninsular Malaysia; *L. P. hirta* Miq.; *Ismail & Arifin BRF 1732; Borneo; L. *P. sp. 1*; *Ambri & Arifin WF 632; 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%28bhhb0445z22hbtbg1ba3a55%29%29/Album/ShowAlbum.aspx?albumid=1cbe615df-1919-4d7b-a4f9-59251c278954&Username=pccsw*

*Pseudovaria fragrans* Y.C.F.Su, Chaowasku & R.M.K.Saunders; *Chaowasku 27*; Thailand; *L. P. setosa* (King) J.Sinclair; *Chaowasku 66*; Thailand; L. *P. sp. 1*; *Ambri & Arifin WF 632; 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%28bhhb0445z22hbtbg1ba3a55%29%29/Album/ShowAlbum.aspx?albumid=1cbe615df-1919-4d7b-a4f9-59251c278954&Username=pccsw*

*Sageraea bracteolata* R.Parker; *Gardner ST 2068*; Thailand; *L. S. elliptica* (A.DC.) Hook.f. & Thomson; *Chaowasku 45*; Thailand; L. *P. sp. 1*; *Chaowasku 27*; Thailand; *L. P. setosa* (King) J.Sinclair; *Chaowasku 66*; Thailand; 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%28bhhb0445z22hbtbg1ba3a55%29%29/Album/ShowAlbum.aspx?albumid=1cbe615df-1919-4d7b-a4f9-59251c278954&Username=pccsw*


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<th>Collection location; Herbarium acronym.</th>
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<td>Alphonsea boniana</td>
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<td>Dendrokingstonia gardneri</td>
<td>TEM; <em>Van Beusekom &amp; Smitinand 2116</em></td>
<td>Thailand; L.</td>
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<td>Desmopis verrucipes</td>
<td>TEM; <em>Chaowasku et al. 102</em></td>
<td>Costa Rica; U.</td>
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<td>Hubera cerasoides</td>
<td>TEM; <em>Larsen et al. 33731</em></td>
<td>Thailand; L.</td>
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<td>Marsypopetalum litorale</td>
<td>TEM; <em>Backer s.n.</em></td>
<td>Java; L. M. sp. 1; TEM; Chaowasku 90; Thailand; L. M. sp. 2; TEM; Chaowasku 102; Thailand; L.</td>
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<td>Miliusa horsfieldii</td>
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<td>Thailand; L. M. macrocarpa (Miq.) Weeras. &amp; R.M.K.Saunders; TEM; <em>Sutrisno 60</em></td>
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<td>Mitrephora keithii</td>
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<td>Phaeanthus ophthalmicus</td>
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**APPENDIX 4.** Selected characters and their states scored for reconstructing the ancestral character states. Char. = character.

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<th>Char. 2</th>
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APPENDIX 4 (continued).

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*a* = Chaowasku *et al.* (2012b)

*b* = Van Setten & Koek-Noorman (1992)

*c* = Mols & Keßler (2000b)

*d* = Weerasooriya & Saunders (2010)

*e* = pers. obs. T. Chaowasku

*f* = Keßler (1996)

*g* = Chaowasku *et al.* (2008)

*h* = Keßler (1988b)

*i* = Chaowasku *et al.* (2012a)

*j* = Chaowasku & Keßler (2013)

*k* = Mols & Keßler (2003)

*l* = Keßler (1988a)

*m* = Mols *et al.* (2004a)

*n* = Xue *et al.* (2011)

*o* = Van Huesden (1997b)

*p* = Su & Saunders (2006)

*q* = Su *et al.* (2010)

*r* = Su & Saunders (2003)

*s* = Xue *et al.* (2012)

*t* = Waha & Morawetz (1988)

*u* = Van Huesden (1992)

*v* = Jessup (2007)

*w* = Van Huesden (1994)

*x* = Thomas *et al.* (2012)

*y* = Schatz & Maas (2010)

*z* = Safford (1916)

*aa* = Schatz (1998)

*ab* = Waha & Hesse (1988)

*ac* = Mols & Keßler (2000a)

*ad* = Chaowasku *et al.* (2011a)

*ae* = Van Huesden (1997a)

*af* = Chaowasku *et al.* (2013b)

*ag* = Van Huesden (1995)

*ah* = inflorescence position undeterminable because of the occurrence of only cauliflory

*i* = Chaowasku (2013)

*ii* = 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.

