



# The Miliuseae revisited

*Phylogenetic, taxonomic, and palynological studies in a major clade of Annonaceae*

**Tanawat Chaowasku**



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**Tanawat Chaowasku**

NATURALIS BIODIVERSITY CENTER  
(Botany Sector)  
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Front cover: Small (from left to right): *Hubera jenkinsii* (Hook.f. & Thomson) Chaowasku, *Dendrokingstonia gardneri* Chaowasku, *Milium* sp., *Milium campanulata* Pierre, *Meiogyne* sp., *Neo-uvaria telopea* Chaowasku; large: *Winitia expansa* Chaowasku. Photographs by K. Aongyong: *Hubera jenkinsii*; T. Chaowasku: *Winitia expansa*; S. Gardner: *Dendrokingstonia gardneri*, *Meiogyne* sp., *Neo-uvaria telopea*; B. Siriphiphat: *Milium* sp., *Milium campanulata*.

Back cover: Ancestral character-state reconstructions of pollen infratectum types (above) and exine diversity found in Miliuseae (below).

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# Chapter 1

## General introduction

### Identifying and describing the diversity of life on Earth

Biodiversity is a term referring to the diversity of life including diversity within species, between species, and of ecosystems (Anonymous 1992). Evolution at all levels of biological organization, e.g. genes, organisms, and ecosystems, is associated with biodiversity (Gaston & Spicer 2004). Disentangling the complex interplay of biotic and abiotic factors, which are underlying the current patterns of biodiversity, is one of the most challenging goals of current ecological and evolutionary research (Pennisi 2005). Even the baseline of many ecological and evolutionary studies on species diversity, i.e. species number in certain taxa or geographic regions, however, is often highly uncertain. Estimates indicate that there are millions of species still unknown to science [e.g. Mora *et al.* 2011: only ca. 14% of terrestrial and ca. 9% of marine species have been described, Costello *et al.* 2013: only 1.5 million (out of  $5 \pm 3$  million) species have been named], mostly in tropical forest ecosystems (Giam *et al.* 2012), emphasizing the continuing need for taxonomists spearheading the efforts to identify and describe species (Bacher 2012).

Taxonomy, an indispensable fundamental biological discipline (see overview in Smith *et al.* 2011), intends to describe biodiversity by classifying, identifying and naming taxa from domain to species, including infraspecific ranks. Phylogenetic systematics, or the cladistic approach, introduced by the German biologist Willi Hennig (Hennig 1950, 1966) aims to identify and define taxa that include all descendents of a common ancestor (clades or monophyla) by reconstructing common ancestry relationships. In phylogenetic systematics ancestral (plesiomorphic) and derived (apomorphic) character states are differentiated. Only shared derived character states (synapomorphies) are used for the identification of clades, whereas shared ancestral character states (symplesiomorphies) are not used. It is not surprising that many groups recognized in pre-cladistic classifications based on shared ancestral character states, e.g. reptiles or bryophytes, are paraphyletic, i.e. derived from a common ancestor, but not including all of its descendents. Other taxa recognized in pre-cladistic classifications were highly artificial because they were not based on common descent, but based on similarities that evolved multiple times (= convergent evolution; e.g. pooling of flowering plants characterized by flowers in catkins and belonging to several distantly related families into a single taxon, the "Amentiferae"; see Stern 1973 for discussion of the widespread use of the amentiferous concept). Similarities resulting from convergent evolution or reversals are called homoplasies. The recognition of taxa primarily based on homoplasious features can result in polyphyletic groups, i.e. taxa that include descendents of only distantly related ancestors and do not include the common ancestor of the whole group (Lipscomb 1998).

There are both quantitative and qualitative limitations in reconstructing phylogenetic relationships using morphological data. Morphological characters are very limited in numbers, states are often difficult to define, and homology of highly modified structures

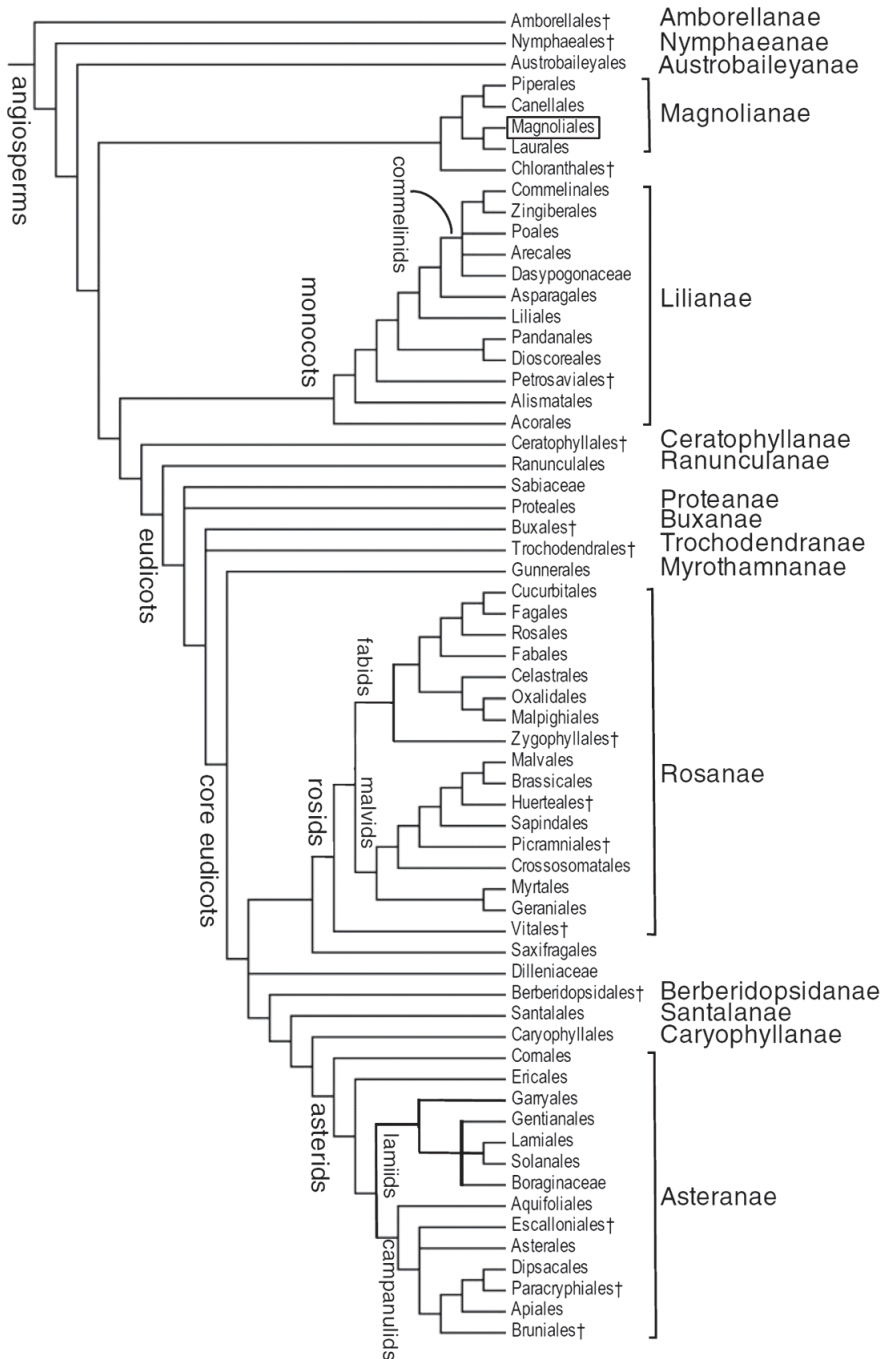
is sometimes difficult to determine. When phylogenetic reconstructions include a large proportion of erroneously included homoplasious character states, the inferred clade memberships will often be no different from the artificial morphological groupings of pre-cladistic classifications. Molecular data (i.e. nucleotides or proteins) then become valuable resources for phylogenetic reconstruction because of the huge number of characters available and the easily definable character states. They are the sole source of data used for phylogenetic reconstructions in this thesis.

One of the goals of taxonomy is to name taxa, of which the species is the basic unit of biological classification. However, delimitation of species is always debatable since several vastly different species concepts have been advanced and scientists working in different branches of the biological science adopt different criteria (see discussion in e.g. De Queiroz 2007, Hausdorf 2011). Although molecular phylogenetic reconstruction is a powerful tool, which I used to investigate deeper phylogenetic relationships, I do not use phylogenetic approaches and molecular data to delimit species in this thesis. The main reason for this is that it would require populational approaches (Avice & Ball 1990, Waters 2003). A sufficient number of accessions were not available, however, and collection of a sufficient number of samples and development of sufficiently informative DNA markers were time- and cost-prohibitive in the framework of this thesis. Therefore, as a taxonomist who has to principally rely on characters observable from herbarium specimens and in the field, I use the morphological species concept after Van Steenis (1957), which does not explicitly consider reproductive cohesion and phylogenetic relationships but is solely based on morphological differences. Van Steenis's concept differentiates two morphologically similar species on the basis of at least two independent differences in morphological character states. The reason for using this simple concept is that distinguishing species using clearly defined morphological differences is practical. It has been argued that the species concept of Van Steenis (1957) is in fact an implementation of inexplicit species concepts, i.e. an attempt to make species concepts operational (Waters 2003).

## **The pantropical flowering plant family Annonaceae**

### *Annonaceae and their diversity, with special reference to the tribe Miliuseae*

The pantropical flowering plant family Annonaceae comprises ca. 108 genera and ca. 2400 species of trees, shrubs and lianas (Rainer & Chatrou 2006, Chatrou *et al.* 2012). It is the largest family in the order Magnoliales (Sauquet *et al.* 2003) of the superorder Magnolianae (Chase & Reveal 2009; Fig. 1). Annonaceae show several diagnostically important features such as vessel elements with simple perforations, a characteristic arrangement of xylem rays and parenchyma bands resulting in secondary xylem that has a 'cobweb-like' appearance in cross-section, a distichous leaf arrangement, a trimerous perianth differentiated into calyx and corolla (Fig. 2), and perichalazal ovules (Keßler 1993, Sauquet *et al.* 2003). Because of these and other highly diagnostic traits such as fruits usually consisting of an aggregate of stalked monocarps and seeds with ruminant endosperm, Annonaceae are easily recognizable both in the field and as herbarium specimens.



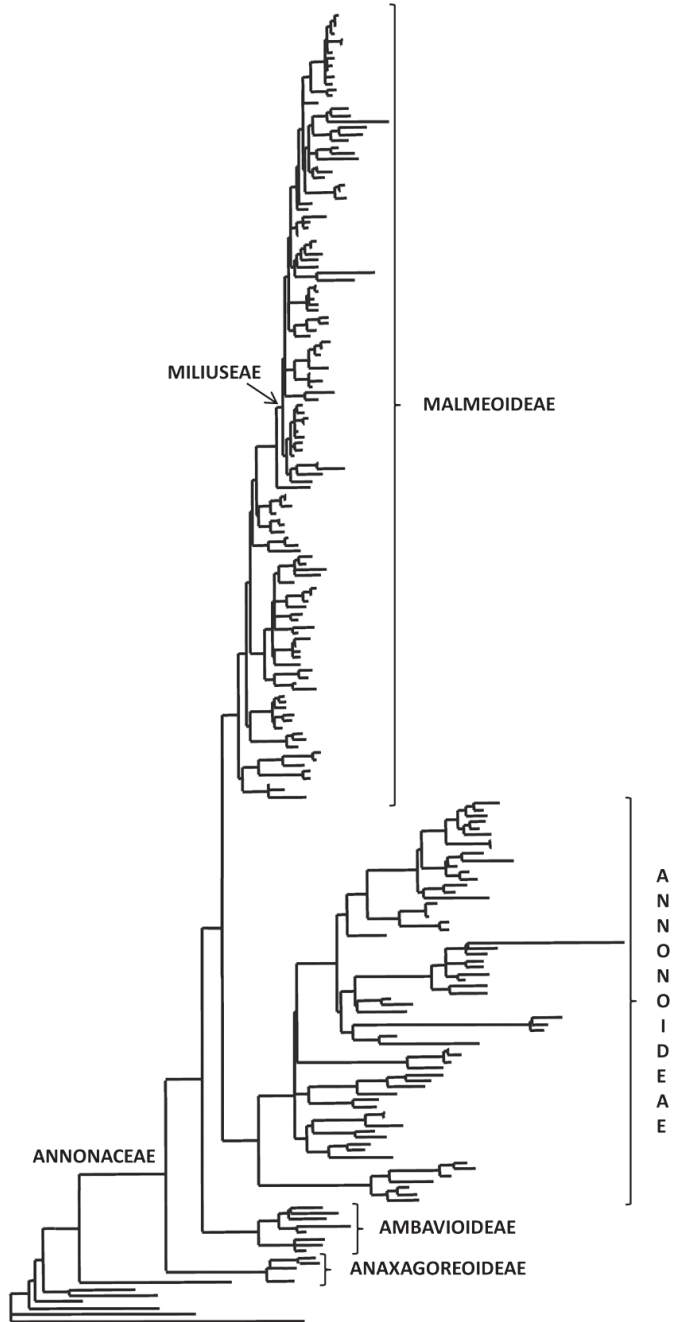
**FIGURE 1.** Phylogenetic position of Magnoliales (rectangle) and Magnolianae in angiosperm phylogeny. Adapted from Chase & Reveal (2009).



**FIGURE 2.** Flower of Annonaceae, showing a trimerous perianth differentiated into calyx and corolla. Photograph: T. Chaowasku.

Recent molecular phylogenetic studies (Richardson *et al.* 2004, Chatrou *et al.* 2012) have defined four major clades (Fig. 3) within the Annonaceae, now named as the sub-families Anaxagoreoideae, Ambavioideae, Annonoideae (formerly known as the ‘long-branch clade’), and Malmeoideae (formerly known as the ‘short-branch clade’). The latter two subfamilies comprise more than 95% of the species diversity of the family. As indicated by the informal names, the average branch lengths of the annonoid and malmeoid clades differ considerably (Richardson *et al.* 2004, Couvreur *et al.* 2011), and substantial differences in molecular evolutionary rates between the two clades have been inferred (Pirie & Doyle 2012).

Chatrou *et al.* (2012) further divided the major clades in the Annonoideae and Malmeoideae into 14 tribes. Tribe Miliuseae consisted traditionally of only six genera: *Alphonsea* Hook.f. & Thomson, *Mezzettia* Becc. (tentatively included), *Milium* Lesch. ex A.DC., *Orophea* Blume, *Phoicanthus* Alston, and *Platymitra* Boerl. (Keßler 1993), which



**FIGURE 3.** Phylogeny of Annonaceae, showing four subfamilies and position of Miliuseae. Adapted from Richardson *et al.* (2004).

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 of ca. 25 genera (= miliusoid clade: e.g. Mols *et al.* 2004a, 2004b). Chatrou *et al.* (2012) recircumscribed the Miliuseae to accommodate all genera of this clade, making it the largest tribe in the subfamily Malmeoideae. The systematics of the recircumscribed Miliuseae, which comprise a substantial part of the species diversity of the Annonaceae (ca. 510 spp.: Chatrou *et al.* 2012), is the main focus of this thesis.

Most genera of Miliuseae are restricted to Asia (including New Guinea, Australia, and the western Pacific islands), but four genera occur in the Neotropics and some African and Malagasy species are present in the genus *Hubera* Chaowasku (Chaowasku *et al.* 2012a). Continental Southeast Asia and Malesia west of Wallace’s Line form the centre of generic diversity of the tribe. For example, based on personal observations (see also Chalermglin 2001), nearly all genera of Miliuseae occur in Thailand, the only exceptions being the four genera endemic to the Neotropics plus *Phoenicanthus* (endemic to Sri Lanka: Huber 1985), *Stelechocarpus* Hook.f. & Thomson (lower Peninsular Malaysia, Sumatra, northwestern Borneo, Java, and Bali: Chaowasku *et al.* 2013b), *Oncodostigma* Diels (Sumatra and New Guinea; note that the interpretation of the type of *Oncodostigma leptoneurum* Diels, which is the type species of the genus, is still problematic, see Chatrou *et al.* 2012 and Thomas *et al.* 2012), and *Wangia* X.Guo & R.M.K.Saunders, a recently described genus endemic to Yunnan, China (Guo *et al.* 2014).

The fact that Thailand consists of different phytogeographical regions (Van Welzen *et al.* 2011), correlating with distinct differences in climates, geology, and altitudes may explain the high diversity of Miliuseae genera in Thailand. For example, certain genera (*Neo-uvaria* Airy Shaw, *Phaeanthus* Hook.f. & Thomson, *Popowia* Endl., and *Winitia* Chaowasku) are restricted to evergreen forests in the southern part of Thailand (Chalermglin 2001; Chaowasku *et al.* 2011a; Chaowasku *et al.* 2013b), and one undescribed genus (see Chaowasku *et al.* 2012a, Chaowasku *et al.* 2013b for its phylogenetic position) is endemic to mountainous deciduous forests with rugged limestone terrain in upper northern Thailand (pers. obs.).

### *Systematics of Annonaceae, with emphasis on subfamily Malmeoideae and tribe Miliuseae: progress and challenges*

Although, as a family, Annonaceae are homogeneous and readily identifiable, generic delimitations within the family based solely on morphology have often been problematic. Many taxa, including most Asian genera, have never received an alpha-taxonomic revision and for some groups clear diagnostic features have not been identified. A prominent example is the notoriously difficult genus *Polyalthia* Blume which has been shown to be highly polyphyletic, necessitating extensive generic realignments and descriptions of several generic segregates (Mols *et al.* 2004a, 2004b, 2008, Saunders *et al.* 2011, Xue *et al.* 2011, 2012, Chaowasku *et al.* 2012a). In other genera, apparently clear diagnostic features are present, but highly specialized traits of a single or a few species regarded as distinct genera nested within larger genera resulted in paraphyly of the latter

(e.g. Su *et al.* 2005, 2010, Zhou *et al.* 2009, 2010, Thomas *et al.* 2012, Xue *et al.* 2012). Finally, high degrees of morphological homoplasy of diagnostic traits within the family and its major clades have sometimes obscured relationships (Saunders 2010, Wang *et al.* 2012, Doyle & Le Thomas 2012), which also explain incongruence between phylogenies based on morphological data (Doyle & Le Thomas 1996) and those based on DNA sequence data (Chatrou *et al.* 2012). Consequently, DNA sequence data have been crucial for phylogenetic reconstructions and as the basis for recircumscriptions and characterizations of genera and higher-level taxa in the family.

In terms of phylogenetic reconstruction, the subfamily Malmeoideae and its largest tribe, Miliuseae, in particular, represent arguably the most recalcitrant major clades within the Annonaceae. Despite the use of up to eight plastid markers, parts of the backbone phylogenies of both Malmeoideae and Miliuseae are still not resolved (e.g. Saunders *et al.* 2011, Chatrou *et al.* 2012). Intertribal relationships of Miliuseae, moreover, are still somewhat obscure. The monogeneric tribe Monocarpieae has consistently been recovered as sister of Miliuseae (e.g. Chatrou *et al.* 2012), but a close relationship of Monocarpieae with another monogeneric tribe (Dendrokingstonieae), hypothesized based on macromorphology and palynological data (Chaowasku *et al.* 2012b), has not been tested in a molecular phylogenetic framework.

Molecular phylogenetic data have clarified several generic circumscriptions in Miliuseae (e.g. Mols *et al.* 2004a, Su *et al.* 2010, Xue *et al.* 2011, 2012, Chaowasku *et al.* 2012a, Thomas *et al.* 2012, Chaowasku *et al.* 2013b), but many genera of the tribe (such as *Hubera*, *Desmopsis* Saff., *Marsypopetalum* Scheff., *Miliusa*, *Monoon* Miq., *Neo-uvaria*, *Polyalthia*, *Popowia*, *Sapranthus* Seem., and *Tridimeris* Baill.) have not been taxonomically revised or studied in greater detail. In addition, the genus *Dendrokingstonia* Rauschert, which is the only member of Dendrokingstonieae, has never been taxonomically revised since its establishment in 1872 (Hooker & Thomson 1872). In order to assess their diversity and provide morphological data crucial for the understanding of character evolution in the tribe Miliuseae, detailed taxonomic studies of these genera are needed. The poor alpha-taxonomic knowledge, the obscure relationships between Miliuseae and related tribes, and particularly the poorly understood generic relationships within Miliuseae have been a major impediment to broad-scale evolutionary studies in the Annonaceae, clearly necessitating further molecular phylogenetic work.

Apart from DNA sequence data, palynological data may provide additional important insights into the inter- and infratribal relationships of Miliuseae. Pollen characters have played a major role in efforts to clarify generic circumscriptions and to infer intergeneric and higher relationships in Annonaceae (Walker 1971a, 1971b, 1972, Le Thomas 1980, 1981, Doyle & Le Thomas, 1994, 1995, 1997, Doyle *et al.* 2000, Mols *et al.* 2004a, Doyle & Le Thomas 2012). Annonaceae pollen is morphologically diverse, especially in the ornamentation, aperture type, and exine/intine stratification (Doyle & Le Thomas 2012). Aperture type and exine/intine stratification of *Dendrokingstonia*, *Monocarpia* Miq., and most genera of Miliuseae were still poorly known at the beginning of this study, however, since only a few genera of Miliuseae had been studied using scanning and transmission electron microscopy (SEM, TEM; e.g. Waha & Hesse 1988, Waha & Morawetz 1988, Su & Saunders 2003), and these techniques have never been applied to the pollen of *Dendrokingstonia* (SEM and

TEM) and *Monocarpia* (TEM). In Miliuseae, TEM is particularly important as the ‘germination zones’ or ‘apertural areas’ are best characterized by the differentiation of the intine sublayers, which can only be observed using TEM (Waha & Hesse 1988, Waha & Morawetz 1988). In order to determine the palynological diversity and its systematic significance, as well as provide additional data for evolutionary inferences, pollen morphological investigations (using SEM/TEM) of *Dendrokingstonia*, *Monocarpia*, and genera of the tribe Miliuseae should be undertaken.

## Aims and structure of the thesis

The research for this thesis addresses the need for comprehensive studies of the phylogenetic relationships and character evolution, clarification of generic circumscriptions, as well as alpha-taxonomic baseline work and detailed studies of the palynological diversity of Miliuseae and putatively closely related tribes. It is divided into the following parts:

**Chapter 2:** This empirical research chapter presents molecular phylogenetic reconstructions of the tribe Miliuseae and related tribes to determine intertribal relationships, clarify intergeneric relationships within Miliuseae, and perform ancestral character-state reconstructions to gain insights into the evolution of these traits.

Chapters 3 to 5 contribute to the clarification of generic delimitations within Miliuseae.

**Chapter 3:** A segregate of the previously highly polyphyletic genus *Polyalthia* is described as a new genus, *Hubera* (= the *Polyalthia cerasoides* group, the sister clade of *Miliusa*), through a combined study of macromorphology, pollen morphology [light microscopy (LM)/SEM/TEM], and molecular phylogenetics.

**Chapter 4:** The generic circumscriptions of *Stelechocarpus* and *Sageraea* Dalzell are assessed using combined evidence from molecular phylogenetics, macromorphology, and pollen morphology (SEM/TEM). Erection of a new genus, *Winitia*, is proposed.

**Chapter 5:** A proposal to conserve the genus name *Meiogyne* Miq. against *Fitzalania* F.Muell. is presented; a consequence of the results of molecular phylogenetic analyses showing the genus *Meiogyne* to be paraphyletic.

Chapters 6 to 9 provide alpha-taxonomic and/or detailed palynological studies of *Dendrokingstonia*, *Monocarpia* and several genera in the tribe Miliuseae.

**Chapter 6:** The poorly known Southeast Asian genus *Dendrokingstonia* is taxonomically revised. In addition, its pollen and that of the supposedly closely related genus *Monocarpia* are studied in detail using SEM/TEM.

**Chapter 7:** Seven new species of the genus *Miliusa* from Thailand are described. Additionally, a complete nomenclature (including lectotypifications, basionyms, and synonyms) of the previously known species and a key to all species of *Miliusa* in Thailand are provided.

**Chapter 8:** The results of comparative palynological studies (LM/SEM/TEM) of *Alphonsea*, *Mezzettia*, *Miliusa*, *Orophea*, *Platymitra* (all included in the tribe Miliuseae *sensu* Keßler 1993), and the *Polyalthia cerasoides* group (= *Hubera*,

the sister clade of *Milium*) are presented.

**Chapter 9:** The poorly known Asian genus *Neo-uvaria* is taxonomically studied. The results of comparative palynological studies (LM/SEM/TEM) of *Neo-uvaria* and its sister group, the genus *Encosanthum* Becc. (now synonymized with *Monoon*), are presented.



## 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 ruminant 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), *Milium* Lesch. ex A.DC., *Orophea* Blume, *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.* 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 & 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 *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 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 1000<sup>th</sup> 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)  $\geq 0.96$ , 0.91–0.95, and  $\leq 0.90$  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 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 in size to inner petals]. (1) =  $\pm$  sepaloid [outer petals approaching sepals in size ( $\leq 2$  times longer and wider than sepals) and considerably smaller than inner petals ( $\geq 2$  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)  $\geq 2$ . 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) Infractectum type: (0) = columellate to coarsely granular. (1) = finely and densely granular. (2) = exine atectate, i.e. exine not to very weakly differentiated into tectum, infractectum, 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 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.* 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 100<sup>th</sup> 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).

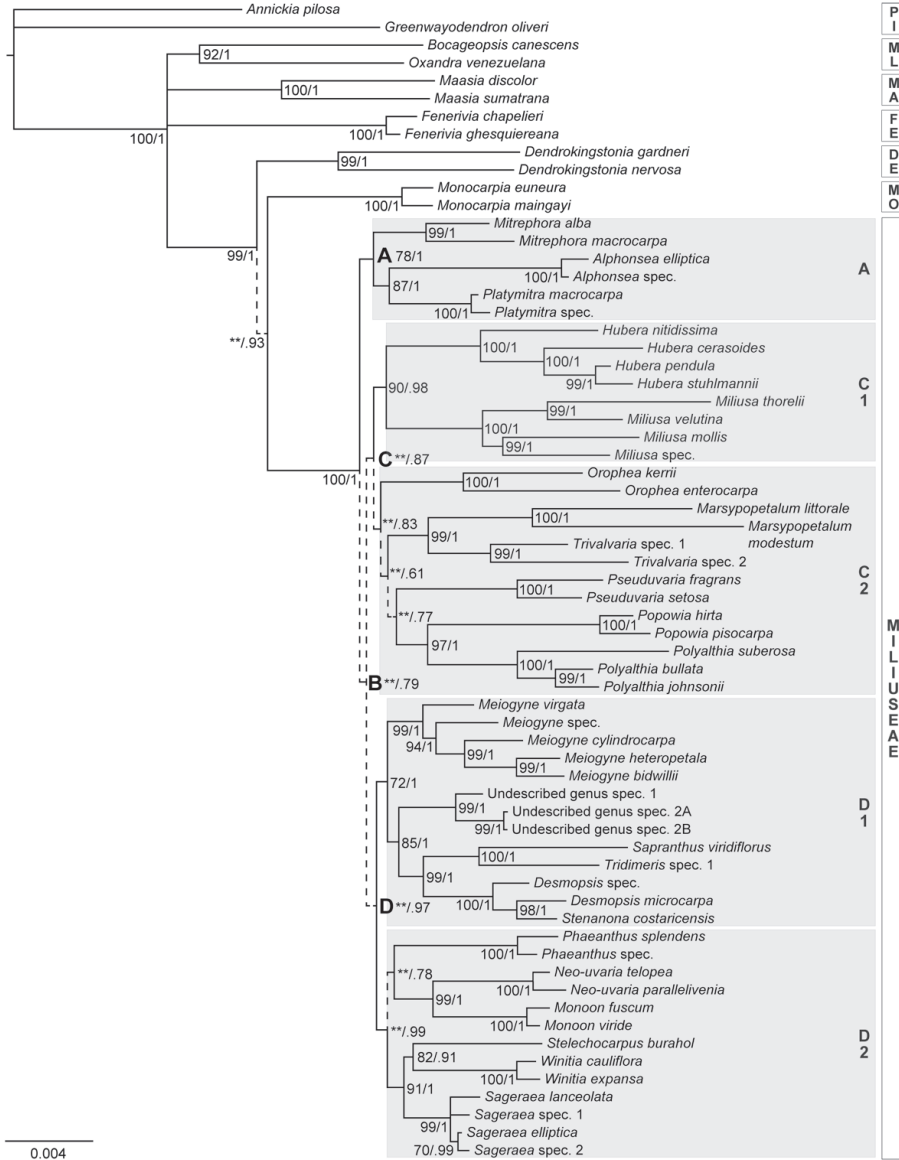
For parsimony ancestral character-state reconstructions in Mesquite, character state changes were treated as unordered. The “trace over trees” option was selected and reconstructions across the input trees were summarized at each node of the pruned 50% majority-rule consensus tree using the “Uniquely 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, Piptostigmatidae (= outgroups); ML, Malmeeae; MA, Maaseiae; FE, Fenerivieae; DE, Dendrokingstonieae; MO, Monocarpieae.