<table>
<thead>
<tr>
<th>Node</th>
<th>Char. 1 (node present: 0/1/eq.)</th>
<th>Char. 2 (node present: 0/1/eq.)</th>
<th>Char. 3 (node present: 0/1/eq.)</th>
<th>Char. 4 (node present: 0/1/eq.)</th>
<th>Char. 5 (node present: 0/1/eq.)</th>
<th>Char. 6 (node present: 0/1/eq.)</th>
<th>Char. 7 (node present: 0/1/eq.)</th>
<th>Char. 8 (node present: 0/1/eq.)</th>
<th>Char. 9 (node present: 0/1/eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miliuseae</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 17993/1/2010)</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 20004/0/0)</td>
<td>(20004: 20004/0/0)</td>
</tr>
<tr>
<td>A</td>
<td>(19997: 19997/0/0)</td>
<td>(19997: 19997/0/0)</td>
<td>(19997: 19997/0/0)</td>
<td>(19997: 19997/0/0)</td>
<td>(19997: 19997/0/0)</td>
<td>(19997: 19997/0/0)</td>
<td>NC</td>
<td>(19997: 19997/0/0)</td>
<td>(19997: 19997/0/0)</td>
</tr>
<tr>
<td>B</td>
<td>(15884: 15883/0/1)</td>
<td>(15884: 15884/0/0)</td>
<td>(15884: 15884/0/0)</td>
<td>(15884: 15884/0/0)</td>
<td>(15884: 15884/0/0)</td>
<td>(15884: 15884/0/0)</td>
<td>NC</td>
<td>(15884: 15884/0/0)</td>
<td>(15884: 15884/0/0)</td>
</tr>
<tr>
<td>C</td>
<td>(17686: 17684/1/0)</td>
<td>(17686: 17686/0/0)</td>
<td>(17686: 17686/0/0)</td>
<td>(17686: 17686/0/0)</td>
<td>(17686: 17686/0/0)</td>
<td>(17686: 17686/0/0)</td>
<td>NC</td>
<td>(17686: 17686/0/0)</td>
<td>(17686: 17686/0/0)</td>
</tr>
<tr>
<td>D</td>
<td>(19523: 19523/0/0)</td>
<td>(19523: 19523/0/0)</td>
<td>(19523: 19523/0/0)</td>
<td>(19523: 19523/0/0)</td>
<td>(19523: 19523/0/0)</td>
<td>(19523: 19523/0/0)</td>
<td>NC</td>
<td>(19523: 19523/0/0)</td>
<td>(19523: 19523/0/0)</td>
</tr>
<tr>
<td>D1</td>
<td>(19986: 19986/0/0)</td>
<td>(19986: 19986/0/0)</td>
<td>(19986: 19986/0/0)</td>
<td>(19986: 19986/0/0)</td>
<td>(19986: 19986/0/0)</td>
<td>(19986: 19986/0/0)</td>
<td>NC</td>
<td>(19986: 19986/0/0)</td>
<td>(19986: 19986/0/0)</td>
</tr>
<tr>
<td>D2</td>
<td>(19859: 19858/0/1)</td>
<td>(19859: 19859/0/0)</td>
<td>(19859: 19859/0/0)</td>
<td>(19859: 19859/0/0)</td>
<td>(19859: 19859/0/0)</td>
<td>(19859: 19859/0/0)</td>
<td>NC</td>
<td>(19859: 19859/0/0)</td>
<td>(19859: 19859/0/0)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Node</th>
<th>Char. 1 (pp 0/1)</th>
<th>Char. 2 (pp 0/1)</th>
<th>Char. 3 (pp 0/1)</th>
<th>Char. 4 (pp 0/1)</th>
<th>Char. 5 (pp 0/1)</th>
<th>Char. 6 (pp 0/1)</th>
<th>Char. 7 (pp 0/1)</th>
<th>Char. 8 (pp 0/1)</th>
<th>Char. 9 (pp 0/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miliuseae</td>
<td>0.999/0.001</td>
<td>0.983/0.017</td>
<td>0.003/0.997</td>
<td>0.938/0.062</td>
<td>0.999/0.001</td>
<td>0.824/0.176</td>
<td>0.996/0.004</td>
<td>0/1</td>
<td>0.999/0.001</td>
</tr>
<tr>
<td>A</td>
<td>0.998/0.002</td>
<td>0.879/0.121</td>
<td>0.006/0.994</td>
<td>0.992/0.008</td>
<td>0.997/0.002</td>
<td>0.554/0.446</td>
<td>0.903/0.097</td>
<td>NC</td>
<td>0.997/0.002/0.001</td>
</tr>
</tbody>
</table>
APPENDIX 6 (continued).

<table>
<thead>
<tr>
<th>Node</th>
<th>Char. 1 (pp 0/1)</th>
<th>Char. 2 (pp 0/1)</th>
<th>Char. 3 (pp 0/1)</th>
<th>Char. 4 (pp 0/1)</th>
<th>Char. 5 (pp 0/1/2)</th>
<th>Char. 6 (pp 0/1)</th>
<th>Char. 7 (pp 0/1)</th>
<th>Char. 8 (pp 0/1)</th>
<th>Char. 9 (pp 0/1/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.994/0.006</td>
<td>0.984/0.016</td>
<td>0.019/0.981</td>
<td>0.832/0.168</td>
<td>0.999/0.001/0</td>
<td>0.855/0.145</td>
<td>0.996/0.004</td>
<td>NC</td>
<td>0.999/0.001/0</td>
</tr>
<tr>
<td>C</td>
<td>0.763/0.237</td>
<td>0.964/0.036</td>
<td>0.296/0.704</td>
<td>0.952/0.048</td>
<td>0.997/0.002/0.001</td>
<td>0.634/0.366</td>
<td>0.995/0.005</td>
<td>NC</td>
<td>0.994/0.004/0.002</td>
</tr>
<tr>
<td>D</td>
<td>0.999/0.001</td>
<td>0.999/0.001</td>
<td>0.005/0.995</td>
<td>0.004/0.996</td>
<td>0.996/0.004/0</td>
<td>0.883/0.117</td>
<td>1/0</td>
<td>NC</td>
<td>0.998/0.001/0.001</td>
</tr>
<tr>
<td>D1</td>
<td>0.997/0.003</td>
<td>0.995/0.005</td>
<td>0.013/0.987</td>
<td>0.05/0.95</td>
<td>0.996/0.002/0.002</td>
<td>0.703/0.297</td>
<td>0.998/0.002</td>
<td>NC</td>
<td>0.996/0.002/0.002</td>
</tr>
<tr>
<td>D2</td>
<td>0.995/0.005</td>
<td>0.999/0.001</td>
<td>0.052/0.948</td>
<td>0.003/0.997</td>
<td>0.878/0.12/0.002</td>
<td>0.877/0.123</td>
<td>1/0</td>
<td>NC</td>
<td>0.977/0.01/0.013</td>
</tr>
</tbody>
</table>