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

DNA region	No. of included characters	No. of accessions (of all 65 accessions included) lacking sequence data (%)	No. of variable characters (%)	No. of parsimony-informative characters (%)
- <i>rbcL</i> exon	1380	24 (36.9)	124 (9.0)	65 (4.7)
- <i>trnL</i> intron + <i>trnL-F</i> spacer	926	0	201 (21.7)	101 (10.9)
- <i>matK</i> exon	828	0	184 (22.2)	84 (10.1)
- <i>ndhF</i> exon	2033	0	436 (21.4)	247 (12.2)
- <i>psbA-trnH</i> spacer	431	0	137 (31.8)	94 (21.8)
- <i>ycf1</i> exon	1429	8 (12.3)	358 (25.1)	177 (12.4)
- Combined data	7027	NA	1440 (20.5)	768 (10.9)

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  $\geq$  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 Fig. 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*, *Neo-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)*

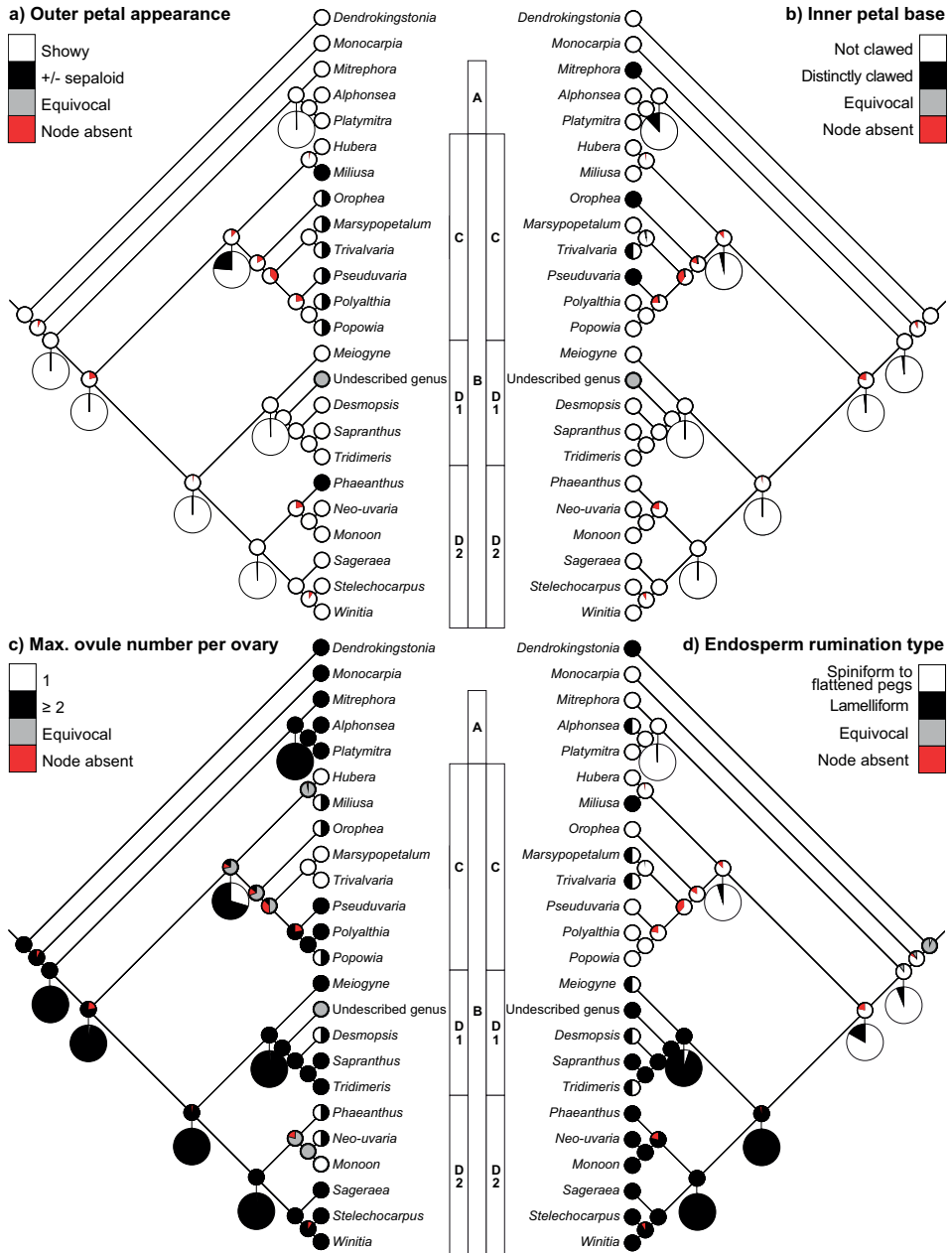
The log-likelihood, RJ hyperprior parameter, acceptance rates and posterior probabilities of each character state at nodes of interest (nodes Miliuseae, A, B, C, D, D1, and D2) all possessed ESS values (after burn-in was discarded) that were greater than 1200, indicating adequate posterior sampling. 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)

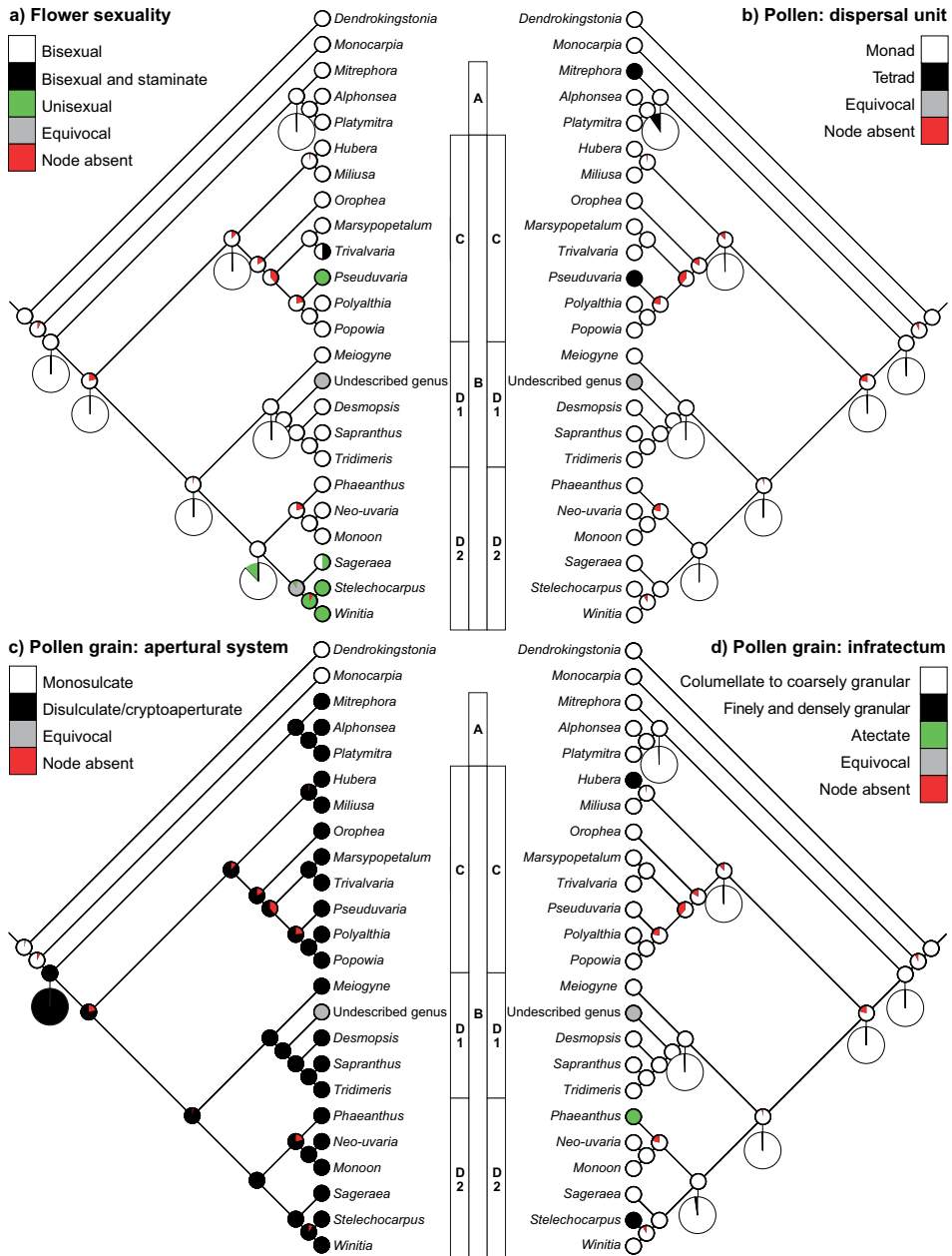
The derived state of outer petals being similar to the sepals in size ( $\leq 2$  times longer and wider than sepals) and considerably smaller than the inner petals ( $\geq 2$  times shorter and narrower than inner petals) (Fig. 4J) is inferred to have evolved multiple times from the ancestral state 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).



**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.



**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  $\geq 2$  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 *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: 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/disulcate 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 Piptostigmatae 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 ruminant 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 form (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  $\pm$  equal and  $\pm$  similar in shape. (J) outer whorl  $\pm$  similar in size to the sepals but the inner whorl much

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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. *Guamia* 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

←  
larger. (K), (L) inner petals distinctly clawed towards 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].

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 *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 multi-ovuled 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 & 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

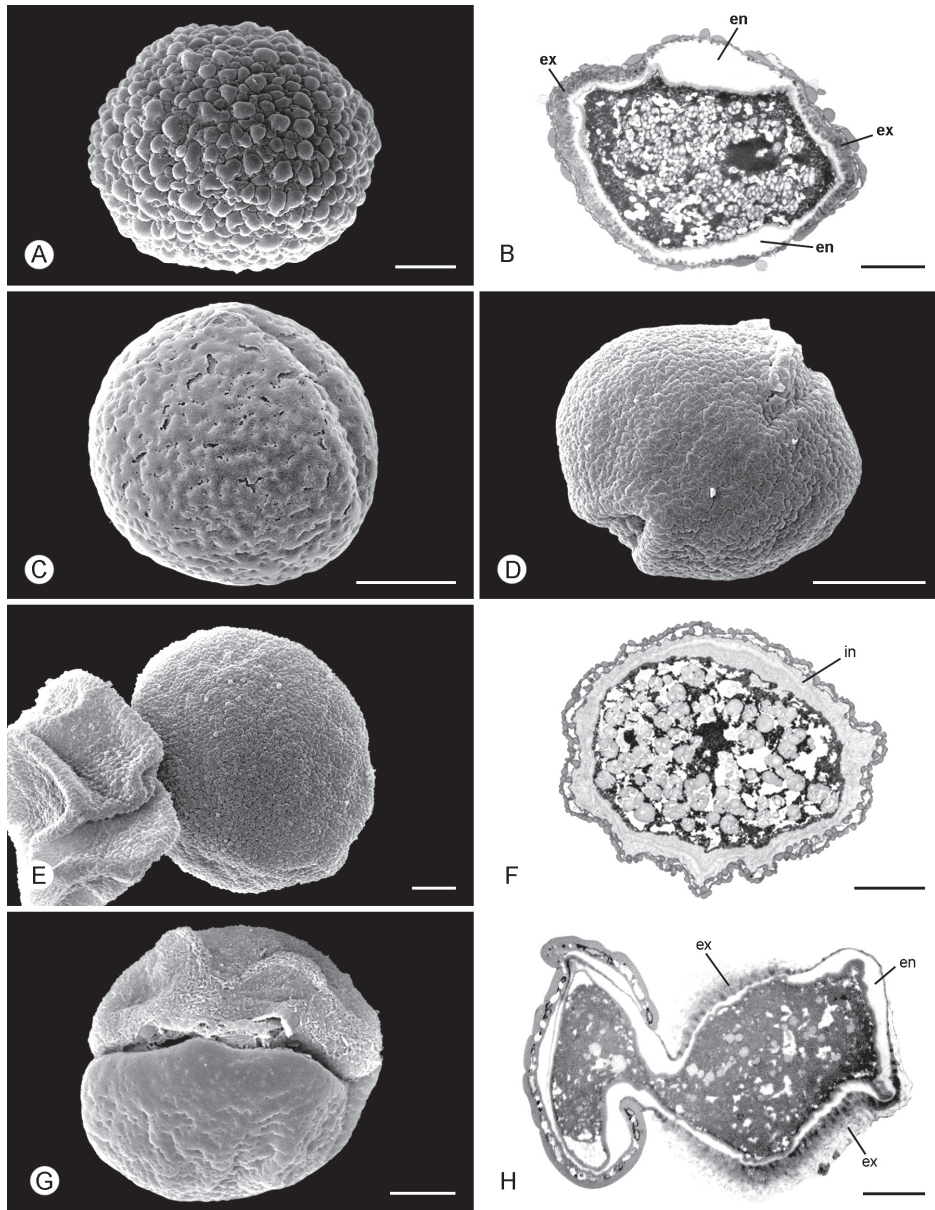
(Van de Pijl 1969). Single-seeded monocarps of certain species of *Neo-uvaria*, e.g. *N. foetida* (Maingay ex Hook.f. & Thomson) Airy Shaw and *N. telopea*, however, are unlikely to be bird-dispersed, as they are relatively large (6.5–7.0 × 5.2–5.5 cm) and have brown, hairy and fetid pericarps (Chaowasku *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 *Mezzettia*, i.e. only differentiated into basal and upper layers, Chaowasku *et al.* 2008).

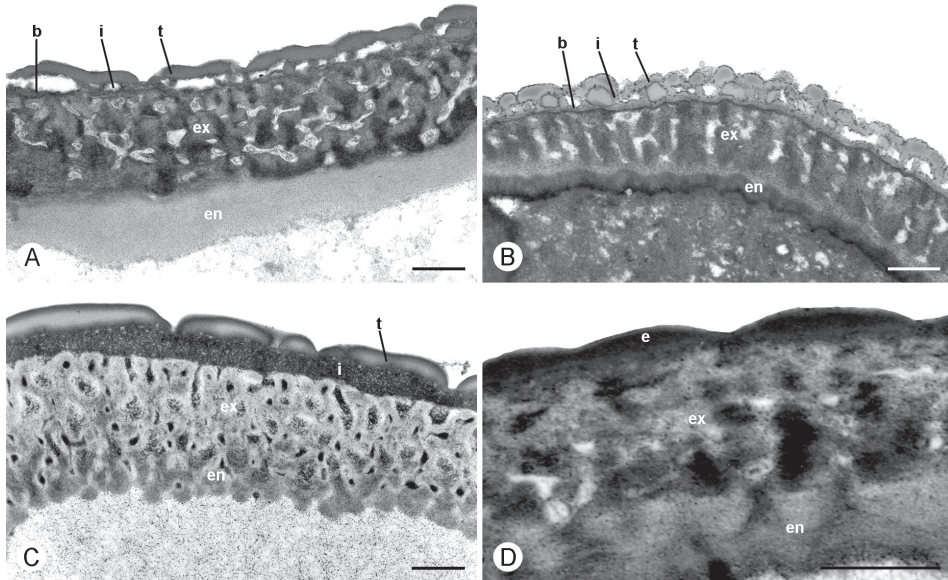
The distribution of major pollen apertural types in the Malmeoideae corroborates the molecular phylogenetic results. The basal grade of the Malmeoideae, including the monogeneric tribes Dendrokingstonieae and Monocarpieae, possesses monosulcate pollen (Fig. 5G, H; Le Thomas 1980, Waha 1985, Waha & Hesse 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). Disulcate pollen may just represent partially collapsed cryptoaperturate pollen; further examination is indispensable to understand the relation of cryptoaperturate and disulcate pollen. Doyle & 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 be also considered “disulcate”. 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 disulcate should be restricted to pollen grains with two sunken exine areas.

(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 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/disulcate apertural system). (G), (H) Dendrokingstonieae (monosulcate apertural system). (A) *Miliusa macropoda* (*Kos-termans 13973*): 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). (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*

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**FIGURE 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.

The pollen grains of *Mitrephora* (pers. obs. T. Chaowasku); *Neo-uvaria* (Chaowasku et al. 2011a); *Pseuduvaria* (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, disulcate 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 disulcate 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.

*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 & Sidsunthorn 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.

*Feneriua 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.

*Miogyne 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\*; KJ418379\*; *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; 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-Thiemchan 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; 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; *Oka-da 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. **Undescribed genus sp. 2A**; JX544752; JX544781; JX544749; JX544770; JX544785; JX544757; *Chaowasku 108*; Thailand; L. **Undescribed sp. 2B**; ---; KC857606; KC857607; KC857608; KC857609; KC857610; *Nakorn-Thiemchan NTC 16*; Thailand; L.

## 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. **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* (Benth.) D.C.Thomas, Chaowasku & R.M.K.Saunders; *Randall 624*; Australia; L. **M. cylindrocarpa** (Burck) Heusden; *Marler s.n.*; Tinian Island; L. **M. heteropetala** (F.Muell.) D.C.Thomas, Chaowasku & R.M.K.Saunders; *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 BR 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. 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.

*Milium horsfieldii* (Benn.) Baill. ex Pierre; TEM; *How 71794*; China; A. *Milium 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; *Phengkklai 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
<i>Dendrokingstonia</i>	0 <sup>b</sup>	0 <sup>a</sup>	1 <sup>a</sup>	1 <sup>a,b</sup>	0 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>b</sup>
<i>Monocarpia</i>	0 <sup>c</sup>	0 <sup>c</sup>	1 <sup>c</sup>	0 <sup>b</sup>	0 <sup>c</sup>	1 <sup>a,c</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
<i>Mitrephora</i>	0 <sup>d</sup>	1 <sup>d</sup>	1 <sup>d</sup>	0 <sup>b</sup>	0 <sup>d</sup>	1 <sup>d</sup>	1 <sup>d,e</sup>	1 <sup>e</sup>	0 <sup>e</sup>
<i>Alphonsea</i>	0 <sup>f</sup>	0 <sup>f</sup>	1 <sup>f</sup>	01 <sup>b</sup>	0 <sup>f</sup>	1 <sup>f</sup>	0 <sup>g</sup>	1 <sup>g</sup>	0 <sup>g</sup>
<i>Platymitra</i>	0 <sup>h</sup>	0 <sup>h</sup>	1 <sup>h</sup>	0 <sup>b</sup>	0 <sup>h</sup>	0 <sup>h</sup>	0 <sup>g</sup>	1 <sup>g</sup>	0 <sup>g</sup>
<i>Hubera</i>	0 <sup>i</sup>	0 <sup>i</sup>	0 <sup>i</sup>	0 <sup>i</sup>	0 <sup>i</sup>	0 <sup>i</sup>	0 <sup>g,i</sup>	1 <sup>g,i</sup>	1 <sup>g,i</sup>
<i>Miliusa</i>	1 <sup>j,k</sup>	0 <sup>j,k</sup>	01 <sup>j,k</sup>	1 <sup>b</sup>	0 <sup>h,i,j,k</sup>	01 <sup>j</sup>	0 <sup>g</sup>	1 <sup>g</sup>	0 <sup>g</sup>
<i>Orophea</i>	01 <sup>l</sup>	1 <sup>l</sup>	01 <sup>l</sup>	0 <sup>b</sup>	0 <sup>l</sup>	01 <sup>l,m</sup>	0 <sup>g</sup>	1 <sup>g</sup>	0 <sup>g</sup>
<i>Marsypopetalum</i>	01 <sup>e</sup>	0 <sup>e</sup>	0 <sup>n</sup>	01 <sup>b,e</sup>	0 <sup>n</sup>	01 <sup>e,n</sup>	0 <sup>e,n</sup>	1 <sup>e</sup>	0 <sup>e</sup>
<i>Trivalvaria</i>	01 <sup>o</sup>	01 <sup>o</sup>	0 <sup>o</sup>	01 <sup>b,e</sup>	02 <sup>e,o</sup>	1 <sup>o</sup>	0 <sup>e,n</sup>	1 <sup>e</sup>	0 <sup>e</sup>
<i>Pseuduvaria</i>	01 <sup>p,q</sup>	1 <sup>p,q</sup>	1 <sup>p,q</sup>	0 <sup>b</sup>	1 <sup>p,q</sup>	0 <sup>p,q,kk</sup>	1 <sup>g,kk</sup>	1 <sup>r</sup>	0 <sup>r</sup>
<i>Polyalthia</i>	01 <sup>e,i</sup>	0 <sup>e,m</sup>	1 <sup>m,s</sup>	0 <sup>i</sup>	0 <sup>e</sup>	01 <sup>m,s</sup>	0 <sup>e,i</sup>	1 <sup>e,t</sup>	0 <sup>e,i</sup>
<i>Popowia</i>	01 <sup>e</sup>	0 <sup>e</sup>	01 <sup>m,u</sup>	0 <sup>b</sup>	0 <sup>u</sup>	1 <sup>e,m</sup>	0 <sup>e</sup>	1 <sup>e</sup>	0 <sup>e</sup>
<i>Meiogyne</i>	0 <sup>v,w,x</sup>	0 <sup>v,w</sup>	1 <sup>v,w</sup>	01 <sup>b</sup>	0 <sup>v,w</sup>	0 <sup>v,w</sup>	0 <sup>t</sup>	1 <sup>t</sup>	0 <sup>t</sup>
Undescribed genus	?	?	?	1 <sup>e</sup>	?	?	?	?	?
<i>Desmopsis</i> (incl. <i>Ste-nanona</i> )	0 <sup>v,z</sup>	0 <sup>v,z</sup>	01 <sup>v,z</sup>	01 <sup>b</sup>	0 <sup>v,z</sup>	1 <sup>v,z</sup>	0 <sup>e</sup>	1 <sup>e</sup>	0 <sup>e</sup>
<i>Sopranthus</i>	0 <sup>e,aa</sup>	0 <sup>e,aa</sup>	1 <sup>m,aa</sup>	1 <sup>b</sup>	0 <sup>u</sup>	1 <sup>e,aa</sup>	0 <sup>e,bb</sup>	1 <sup>e,bb</sup>	0 <sup>e</sup>
<i>Tridimeris</i>	0 <sup>e</sup>	0 <sup>e</sup>	1 <sup>e</sup>	01 <sup>b,e</sup>	0 <sup>u</sup>	0 <sup>e</sup>	0 <sup>e</sup>	1 <sup>e</sup>	0 <sup>e</sup>
<i>Phaeanthus</i>	1 <sup>cc</sup>	0 <sup>cc</sup>	01 <sup>cc</sup>	1 <sup>b</sup>	0 <sup>cc</sup>	1 <sup>cc</sup>	0 <sup>e</sup>	1 <sup>e</sup>	2 <sup>e</sup>
<i>Neo-uvaria</i>	0 <sup>dd</sup>	0 <sup>dd</sup>	01 <sup>dd</sup>	1 <sup>b,dd</sup>	0 <sup>dd</sup>	0 <sup>dd</sup>	0 <sup>dd</sup>	1 <sup>dd</sup>	0 <sup>dd</sup>
<i>Monoon</i>	0 <sup>nn</sup>	0 <sup>nn</sup>	0 <sup>s</sup>	1 <sup>b,s</sup>	0 <sup>dd</sup>	0 <sup>s</sup>	0 <sup>dd</sup>	1 <sup>e,dd</sup>	0 <sup>e,dd</sup>
<i>Sageraea</i>	0 <sup>ee</sup>	0 <sup>e,ee</sup>	1 <sup>ee</sup>	1 <sup>b</sup>	01 <sup>ee</sup>	0 <sup>e,ee</sup>	0 <sup>f</sup>	1 <sup>e</sup>	0 <sup>f</sup>



**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: 19859/0/0)	(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

## APPENDIX 6 (continued).

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)
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



# Chapter 3

## Characterization of *Hubera* (Annonaceae), a new genus segregated from *Polyalthia* and allied to *Miliusa*

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### Abstract

On the basis of molecular phylogenetics, pollen morphology and macromorphology, a new genus of the tribe Miliuseae, *Hubera*, segregated from *Polyalthia* and allied to *Miliusa*, is established and described. It is characterized by the combination of reticulate tertiary venation of the leaves, axillary inflorescences, a single ovule per ovary and therefore single-seeded monocarps, seeds with a flat to slightly raised raphe, spiniform(-flattened peg) ruminations of the endosperm, and pollen with a finely and densely granular infratectum. Twenty-seven species are accordingly transferred to this new genus.

**Key words:** Malmeoideae, molecular systematics, Old World floristics, Paleotropics, palynology

### Introduction

The large magnoliid angiosperm family Annonaceae is prominent in lowland forests across the tropics (Gentry 1988, Slik *et al.* 2003). Circumscription of genera within the family was initially founded on characters emphasizing the diversity of floral morphologies represented in the family, which recapitulates many trends found with angiosperm evolution at large (Johnson & Murray 1995, Endress & Doyle 2009, Endress 2011): apocarp/syncarp, polypetal/sympetal, bisexual/unisexual flowers, reductions in stamen and carpel number, and changes in ovule number.

At the same time that molecular and other analyses have shown the homoplasious evolution of similar perianth specializations (e.g. Mols *et al.* 2004a, Saunders 2010), it is nevertheless true that such specializations still have value as morphological markers of particular clades within the family, usually at the generic level. However, reliance on perianth modifications for classification in Annonaceae has also resulted in genera defined by lack of such specializations, i.e. they are defined on the basis of symplesiomorphic perianth features (Johnson & Murray 1999). This situation is best exemplified in the family by the Paleotropical genus *Polyalthia* Blume (1830: 68), which has been defined by similarity of petals in its two perianth whorls and little else; it is thus not surprising that several analyses (e.g. Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004, Xue *et al.* 2011) have shown the genus to be polyphyletic. Even before the advent of molecular analyses,

species had been removed from the genus on morphological grounds. For example, the new genus *Greenwayodendron* Verdcourt (1969: 89) was established to accommodate two African species now known to be closer to a suite of African genera (Couvreur *et al.* 2009), Johnson (1989) removed two species to the genus *Disepalum* Hooker (1860: 156), following the pollen morphology studied by Walker (1971b) and *Maasia* Mols, Kessler & Rogstad in Mols *et al.* (2008: 493) was recognized following Rogstad's (1989) systematic studies, although monophyly of the last genus had been demonstrated in phylogenetic analyses by Mols *et al.* (2004b). Phylogenetic analyses have led to removal of additional species to *Marsypopetalum* Scheffer (1870: 342; Xue *et al.* 2011), *Fenerivia* Diels (1925: 355; Saunders *et al.* 2011), and *Monoon* Miquel (1865: 15; Xue *et al.* 2012). On the other hand, *Haplostichanthus* Mueller (1891: 180) has been found to be congeneric with *Polyalthia sensu stricto* (i.e. a clade consisting of the type species of *Polyalthia*; Xue *et al.* 2012).

Molecular phylogenetics has played a crucial role in elucidating evolutionary relationships of Annonaceae and provided a new foundation for classification of genera within the family (e.g. Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004, Pirie *et al.* 2006, Couvreur *et al.* 2008, 2009, Zhou *et al.* 2009, 2010, Chatrou *et al.* 2012). Following Chatrou *et al.* (2012), Annonaceae now includes four subfamilies: Anaxagoreoideae, Ambavioideae, Annonoideae (also known as long-branch clade, LBC) and Malmeoideae (also known as short-branch clade, SBC). Previous analyses (e.g. Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004, Xue *et al.* 2011) have suggested that one group of species within subfamily Malmeoideae, tribe Miliuseae *sensu* Chatrou *et al.* (2012), currently residing in *Polyalthia*, represents the sister group to the Asian genus *Miliusa* Leschenault ex De Candolle (1832: 213). This group of *Polyalthia* species, characterized by reticulate tertiary veins of the leaves, axillary inflorescences, uniovulate carpels, seeds with a flat to slightly raised raphe, and spiniform(-flattened peg) endosperm ruminations, is widespread in the Paleotropics, including East Africa and Madagascar, southern and southeastern Asia, Malesia, and the southwestern Pacific. The purpose of the present study was to investigate additional *Polyalthia* species possibly belonging to this sister clade of *Miliusa*. We proposed to examine these species with an expanded molecular dataset compared to previous analyses (e.g. Saunders *et al.* 2011) and then evaluate the systematic value of pollen and macromorphological characters with this result to determine whether or not such a grouping might be characterized using these traits. In Chaowasku *et al.* (2008), three species of this *Polyalthia* clade sister to *Miliusa* were described as having pollen with a finely and densely granular infratectum. It is worth investigating if all other species in this clade also possess the same type of pollen infratectum and thus if this character could be a potential synapomorphy of this clade. Additionally, in order to distinguish this clade from the other two unrelated but morphologically similar clades, *Monoon* and *Polyalthia s.s.*, the macromorphology and pollen morphology of these two clades will be compared. As no obvious macromorphological similarities have been found between *Miliusa* and this group of *Polyalthia* species, a survey and comparison of vegetative, floral, fruit/seed, and pollen characters will be made as well.

## Materials and methods

### Materials

Voucher specimens for macromorphological observations, pollen morphological and molecular phylogenetic studies, including GenBank accession numbers, are indicated in Appendix 1.

### Methods

#### Molecular phylogenetics

All 56 accessions sampled (Appendix 1) belong to Malmeoideae; 54 accessions comprise the ingroup: Monocarpieae and Miliuseae. Besides accessions of (former) *Polyalthia* species, accessions of representatives of nearly all other genera in the Miliuseae were included; the genera *Oncodostigma* Diels (1912b: 143) and *Phoenicanthus* Alston in Trimen (1931: 6) were not included because available material was not suitable for DNA extraction. However, they are unlikely to be nested in a clade sister to *Miliusa* because they do not possess all morphological characters diagnostic for this clade, e.g. there are  $\geq 2$  ovules (1 in *Miliusa*'s sister clade) per ovary in *Oncodostigma* and *Phoenicanthus*. The outgroups are species of *Bocageopsis* Fries (1931: 143) and *Oxandra* Richard (1841: 45), both from Malmeae. Seven plastid markers (*rbcl* exon, *trnL* intron, *trnL-F* spacer, *matK* exon, *ndhF* exon, *psbA-trnH* spacer, *ycf1* exon) were amplified. In total, 7044 characters, including 10 separately coded indels were included in the analyses. Indel coding follows Simmons & Ochoterena (2000). For a 15-nucleotide stretch in the *psbA-trnH* marker, the reverse complement was present in roughly half the accessions sequenced, and we converted this into the reverse complement in line with Pirie *et al.* (2006).

DNA extractions were performed using a CTAB method (Doyle & Doyle 1987) modified following Bakker *et al.* (1998). A standard PCR protocol was used throughout, with the addition of 50  $\mu\text{g}$  of bovine serum albumin (BSA) in 50  $\mu\text{l}$  PCR reactions. The PCR program used comprises 35 cycles of 94 °C: 30 sec., 53 °C–65 °C: 1 min. (annealing temperatures depending on each primer pair), 72 °C: 2 min., with the initial denaturation for 4 min. at 94 °C and the final extension for 7 min. at 72 °C. The primer sequences of the seven plastid regions were taken from the literature or newly designed (see Table 1). All PCR products were cleaned by the MinElute<sup>®</sup> PCR Purification Kit (Qiagen), and then sequenced using the Dye ET terminator sequencing kit (Amersham Pharmacia Biotech), running on the ABI Prism 3700 (Greenomics, Wageningen).

Sequences were edited using the program Staden version 1.7.0 (<http://staden.sourceforge.net/>) and subsequently manually aligned. Some sequences were obtained from previous studies (Mols *et al.* 2004a, 2004b, Pirie *et al.* 2006, Su *et al.* 2008). Maximum parsimony analyses were performed in TNT version 1.1 (Goloboff *et al.* 2008). All characters were equally weighted and unordered. Multiple most parsimonious trees were generated by a heuristic search of the combined data, with 6000 replicates of random sequence additions, saving 10 trees per replicate, and using tree bisection and reconnection (TBR) branch swapping. Clade support was measured by

symmetric resampling (SR), which is not affected by a distortion (resulting in incorrectly estimated percentages) as 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 additions, saving one tree per replicate. Groups with SR of  $\geq 85\%$ , 70–84 %, and  $\leq 69\%$  were considered strongly, moderately, and weakly supported, respectively.

Bayesian analyses were performed in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003). Eight MCMC chains (two independent runs) were simultaneously run for  $2 \times 10^7$  generations. The data matrix was divided into seven partitions (*trnL* intron and *trnL-F* spacer were included in the same partition), including a set of binary indel coding. The most appropriate model of sequence evolution for each partition was selected by AIC scores, using FindModel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>). The default prior settings were used except for the ratepr (=variable) and brlenspr [=unconstrained:exp(100)]. The latter prior setting was used to prevent 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 0.15. Trees and all parameter values were sampled every 1000<sup>th</sup> generation. Convergence of the runs was checked by the values for effective sample sizes (ESS) using Tracer version 1.5 (Rambaut & Drummond 2009). The posterior probability tree was generated from the two runs combined, with 10% of the first trees removed as the burn-in. Groups with posterior probabilities (PP) of  $\geq 0.96$ , 0.91–0.95, and  $\leq 0.90$  were considered strongly, moderately, and weakly supported, respectively.

### Pollen morphology

Pollen data of 18 *Polyalthia* species expected or known to be members of a clade sister to *Miliusa* are available, including 11 out of the 16 species included in the phylogenetic analysis. Thirteen species were investigated with light microscopy (LM), scanning electron microscopy (SEM) and/or transmission electron microscopy (TEM) (Appendix 1, Table 2). Pollen data (SEM or TEM) for five other species (Table 2) were taken from the literature (Le Thomas 1980, 1988, Schatz & Le Thomas 1990). Pollen data (SEM and/or TEM) for *Monoon* and *Polyalthia s.s.* were available for comparisons (Appendix 1). The pollen material for SEM was not acetolysed, following Chaowasku *et al.* (2008) and Couvreur *et al.* (2009). The material for TEM was prepared following Van der Ham (1990). Subdivision of the exine into tectum, infratectum and basal layer (Le Thomas 1980) was used. Further pollen terminology follows Punt *et al.* (2007).

## Results

### *Molecular phylogenetics*

Due to poor quality of extracted DNA or unavailability of leaf material, we could not produce seven markers for all accessions (see Appendix 1). The maximum parsimony

**TABLE 1.** Primers used for DNA amplification in this study.

Marker	Primer	Sequence (5'→3')	Reference
<i>rbcL</i>	1F	ATGTCACCACAACAGAAAC	Olmstead <i>et al.</i> (1992)
	724R	TCGCATGTACCTGCAGTAGC	Fay <i>et al.</i> (1997)
	636F	GCGTTGGAGAGATCGTTTCT	Fay <i>et al.</i> (1997)
	1460R	TCCTTTTAGTAAAAGATTGGGCCGAG	Olmstead <i>et al.</i> (1992)
<i>trnLF</i>	C	CGAAATCGGTAGACGCTACG	Taberlet <i>et al.</i> (1991)
	D	GGGGATAGAGGGACTTGAAC	Taberlet <i>et al.</i> (1991)
	E	GGTTCAAGTCCCTCTATCCC	Taberlet <i>et al.</i> (1991)
	F	ATTGAACTGGTGACACGAG	Taberlet <i>et al.</i> (1991)
<i>matK</i>	390F	CGATCTATTCATTCAATATTTTC	Cuénoud <i>et al.</i> (2002)
	Mint-F	TCCTTTGGAAGTGTCTTGAGC	Pirie <i>et al.</i> (2005)
	Mint-R	GATCCTGTGCGTTGAGACC	Pirie <i>et al.</i> (2005)
	1326R	TCTAGCACACGAAAGTCGAAGT	Cuénoud <i>et al.</i> (2002)
<i>ndhF</i>	1F	ATGGAACAKACATATSAATATGC	Olmstead & Sweere (1994)
	54F	GCTCGTCGTATGTGGGCTTTTC	Present study
	660R	GTGCAGATTTAGCAACTGCACC	Present study
	481F	CGTTTGTAAACGAATCGGTAGGGG	Present study
	1089R	CCACCATAAGAACCATGTTCTG	Present study
	972F	GTCTCAATTGGGTTATATGATG	Olmstead & Sweere (1994)
	1087F	GTCAGAACATGGTTCTTATGGG	Present study
	1650R	CGAAGGGAATTCCTATGGACCC	Present study
	1550F	CTCTGACAATAAAGACACTTCC	Present study
	2110R	CCCCCTAYATATTGATACCTTCTCC	Olmstead & Sweere (1994)
<i>psbA-trnH</i>	F	CGAAGCTCCACTACAAATGG	Hamilton (1999)
	R	ACTGCCTTGATCCACTTGGC	Hamilton (1999)
<i>ycf1</i>	72F	GTGGACACTAGGAATATTGGATGC	Present study
	1674R	CCGCGGAATCAACAAGAAGATC	Present study
	914F	GGATGGGAATGAATGAAGAAATGC	Present study
	2323R	CCGTATCAATATGCTTGTCCTC	Present study

**TABLE 2.** Pollen morphological observations of species in clade A. -, data not available.

Taxon	Size (µm; LM)	Ornamentation (SEM)	Infratectum (TEM)	Germ. zone(s) (TEM)
<i>Polyalthia cerasoides</i>	42	Verrucate(-rugulate)	Finely, densely granular	1?
<i>Polyalthia decora</i> <sup>1</sup>	-	Verrucate-rugulate	-	-
<i>Polyalthia forbesii</i>	29	Fossulate-perforate	Finely, densely granular	2
<i>Polyalthia henrici</i>	31	Rugulate	Finely, densely granular	2
<i>Polyalthia jenkinsii</i>	33	(Weakly) coarsely rugulate to fossulate	Finely, densely granular	2
<i>Polyalthia keraudreniae</i> <sup>1</sup>	-	± Fossulate-perforate	-	-
<i>Polyalthia korinti</i>	33	(Verrucate-)rugulate	Finely, densely granular	?

TABLE 2 (continued).

Taxon	Size ( $\mu\text{m}$ ; LM)	Ornamentation (SEM)	Infratectum (TEM)	Germ. zone(s) (TEM)
<i>Polyalthia multistamina</i> <sup>1</sup>	-	Fossulate-perforate	-	-
<i>Polyalthia nitidissima</i>	-	Verrucate-rugulate, with two $\pm$ psilate areas opposite each other	Finely, densely granular	?
<i>Polyalthia pendula</i>	43	Verrucate	Finely, densely granular	2
<i>Polyalthia perrieri</i>	30	Verrucate(-rugulate)	Finely, densely granular	2
<i>Polyalthia sambiranensis</i> <sup>1</sup>	-	Verrucate-rugulate	-	-
<i>Polyalthia stuhlmannii</i> <sup>2</sup>	-	-	Finely, densely granular	1?
<i>Polyalthia tanganyigensis</i>	32	Verrucate(-rugulate)	Finely, densely granular	1?
<i>Hubera</i> sp. 2	43	Weakly coarsely rugulate to fossulate	Finely, densely granular	2
<i>Hubera</i> sp. 6	46	$\pm$ Fossulate-perforate	Finely, densely granular	?
<i>Hubera</i> sp. 7	43	$\pm$ Fossulate-perforate	Finely, densely granular	2
<i>Hubera</i> sp. 8	43	$\pm$ Fossulate-perforate	Finely, densely granular	1?

<sup>1</sup>(Schatz & Le Thomas 1990); <sup>2</sup>(Le Thomas 1980, 1988); Germ. = Germination

analysis of combined datasets resulted in > 30,000 most parsimonious trees of 1587 steps (results not shown). The consistency and retention indices were 0.77 and 0.76, respectively. For Bayesian analysis, the substitution model was GTR + G for all partitions except for *trnLF* (= *trnL* intron + *trnL-F* spacer) and *psbA-trnH*, which had the HKY + G model. Twenty thousand and two trees were sampled from  $2 \times 10^7$  generations of two independent runs. For burn-in, 2000 trees were discarded, resulting in 18002 trees left for calculation of the posterior probability tree (Fig. 1). All effective sample size (ESS) values after discarding the burn-in were larger than 1400, indicating convergence of the runs.

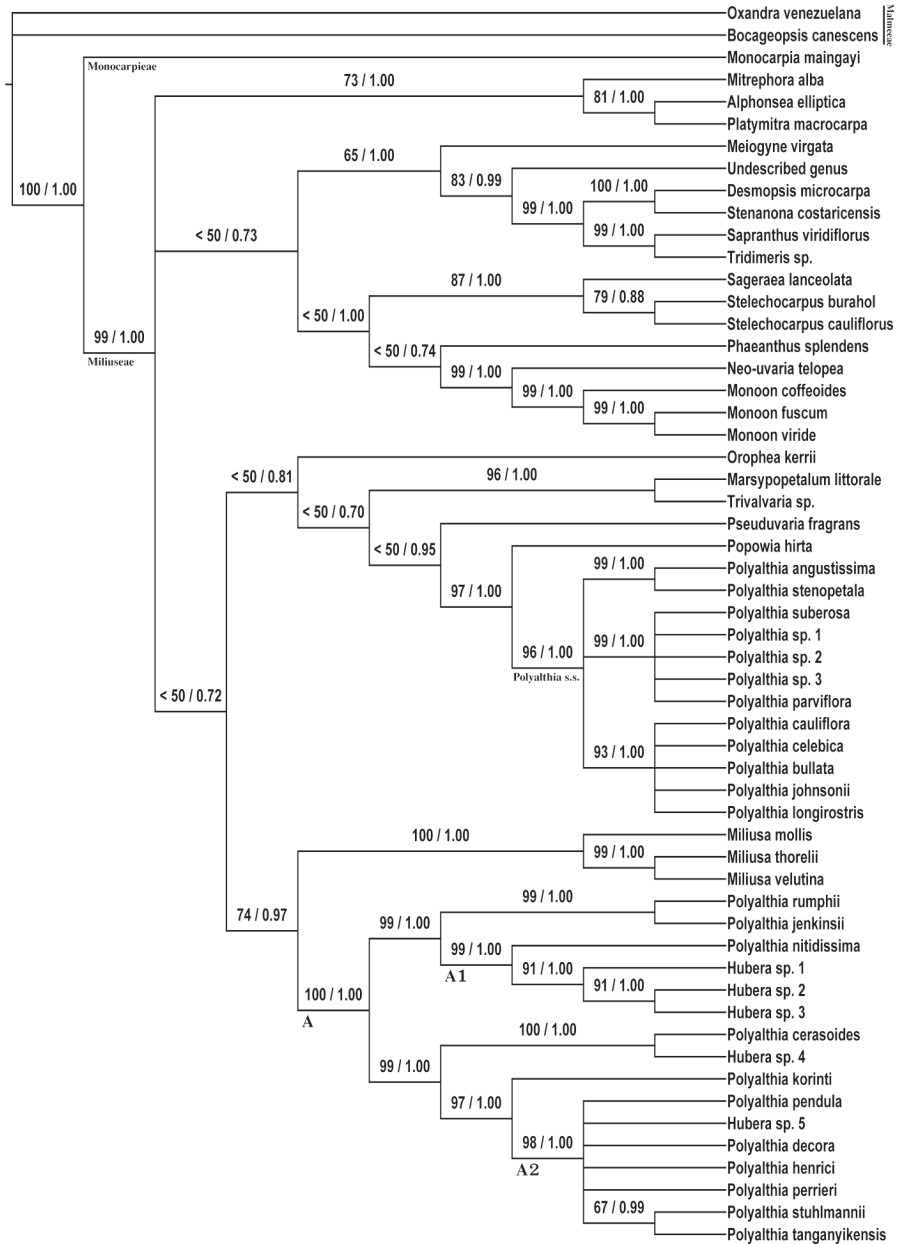
A clade comprising 11 species currently known as *Polyalthia* and five most likely undescribed species is maximally supported (clade A: SR 100%; PP 1.00; see Fig. 2A–C, G for representatives). It is sister to the genus *Miliusa* with moderate to strong support (SR 74%; PP 0.97).

### Pollen morphology (Table 2; Fig. 3A–O)

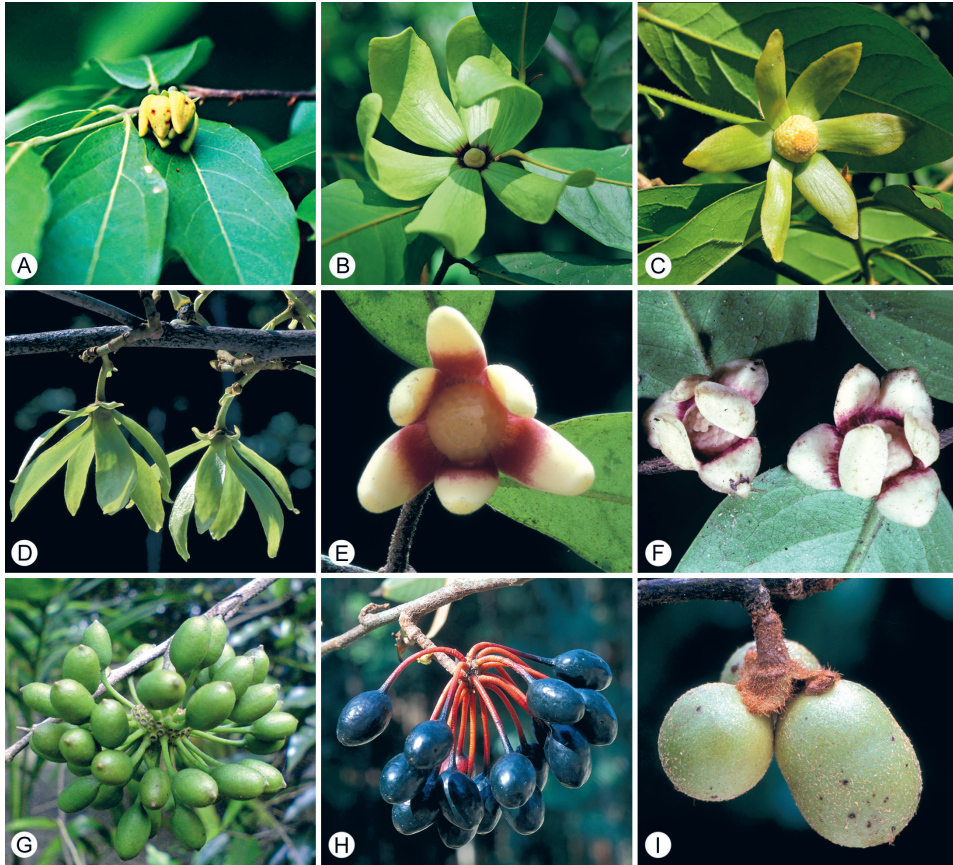
**LM:** Pollen grains apolar, (sub)spheroidal, inaperturate monads, longest axis 29–46  $\mu\text{m}$ .

**SEM (Fig. 3A, B, D, E, G, H):** Exine ornamentation verrucate to rugulate to fossulate (-perforate). Two opposite,  $\pm$  psilate exine areas (exine “plates” *sensu* Waha & Hesse 1988), surrounded by verrucate-rugulate ornamentation, were observed in the pollen of *Polyalthia nitidissima* (Dunal 1817: 109) Bentham (1863: 51) (pollen not shown).

**TEM (Fig. 3C, F, I):** Exine inaperturate, tectate. Infratectum finely and densely granular, 0.3–1.8 as thick as tectum. Basal layer mostly indistinct [distinct and  $\pm$  lamellate in *Polyalthia perrieri* Cavaco & Keraudren (1957: 75) and *P. tanganyikensis* Vollesen (1980b: 56)]. Intine consisting of a thick tubular exintine and a thin homogeneous endintine,



**FIGURE 1.** Bayesian posterior probability tree, with support indicated above branches: Bayesian posterior probabilities right of slash, maximum parsimony's symmetric resampling values left of slash.



**FIGURE 2.** Flowers/fruits of representative species of clade A, *Monoon*, and *Polyalthia sensu stricto*. A–C, G. Clade A. A. *Polyalthia cerasoides*. B, G. *Polyalthia jenkinsii*. C. *Hubera* sp. 4. D, H. *Monoon*. *Monoon* sp. E, F, I. *Polyalthia sensu stricto*. E, I. *Polyalthia parviflora*. F. *Polyalthia submontana*. Photographs: A, Mr. Outlander from siamensis.org; B, G, K. Aongyong; C, U. Treesucon; D, E, H, I, S. Gardner; F, L. Jessup.

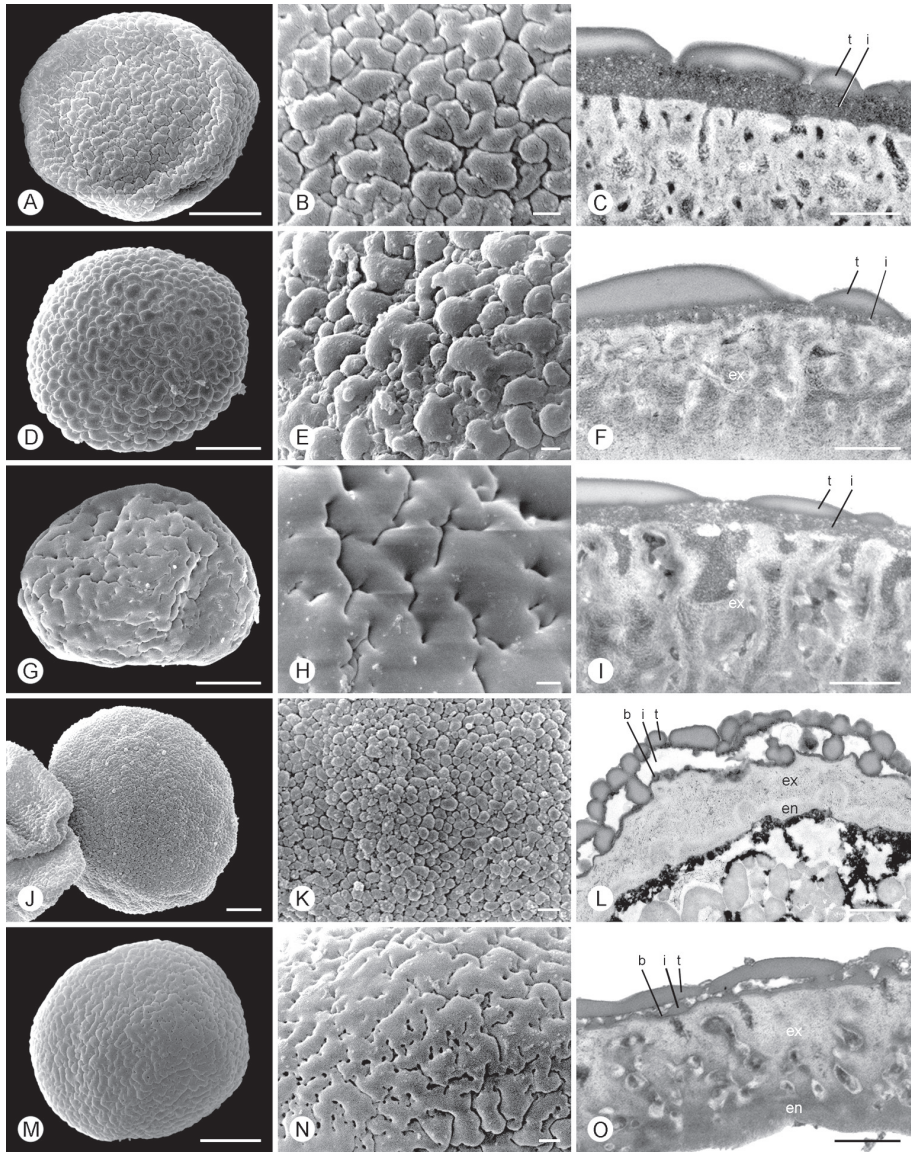
which is sometimes indistinguishable from the tubular exintine, without (?) or with one (?) or two germination zone(s).

The infratectum of species belonging to *Monoon* and *Polyalthia s.s.* thus far investigated using TEM (Appendix 1) is  $\pm$  columellate or coarsely granular (Fig. 3L, O).

### Macromorphology

All species of clade A share the following characters [contrasted with characters of *Monoon* (see Fig. 2D, H for representatives) and *Polyalthia s.s.* (see Fig. 2E, F, I for

showing coarsely granular (to  $\pm$  columellate) infratectum and tubular exintine. Scale bars: 10  $\mu$ m (D, G, M), 5  $\mu$ m (A, J), 1  $\mu$ m (B, C, E, F, H, I, K, L, N, O). Abbreviations: b, basal layer; en, endintine; ex, exintine; i, infratectum; t, tectum.



**FIGURE 3.** Pollen of representative species of clade A, *Monoon*, and *Polyalthia sensu stricto*.; SEM (columns 1 and 2) and TEM (column 3). A–I. Clade A. A–C. *Polyalthia cerasoides* (A, B, *Vajravelu* 36762; C, *Larsen et al.* 33731). A. Pollen grain. B. Detail showing verrucate(-rugulate) ornamentation. C. Detail of pollen wall with finely and densely granular infratectum and tubular exintine. D–F. *Polyalthia pendula* (*Rabevohitra* 2386). D. Pollen grain. E. Detail showing verrucate ornamentation. F. Detail of pollen wall with finely and densely granular infratectum and tubular exintine. G–I. *Hubera* sp. 2 (*Darbyshire* 981). G. Pollen grain. H. Detail showing weakly coarsely rugulate to fossulate ornamentation. I. Detail of pollen wall with finely and densely granular infratectum and tubular exintine. J–L. *Monoon*. *Monoon paradoxum* (*Ambriansyah & Arifin* B 1520). J. Pollen grain. K. Detail showing scabrate ornamentation. L. Detail of pollen wall showing (coarsely granular to)  $\pm$  columellate infratectum and homogeneous exintine. M–O. *Polyalthia sensu stricto*. *Polyalthia cauliflora* (S 24388). M. Pollen grain. N. Detail showing rugulate to fossulate(-perforate) ornamentation. O. Detail of pollen wall

...continued on page 48

representatives) in square brackets]: reticulate [usually percurrent in *Monoon*] tertiary venation of the leaves, axillary [often terminal in *Polyalthia s.s.*] inflorescences, six petals of  $\pm$  equal size (Fig. 2A–C) [sometimes outer petals considerably reduced in *Polyalthia s.s.*], a single ovule/seed per ovary/monocarp (Fig. 2G) [usually two or more uniseriate ovules per ovary and thus often multi-seeded monocarps (Fig. 2I) in *Polyalthia s.s.*], seeds with a flat to slightly raised [distinctly grooved in *Monoon* (Fig. 4c); generally slightly grooved in *Polyalthia s.s.* (Fig. 4b)] raphe (Fig. 4a) and spiniform(-flattened peg) [four-parted lamelliform in *Monoon*] ruminations of the endosperm. Table 3 summarizes important diagnostic characters of clade A, *Monoon* and *Polyalthia s.s.*, and figure 2 shows the similarities and differences in flower and fruit morphology of these three clades.

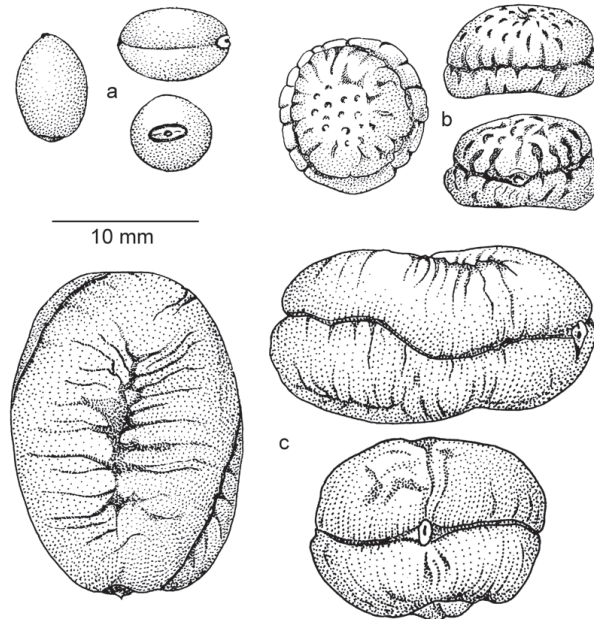
**TABLE 3.** Comparisons of important pollen and morphological characters of clade A, *Monoon*, and *Polyalthia sensu stricto*.

Character	Clade A	<i>Monoon</i>	<i>Polyalthia sensu stricto</i>
- Tertiary venation of leaves	Reticulate	Usually percurrent	Reticulate
- Leaf base	Generally attenuate, wedge-shaped or obtuse; rarely unequal	Generally wedge-shaped or obtuse; rarely unequal	Generally $\pm$ (sub) cordate; generally $\pm$ unequal
- Domatia on lower leaf surface	Present or absent	Absent	Absent
- Position of inflorescences	Axillary	Axillary	Axillary or terminal
- Number of ovule(s) per ovary	1	1	Usually 2–6
- Seed raphe	Flat to slightly raised	Distinctly grooved	Generally slightly grooved
- Endosperm ruminations	Spiniform(-flattened peg)	Four-parted lamelliform	Spiniform(-flattened peg)
- Pollen infratectum	Finely, densely granular	$\pm$ Columellate or coarsely granular	$\pm$ Columellate or coarsely granular

## Discussion

Clade A is maximally supported in both the maximum parsimony and Bayesian analyses (see Fig. 1), which was reported previously (Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004), but those analyses included only three species. Besides being maximally supported by molecular phylogenetic analyses, their morphology is also highly uniform, i.e. all known species share 1) reticulate tertiary leaf venation, 2) a single ovule/seed per ovary/monocarp, 3) seeds with a flat to slightly raised raphe (Fig. 4a), and 4) spiniform (-flattened peg) ruminations of the endosperm.

Pollen of species recovered in clade A shows a characteristic infratectum, which is one of the putative synapomorphies of the clade and can be readily distinguishable from that of *Monoon* and *Polyalthia s.s.* It is finely and densely granular (Fig. 3C, F, I), whereas



**FIGURE 4.** Seeds of representative species of clade A, *Monoon*, and *Polyalthia sensu stricto*. Clade A. a. *Polyalthia cerasoides*. *Monoon*. c. *Monoon fuscum*. *Polyalthia sensu stricto*. b. *Polyalthia subcordata*. All reproduced from Van Setten & Koek-Noorman (1992).

it is  $\pm$  columellate or coarsely granular in *Monoon* (Fig. 3L) and *Polyalthia s.s.* (Fig. 3O). As discussed in Chaowasku *et al.* (2008), there are probably two germination zones in the pollen of clade A, although this is not always clear in the present study. Possibly, the TEM sections did not cross the germination zones.

*Miliusa*, the sister of clade A, possesses more or less similar pollen features of ornamentation and presence of the germination zone(s); however, its pollen infratectum differs from that of clade A in being  $\pm$  columellate or coarsely granular (Chaowasku *et al.* 2008). As clade A is not only phylogenetically unrelated to *Monoon* and *Polyalthia s.s.*, but also morphologically and palynologically distinguishable from these two clades, we propose that this clade be recognized at the generic level.

***Hubera*** Chaowasku, *gen. nov.*

**Type:**—*Hubera cerasoides* (Roxburgh 1795: 30) Chaowasku.

Shrubs, treelets or medium-sized trees, evergreen or rarely deciduous (so far observed only in *H. cerasoides*). Young twigs glabrous or hairy. Leaves petiolate; elliptic(-oblong), oblong or seldom obovate, base equal or rarely unequal, attenuate, wedge-shaped, obtuse or rarely (sub)cordate, apex usually obtuse, acute(-acuminate) or (caudate-) acuminate, rarely rounded or emarginate; upper surface of midrib (slightly) sunken, flat, or (both edges) slightly raised [with one or two groove(s) in the middle]; domatia of aggregated tuft of hairs present in some species, at the axils where secondary veins

meet primary veins; tertiary venation reticulate. Inflorescences usually on branches and axillary, one-flowered or seldom two-flowered, rarely cauliflorous and then > 3-flowered; pedicels often articulated above the base; bracts (and bracteoles) present or sometimes not seen (absent?). Flowers bisexual; sepals free or rarely basally connate, triangular to ovate; petals of the outer and inner whorls equal or slightly subequal, ovate, elliptic (-ovate) or (elliptic-)tongue-shaped, erect or spreading at anthesis; in dried condition often marked by yellow powdery material. Stamens generally  $\geq 40$  per flower, apex of anther connective flat-topped, covering the thecae. Carpels generally  $\geq 10$  per flower, cylindrical or occasionally flask-shaped, peripheral ones sometimes slightly incurved; stigmas  $\pm$  globose, (ellipsoid-)obovoid, ellipsoid(-ovoid) or ellipsoid-cylindrical, generally  $\pm$  hairy; ovaries hairy, 1 ovule per ovary, sub-basal. Torus (nearly) flat or (slightly) raised with flat-topped (or with rarely rounded) apex, generally  $\pm$  hairy, often  $\pm$  enlarged in fruit. Monocarps per fruit few to many, red *in vivo*, stipitate or rarely (sub)sessile; seed-containing portion (sub)globose, ellipsoid(-cylindrical), cylindrical or rarely  $\pm$  obovoid, apex sometimes (slightly) apiculate. Seed per monocarp 1, (sub)globose, ellipsoid, cylindrical or rarely  $\pm$  obovoid; seed coat smooth; raphe flat or slightly raised; endosperm ruminations spiniform(-flattened peg).

**Etymology:**—Named in honour of Prof. Herbert Huber (1931-2005), who was the first to distinguish the three clades discussed in the present paper: *Hubera* (clade A), *Monoon*, and *Polyalthia* s.s. as informal groups of *Polyalthia sensu lato* based solely on morphology (Huber 1985).

**Distribution:**—Twenty-seven species are formally transferred here (see below); they are distributed from East Africa and Madagascar through southern and southeastern Asia to Malesia and the southwestern Pacific. It is anticipated that when the species of *Hubera* are thoroughly revised, an additional 10–20 species will be added.

Given the morphological criteria mentioned above, the following species are transferred to the new genus:

**1. *Hubera amoena* (A.C.Sm.) Chaowasku, *comb. nov.***

Basionym: *Polyalthia amoena* Smith (1950: 159).

**Distribution:**—Fiji.

**Notes:**—Study of the type specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**2. *Hubera capillata* (A.C.Sm.) Chaowasku, *comb. nov.***

Basionym: *Polyalthia capillata* Smith (1950: 158).

**Distribution:**—Fiji.

**Notes:**—Study of the type specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**3. *Hubera ceramensis* (Boerl.) Chaowasku, *comb. nov.***

Basionym: *Polyalthia ceramensis* Boerlage (1899b: t. 65<sup>1</sup>).

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<sup>1</sup> Incorrectly indicated as page 106 (1899: 106) in the original publication of this chapter.

**Distribution:**—Ceram.

**Notes:**—Study of a drawing and description of this species in Boerlage (1899b) was the basis for this transfer; no DNA was available for this species.

**4. *Hubera cerasoides* (Roxb.) Chaowasku, *comb. nov.***

Basionym: *Uvaria cerasoides* Roxburgh (1795: 30).

Homotypic synonyms: *Guatteria cerasoides* (Roxb.) Dunal (1817: 127). *Unona cerasoides* (Roxb.) Baillon (1868: 272). *Polyalthia cerasoides* (Roxb.) Beddome (1869: t. 1).

**Distribution:**—Mainland Asia except southern Thailand and Peninsular Malaysia.

**5. *Hubera decora* (Diels) Chaowasku, *comb. nov.***

Basionym: *Polyalthia decora* Diels (1925: 342).

**Distribution:**—Madagascar.

**6. *Hubera forbesii* (F.Muell. ex Diels) Chaowasku, *comb. nov.***

Basionym: *Polyalthia forbesii* Mueller ex Diels (1912b: 131).

**Distribution:**—New Guinea (to Kei Islands?).

**Notes:**—Study of herbarium specimens including the type (see Appendix 1) and pollen morphology of this species (Table 2) was the basis for this transfer; no DNA was available for this species.

**7. *Hubera gracilis* (Burck) Chaowasku, *comb. nov.***

Basionym: *Polyalthia gracilis* Burck (1911: 430)

**Distribution:**—New Guinea.

**Notes:**—Study of the type specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**8. *Hubera henrici* (Diels) Chaowasku, *comb. nov.***

Basionym: *Polyalthia henrici* Diels (1925: 342).

**Distribution:**—Madagascar.

**9. *Hubera hirta* (Miq.) Chaowasku, *comb. nov.***

Basionym: *Monoon hirtum* Miquel (1865: 16).

Homotypic synonym: *Polyalthia hirta* (Miq.) Mueller (1877: 95).

**Distribution:**—New Guinea.

**Notes:**—Study of the type specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**10. *Hubera humblotii* (Drake ex Cavaco & Keraudren) Chaowasku, *comb. nov.***

Basionym: *Polyalthia humblotii* Drake ex Cavaco & Keraudren (1957: 77).

**Distribution:**—Comoro Islands.

**Notes:**—This species was transferred to *Hubera* based on the fact that related species [e.g. *H. henrici*, *H. perrieri* (Cavaco & Keraudren) Chaowasku] with similar morphology (presence of domatia on the lower leaf surface, Schatz & Le Thomas 1990) belong to *Hubera* (Fig. 1); no DNA was available for this species.

**11. *Hubera jenkinsii* (Hook.f. & Thomson) Chaowasku, *comb. nov.***

Basionym: *Guatteria jenkinsii* Hooker & Thomson (1855: 141).

Homotypic synonym: *Polyalthia jenkinsii* (Hook.f. & Thomson) Hooker & Thomson (1872: 64).

**Distribution:**—Southeastern Asia (west of Wallace’s Line).

**12. *Hubera keraudreniae* (Le Thomas & G.E.Schatz) Chaowasku, *comb. nov.***

Basionym: *Polyalthia keraudreniae* Le Thomas & Schatz in Schatz & Le Thomas (1990: 124).

**Distribution:**—Madagascar.

**Notes:**—This species was transferred to *Hubera* based on the fact that a related species (*H. decora*) with similar morphology (absence of domatia on the lower leaf surface, Schatz & Le Thomas 1990), belongs to *Hubera* (Fig. 1); no DNA was available for this species.

**13. *Hubera korinti* (Dunal) Chaowasku, *comb. nov.***

Basionym: *Guatteria korinti* Dunal (1817: 133).

Homotypic synonym: *Polyalthia korinti* (Dunal) Thwaites (1864: 398).

**Distribution:**—Southern/southwestern India to Sri Lanka.

**14. *Hubera leptopoda* (Diels) Chaowasku, *comb. nov.***

Basionym: *Polyalthia leptopoda* Diels (1915: 179).

**Distribution:**—New Guinea.

**Notes:**—Study of the type specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**15. *Hubera loriformis* (Gillespie) Chaowasku, *comb. nov.***

Basionym: *Polyalthia loriformis* Gillespie (1931: 4).

**Distribution:**—Fiji.

**Notes:**—Study of herbarium specimens including the type of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**16. *Hubera mossambicensis* (Vollesen) Chaowasku, *comb. nov.***

Basionym: *Polyalthia mossambicensis* Vollesen (1980a: 403).

**Distribution:**—East Africa.

**Notes:**—Study of herbarium specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**17. *Hubera multistamina* (G.E.Schatz & Le Thomas) Chaowasku, *comb. nov.***

Basionym: *Polyalthia multistamina* Schatz & Le Thomas (1990: 126).

**Distribution:**—Madagascar.

**Notes:**—This species was transferred to *Hubera* based on the fact that a related species (*H. decora*) with similar morphology (absence of domatia on the lower leaf surface, Schatz & Le Thomas 1990), belongs to *Hubera* (Fig. 1); no DNA was available for this species.

**18. *Hubera nitidissima* (Dunal) Chaowasku, *comb. nov.***

Basionym: *Unona nitidissima* Dunal (1817: 109).

Homotypic synonym: *Polyalthia nitidissima* (Dunal) Bentham (1863: 51).

**Distribution:**—New Guinea and northern/northeastern Australia to New Caledonia.

**19. *Hubera pendula* (Capuron ex G.E.Schatz & Le Thomas) Chaowasku, *comb. nov.***

Basionym: *Polyalthia pendula* Capuron ex Schatz & Le Thomas (1990: 128).

**Distribution:**—Madagascar.

**20. *Hubera perrieri* (Cavaco & Keraudren) Chaowasku, *comb. nov.***

Basionym: *Polyalthia perrieri* Cavaco & Keraudren (1957: 75).

**Distribution:**—Madagascar.

**21. *Hubera rumphii* (Blume ex Hensch.) Chaowasku, *comb. nov.***

Basionym: *Guatteria rumphii* Blume ex Henschel (1833: 153).

Homotypic synonym: *Polyalthia rumphii* (Blume ex Hensch.) Merrill (1923: 162).

**Distribution:**—Southeastern Asia (west of Wallace's Line) to Ambon (and New Guinea?).

**Notes:**—This and the other two morphologically similar species, *H. ceramensis* and *H. jenkinsii*, constitute a species complex (Turner 2011a). To resolve this complex, detailed revisionary and phylogenetic studies are required.

**22. *Hubera sambiranensis* (Capuron ex Le Thomas & G.E.Schatz) Chaowasku, *comb. nov.***

Basionym: *Polyalthia sambiranensis* Capuron ex Le Thomas & Schatz in Schatz & Le Thomas (1990: 116).

**Distribution:**—Madagascar.

**Notes:**—This species was transferred to *Hubera* based on the fact that related species (e.g. *H. henrici*, *H. perrieri*) with similar morphology (presence of domatia on the lower leaf surface, Schatz & Le Thomas 1990), belong to *Hubera* (Fig. 1); no DNA was available for this species.

**23. *Hubera stuhlmannii* (Engl.) Chaowasku, *comb. nov.***

Basionym: *Unona stuhlmannii* Engler (1895: 179).

Homotypic synonym: *Polyalthia stuhlmannii* (Engl.) Verdcourt (1969: 94).

**Distribution:**—East Africa.

**24. *Hubera tanganyikensis* (Vollesen) Chaowasku, *comb. nov.***

Basionym: *Polyalthia tanganyikensis* Vollesen (1980b: 56).

**Distribution:**—East Africa.

**25. *Hubera trichoneura* (Diels) Chaowasku, *comb. nov.***

Basionym: *Polyalthia trichoneura* Diels (1912a: 871).

**Distribution:**—New Guinea.

**Notes:**—Study of the type specimen of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

**26. *Hubera verdcourtii* (Vollesen) Chaowasku, *comb. nov.***

Basionym: *Polyalthia verdcourtii* Vollesen (1980b: 56).

**Distribution:**—East Africa.

**Notes:**—This species was transferred to *Hubera* based on the fact that a related species (*H. stuhlmannii*) with similar morphology (presence of domatia on the lower leaf surface, Vollesen 1980b), belongs to *Hubera* (Fig. 1); no DNA was available for this species.

### **27. *Hubera vitiensis* (Seem.) Chaowasku, comb. nov.**

Basionym: *Polyalthia vitiensis* Seemann (1865: 4).

**Distribution:**—Fiji.

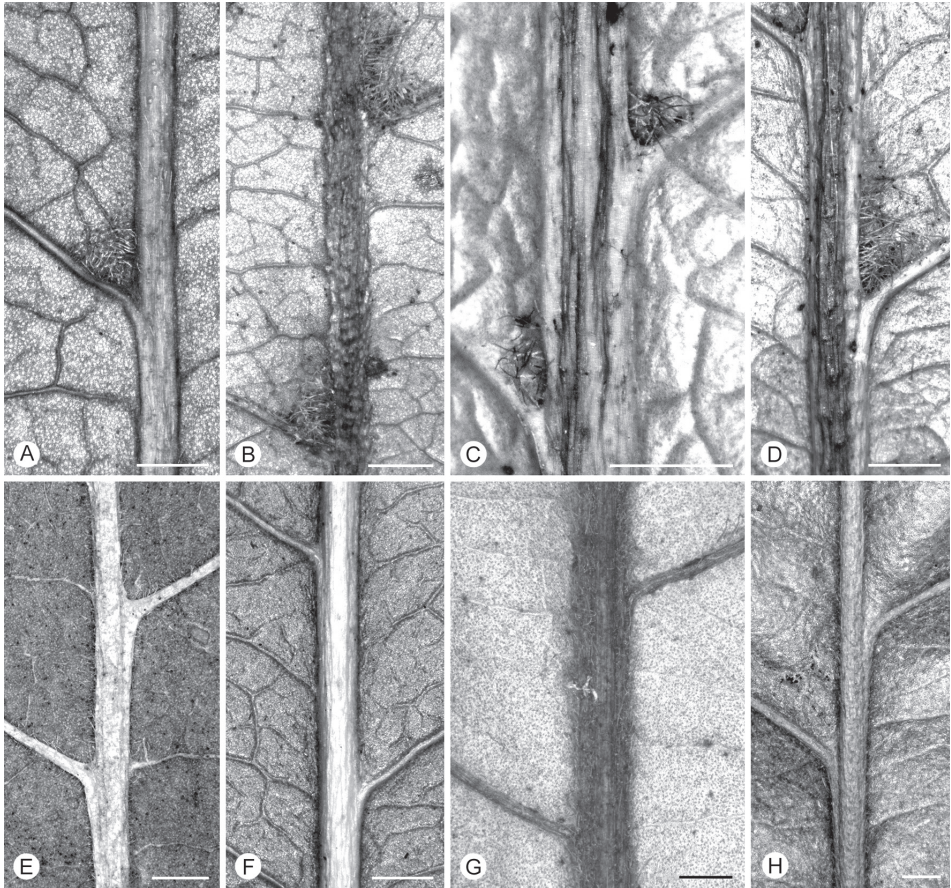
**Notes:**—Study of herbarium specimens of this species (see Appendix 1) was the basis for this transfer; no DNA was available for this species.

Several species (e.g. *H. henrici*, *H. korinti*, *H. nitidissima*, *H. perrieri*, *H. stuhlmannii*, *H. vitiensis*) exhibit domatia on the lower leaf surface at the axils where the secondary veins meet the primary veins (Fig. 5A–D; see comparisons of lower leaf surface without domatia in Fig. 5E–H). They are characterized by a tuft of aggregated hairs visible to the naked eye. In Annonaceae, this kind of domatium has been reported to occur in *Mitrephora* Hooker & Thomson (1855: 112) (Weerasooriya & Saunders 2010), which is also a member of the Miliuseae, and *Annona* Linnaeus (1753: 536) [including *Rollinia* Saint-Hilaire (1824: 28)] (Van den Bos *et al.* 1989), a member of the subfamily Annonoideae (Chatrou *et al.* 2012).

The genus *Miliusa* was recovered as sister to *Hubera*. So far, no morphological synapomorphy linking these genera has been observed. They only share some characters considered as symplesiomorphies, such as reticulate tertiary leaf venation and pollen with verrucate to rugulate ornamentation (cerebroid *sensu* Mols *et al.* 2004a) and germination zone(s) characterized by enlargements/reductions of the intine sublayers (Chaowasku *et al.* 2008).

Among genera of Malmeoideae, *Hubera* exhibits the widest distribution, ranging from East Africa and Madagascar across southern and southeastern Asia through Malesia and the southwestern Pacific. It is the only genus of Miliuseae that occurs in Madagascar and East Africa. Phylogenetic analysis of *Hubera* (Fig. 1) shows some clear biogeographic patterns. The Afro-Madagascan species are grouped together in a strongly supported clade (clade A2), as do the species occurring in the Austro-Papuasian area, which are clustered, with strong support, in clade A1. The biogeographic scenario explaining this distribution will be the focus of another study.

Schatz & Le Thomas (1990) revised *Polyalthia* species occurring in Madagascar and distinguished five informal groups (groups A–E) based on macromorphological and pollen characters. Species of groups B and C possess monosulcate pollen and were found to form a strongly supported clade (now transferred to *Fenerivia*) recovered outside Miliuseae (Saunders *et al.* 2011), which is congruent with the phylogenetic results. Genera outside Miliuseae exhibit monosulcate pollen, whereas genera belonging to Miliuseae possess cryptoaperturate/disulcate pollen (Chaowasku *et al.* 2012b). Species of groups A, D, and E have cryptoaperturate pollen, and their membership in *Hubera*, which is a member of the Miliuseae, was thus not unexpected.



**FIGURE 5.** Lower leaf surface of representative species of *Hubera* with domatia (A–D) and *Hubera* (E, F), *Monoon* (G), and *Polyalthia sensu stricto* (H) without domatia. A. *Hubera nitidissima* (Forster PIF 28246). B. *Hubera vitiensis* (Smith 8203). C. *Hubera korinti* (Kostermans 24916). D. *Hubera stuhlmannii* (Luke & Robertson 1424). E. *Hubera cerasoides* (Maxwell 90-744). F. *Hubera pendula* (Rabevohitra 2386). G. *Monoon fuscum* (Maxwell 87-536). H. *Polyalthia longirostris* (Brass 24039). Scale bars: 1 mm.

It is generally difficult to distinguish *Hubera* from *Fenerivia* using only macromorphology. Both genera share some similar morphological features, e.g. axillary inflorescences, uniovulate carpels, and spiniform(-flattened peg) endosperm ruminations (Schatz & Le Thomas 1990). However, *Fenerivia* possesses a more pronounced seed raphe that is rib-like (Saunders *et al.* 2011), whereas that of *Hubera* is flat to slightly raised (Fig. 4a). In addition, *Fenerivia* exhibits a pronounced ( $\pm$  thickened) receptacle rim (vestigial calyx flange *sensu* Saunders *et al.* 2011). This feature is considered one of the diagnostic characters of *Fenerivia*. It is absent (or rarely slightly observed) in *Hubera*. Nevertheless, the presence of domatia on the lower leaf surface should be a primary character in distinguishing Madagascan *Hubera* from *Fenerivia* because the latter does not possess this character, whereas *Hubera* species formerly known as *Polyalthia* group A

of Schatz & Le Thomas (1990) do. Domatia on the lower leaf surface can also be used to quickly distinguish certain Afro-Asian species of *Hubera* from *Polyalthia* s.s. and other genera formerly known as *Polyalthia*. Another consequence of this study is elimination of *Polyalthia* s.s. from the floras of Africa and Madagascar; thus, it is strictly a genus of Asia-Australasia.

### **Appendix 1 (next page)**

**APPENDIX 1.** Voucher specimens for macromorphological observations, pollen morphological (with applied techniques), and molecular phylogenetic studies (with GenBank accession numbers). Symbols: #, macromorphologically observed for this study; \*, type specimen; {...}, pollen sample taken, with applied techniques indicated; ---, sequence not available for this study.

Taxon	Molecular phylogenetic study (GenBank accession numbers)					
	<i>rbcl</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b><i>Alphonsea elliptica</i></b>						
- Van Balgooy 5141, Bogor Bot. Gard. (L)	AY318966	AY319078	AY519907	JQ690401	JQ690402	JQ690403
<b><i>Bocageopsis canescens</i></b>						
- Maas et al. 9243, Brazil (U)	JQ690407	JQ690408	JQ690409	JQ690410	JQ690411	JQ690412
<b><i>Desmopsis microcarpa</i></b>						
- Chatrou et al. 85, Costa Rica (U)	AY319059	AY319173	AY518804	JX544771	AY841461	JX544758
<b><i>Hubera amoena</i></b>						
- Smith 6423, Fiji (BISH)#						
<b><i>Hubera capillata</i></b>						
- Smith 4581, Fiji (BISH)#						
<b><i>Hubera cerasoides</i></b>						
- Chalermglin 440214-4, Thailand (L)#	AY319017	AY319131	AY518854	JQ889985	JQ889980	JQ889975
<b><i>Hubera decora</i></b>						
- Phillipson 2971, Madagascar (WAG)#	---	JX544869	---	JX544879	JX544859	JX544849
<b><i>Hubera forbesii</i></b>						
- Forbes 836 [B (photograph)]#						
- Kostermans 2787, New Guinea (L)# {LM, SEM, TEM}						
<b><i>Hubera gracilis</i></b>						
- Versteeg 1408 [B (photograph)]#						
<b><i>Hubera henrici</i></b>						
- Dorr & Koenders 3033, Madagascar (WAG)#	---	JX544870	---	JX544880	JX544860	JX544850
- Dorr 3039, Madagascar (WAG)# {LM, SEM, TEM}						

## APPENDIX 1 (continued).

Taxon - Voucher, location (herb.)	Molecular phylogenetic study (GenBank accession numbers)					
	<i>rbcl</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b><i>Hubera hirta</i></b>						
- Zippelius s.n. [B (photograph)] <sup>#</sup>						
<b><i>Hubera jenkinsii</i></b>						
- Chaowasku 60, Thailand (L) <sup>#</sup>	---	JX544803	---	JX544842	JX544812	JX544821
- Gardner & Sidsunthorn ST 0974 (L) <sup>#</sup>						
- Geesink et al. 7761, Thailand (L) <sup>#</sup> {LM, SEM, TEM}						
<b><i>Hubera korinti</i></b>						
- Ratnayake 2/03, Sri Lanka (HKU)	---	EU522179	---	JX544877	EU522124	JX544847
- Kostermans 24580, Sri Lanka (L) <sup>#</sup> {LM, SEM, TEM}						
- Kostermans 24916, Sri Lanka (L) <sup>#</sup>						
- Kostermans 25503, Sri Lanka (L) <sup>#</sup>						
- Kostermans 27618, Sri Lanka (L) <sup>#</sup>						
<b><i>Hubera leptopoda</i></b>						
- Ledermann 6585 [B (photograph)] <sup>#</sup>						
<b><i>Hubera loriformis</i></b>						
- Gillespie 3639, Fiji (BISH) <sup>#</sup>						
- Greenwood 1125, Fiji (BISH) <sup>#</sup>						
- Parham 7182, Fiji (BISH) <sup>#</sup>						
- Smith 601, Fiji (BISH) <sup>#</sup>						
<b><i>Hubera mossambicensis</i></b>						
- Torre & Correia 17062, Mozambique (P) <sup>#</sup>						
<b><i>Hubera nitidissima</i></b>						
- Ford AF 4967, Australia (L)	---	JQ889988	JQ889989	JQ889986	JQ889981	JQ889976
- Forster PIF 28246, Australia (L) <sup>#</sup> {SEM, TEM}						
- Gray 1959, Australia (L) <sup>#</sup>						
<b><i>Hubera pendula</i></b>						
- Rabevohitra 2386, Madagascar (WAG) <sup>#</sup> {LM, SEM, TEM}	---	AY319144	AY518852	JQ889987	JQ889982	JQ889977

## APPENDIX 1 (continued).

Taxon - Voucher, location (herb.)	Molecular phylogenetic study (GenBank accession numbers)					
	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b>Hubera perrieri</b> - Copuron 20.977-SF, Madagascar (K) <sup>#</sup> {LM, SEM, TEM}	---	JX544871	---	JX544881	JX544861	JX544851
<b>Hubera rumphii</b> - Slik 2821, Borneo (L) <sup>#</sup>	---	JX544802	---	JX544841	JX544811	JX544820
<b>Hubera stuhlmannii</b> - Luke & Robertson 1424, Kenya (K) <sup>#</sup>	---	AY319149	AY518853	JX544882	JX544862	JX544852
<b>Hubera tanganyikensis</b> - Couvreur 66, Tanzania (WAG) <sup>#</sup> {LM, SEM, TEM}	---	JX544872	---	JX544883	JX544863	JX544853
<b>Hubera trichoneura</b> - Von Römer s.n. [B (photograph)] <sup>#</sup>	---					
<b>Hubera vitensis</b> - Smith 647, Fiji (BISH) <sup>#</sup> - Smith 8203, Fiji (L) <sup>#</sup>	---					
<b>Hubera sp. 1</b> - Polak 1211A, New Guinea (L) <sup>#</sup>	---	JX544864	---	JX544873	JX544854	JX544843
<b>Hubera sp. 2</b> - Darbyshire 981, New Guinea (L) <sup>#</sup> {LM, SEM, TEM}	---	JX544865	JX544884	JX544874	JX544855	JX544844
<b>Hubera sp. 3</b> - Takeuchi & Ama 15543, New Guinea (L) <sup>#</sup>	---	JX544866	---	JX544875	JX544856	JX544845
- Conn et al. 45, New Guinea (L) <sup>#</sup> - Takeuchi & Ama 16263, New Guinea (L) <sup>#</sup> - Takeuchi & Ama 16284, New Guinea (L) <sup>#</sup>						
<b>Hubera sp. 4</b> - Treesucon 2009-4, Vietnam (L) <sup>#</sup>	---	JX544867	---	JX544876	JX544857	JX544846
<b>Hubera sp. 5</b> - Schatz & Modeste 2886, Madagascar (WAG) <sup>#</sup>	---	JX544868	---	JX544878	JX544858	JX544848
<b>Hubera sp. 6</b> - Katik NGF 46922, New Guinea (L) <sup>#</sup> {LM, SEM, TEM}	---					

APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)

Taxon	<i>rbcL</i>	<i>trnL</i> F	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b>Hubera sp. 7</b>						
- Takeuchi 10122, New Guinea (L) <sup>#</sup> {LM, SEM, TEM}						
- Takeuchi & Regalado 10210, New Guinea (L) <sup>#</sup>						
<b>Hubera sp. 8</b>						
- Pullen 1858, New Guinea (L) <sup>#</sup> {LM, SEM, TEM}						
<b>Hubera sp. 9</b>						
- Kairo NGF 24405, New Guinea (L) <sup>#</sup>						
<b>Marsypopetalum littorale</b>						
- Rastini 153, Bogor Bot. Gard. (L)	AY319026	AY319140	AY518835	JX544827	JX544804	JX544813
<b>Meiogyne virgata</b>						
- Keßler PK 2751, Borneo (L)	AY318982	AY319094	AY518798	JX544769	JX544784	JX544756
<b>Mililusa mollis</b>						
- Keßler PK 3207, Thailand (L)	---	AY319102	AY518851	JQ690503	JQ690504	JQ690505
<b>Mililusa thorelii</b>						
- Keßler PK 3184, Thailand (L)	---	AY319104	AY518846	JQ690519	JQ690520	JQ690521
<b>Mililusa velutina</b>						
- Pholsena & Koonthunthod 2842, Thailand (L)	AY318993	AY319105	AY518847	JQ690536	JQ690537	JQ690538
<b>Mitrephora alba</b>						
- Chalermglin 440304-1, Thailand (TISTR, Bangkok)	AY318994	AY319106	AY518855	JQ889983	JQ889978	JQ889973
<b>Monocarpia maingayi</b>						
- Kaewruang 1, Thailand (L)	JQ690395	JQ690396	JQ690397	JQ690398	JQ690399	JQ690400
<b>Monoon coffeoides</b>						
- Ratnayake J/03, Sri Lanka (HKU)	EU522288	EU522178	EU522233	---	EU522123	---
<b>Monoon fuscum</b>						
- Keßler PK 3222, Thailand (L)	AY318973	AY319085	AY518787	JX544779	JX544792	JX544767
- Kostermans 774, Thailand (L) {TEM}						
- Maxwell 87-536, Thailand (L) <sup>#</sup>						

## APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)						
Taxon	<i>rbcl</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b>Monoon paradoxum</b>						
- Ambriansyah & Arifin B 1520, Borneo (L) {SEM, TEM}						
<b>Monoon viride</b>						
- Chalermglin 440214-3, Thailand (L)	AY319040	AY319154	AY518784	JX544780	JX544793	JX544768
- Phengkjai et al. 4244, Thailand (L) {TEM}						
<b>Neo-uvaria telopea</b>						
- Chaowasku 77, Thailand (L)	JX544755	JX544783	JX544751	JX544778	JX544791	JX544766
<b>Orophea kerrii</b>						
- Chalermglin 440416-1, Thailand (L)	AY319008	AY319121	AY518818	JQ690419	JQ690420	JQ690421
<b>Oxandra venezuelana</b>						
- Chatrou et al. 120, Costa Rica (U)	AY841645	AY841723	JQ690413	JQ690414	AY841495	JQ690415
<b>Phaeanthus splendens</b>						
- Keßler B 1564, Borneo (L)	JX544754	AY319126	AY518864	JX544777	JX544790	JX544765
<b>Platymitra macrocarpa</b>						
- Okada 3457, Bogor Bot. Gard. (L)	AY319013	AY319127	AY518812	JQ690422	JQ690423	JQ690424
<b>Polyalthia angustissima</b>						
- Maxwell 82-168, Singapore Bot. Gard. (L) <sup>#</sup>	---	JX544795	---	JX544831	JX544807	---
<b>Polyalthia bullata</b>						
- Chaowasku 34 (L) <sup>#</sup>	---	JX544800	JX544825	JX544839	JX544809	JX544818
<b>Polyalthia cauliflora</b>						
- Keßler PK 3114, Singapore Bot. Gard. (L)	AY319015	AY319129	AY518823	JX544837	---	---
<b>-S 24388, Borneo (L) {SEM, TEM}</b>						
<b>Polyalthia celebica</b>						
- Mols 9, Bogor Bot. Gard. (IV-C-97) (L) <sup>#</sup> {TEM}	AY319016	AY319130	AY518827	JX544838	JX544808	---
<b>Polyalthia flagellaris</b>						
-S 57495, Borneo (L) {TEM}						

APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)

Taxon	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
- Voucher, location (herb.)						
<b><i>Polyalthia johnsonii</i></b>						
- Ford AF 3625, Australia (CNS)	---	JX544801	JX544826	JX544840	JX544810	JX544819
- Forster PIF 24658, Australia (L) <sup>#</sup>						
<b><i>Polyalthia longirostris</i></b>						
- Takeuchi & Ama 15656, New Guinea (L)	AY318979	AY319091	AY518826	---	---	---
- Brass 24039, New Guinea (L) <sup>#</sup>						
- Van Royen & Sleumer 7093, New Guinea (L) <sup>#</sup> {TEM}						
<b><i>Polyalthia parviflora</i></b>						
- Chaowasku 24, Thailand (L) <sup>#</sup> {TEM}	---	JX544799	---	JX544836	---	---
<b><i>Polyalthia stenopetala</i></b>						
- Johnson & Chalermglin 2040, Thailand (OWU) <sup>#</sup>	JX544823	JX544796	---	JX544832	---	---
- KEP/FRI 13402, Peninsular Malaysia (L) {TEM}						
<b><i>Polyalthia subcordata</i></b>						
- Gravendeel et al. 678, Java (L) {TEM}						
<b><i>Polyalthia suberosa</i></b>						
- Chatrou 480, Utrecht Univ. Bot. Gard. (U)	AY238956	AY231289	AY238965	AY841417	AY841502	JX544817
		AY238949				
- Maxwell 93-1463, Thailand (L) <sup>#</sup> {TEM}						
<b><i>Polyalthia</i> sp. 1</b>						
- Punnadee 1, Thailand (L) <sup>#</sup> {TEM}	---	JX544797	---	JX544833	---	---
<b><i>Polyalthia</i> sp. 2</b>						
- Chaowasku 50, Thailand (L) <sup>#</sup> {TEM}	---	JX544798	---	JX544834	---	---
<b><i>Polyalthia</i> sp. 3</b>						
- Kefšler PK 3228, Thailand (L) <sup>#</sup>	AY319020	AY319134	AY518832	JX544835	---	---
<b><i>Popowia hirta</i></b>						
- Kefšler B 1628, Borneo (L)	AY319042	AY319156	AY518860	JX544830	JX544806	JX544816

## APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)							
Taxon	<i>rbcl</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>	
<b><i>Pseuduvaria fragrans</i></b>							
- Chaowasku 27, Thailand (L)	EU522341	EU522231	EU522286	JX544829	EU522176	JX544815	
<b><i>Sageraea lanceolata</i></b>							
- Ridsdale DV-M2-1692, Borneo (L)	AY319050	AY319164	AY518799	JX544774	JX544787	JX544762	
<b><i>Sapranthus viridiflorus</i></b>							
- Chatrou et al. 55, Costa Rica (U)	AY319051	AY319165	AY743493	AY841422	AY841515	JX544760	
<b><i>Stelechocarpus burahol</i></b>							
- Mols 13, Bogor Bot. Gard. (L)	AY319053	AY319167	AY518803	JX544775	JX544788	JX544763	
<b><i>Stelechocarpus cauliflorus</i></b>							
- Unknown s.n., Bogor Bot. Gard. (XV-A-196) (L)	AY319054	AY319168	AY518800	JX544776	JX544789	JX544764	
<b><i>Stenanona costaricensis</i></b>							
- Chatrou et al. 67, Costa Rica (U)	AY319069	AY319183	AY518801	JX544772	AY841516	JX544759	
<b><i>Tridimeris</i> sp.</b>							
- Maas 8646, Missouri Bot. Gard. (U)	JX544753	JX544782	JX544750	JX544773	JX544786	JX544761	
<b><i>Trivalvaria</i> sp.</b>							
- Chaowasku 35, Thailand (L)	JX544822	JX544794	JX544824	JX544828	JX544805	JX544814	
<b>Undescribed genus</b>							
- Chaowasku 108, Thailand (L)	JX544752	JX544781	JX544749	JX544770	JX544785	JX544757	

herb. = herbarium



# Chapter 4

## **Integrative systematics supports the establishment of *Winitia*, a new genus of Annonaceae (Malmeoideae, Miliuseae) allied to *Stelechocarpus* and *Sageraea***

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### **Abstract**

The generic circumscriptions of *Stelechocarpus* and *Sageraea* (Annonaceae) are assessed using molecular phylogenetic, macromorphological, and pollen morphological evidence. For molecular phylogenetic analysis the combined seven plastid markers: *rbcL* exon, *trnL* intron, *trnL-F* spacer, *matK* exon, *ndhF* exon, *psbA-trnH* spacer, and *ycf1* exon constituting ca. 7 kb are used. The results corroborate the recognition of a maximally supported clade as a new genus, *Winitia*. It is weakly to moderately supported as sister to *Stelechocarpus burahol*, the type and only species of *Stelechocarpus*. A clade consisting of *Winitia* and *Stelechocarpus* is strongly supported as sister to *Sageraea*, which is monophyletic with strong support. *Winitia* consists of two species, one of which (*W. expansa*) is proposed as a new species endemic to Thailand, whereas one new combination (*W. cauliflora*) is made. The new genus is primarily characterized by (1) multicolumellar stigmas ( $\geq 5$  columns per stigma) and (2) pollen grains with a very thin tectum, a more or less columellate/coarsely granular infratectum, and a very distinct basal layer. The macromorphology and pollen morphology of the three genera (*Stelechocarpus*, *Winitia*, and *Sageraea*) are highlighted.

**Keywords:** Annonaceae, Miliuseae, molecular phylogenetics, morphology, nomenclature, palynology, *Sageraea*, *Stelechocarpus*, taxonomy, *Winitia*

### **Introduction**

Annonaceae are a pantropical angiosperm family consisting of ca. 2400 species in ca. 108 genera, which have been classified into four subfamilies, i.e. Anaxagoreoideae, Ambavioideae, Annonoideae, and Malmeoideae (Chatrou *et al.* 2012). Recent expeditions in southern Thailand have yielded several new species of Annonaceae belonging to various genera [i.e. *Dasymaschalon* (Hook.f. & Thomson) Dalla Torre & Harms: Wang *et al.* 2009; *Pseuduvaria* Miq.: Su *et al.* 2010; *Neo-uvaria* Airy Shaw: Chaowasku *et al.* 2011a; *Dendrokingstonia* Rauschert: Chaowasku *et al.* 2012b; *Milium* Lesch. ex A.DC.: Chaowasku & Keßler 2013]. There are still a number of undescribed Annonaceae species

collected during these expeditions, including one species having conspicuously thick and fleshy, pink-orange petals. When only flowers were seen at first, this plant was quickly identified as an undescribed species of *Sageraea* Dalzell, a member of Miliuseae, which are the largest tribe of the subfamily Malmeoideae (Chatrou *et al.* 2012). This tribe is predominantly Asian (including New Guinea, Australia, and the western Pacific islands), with a strongly supported clade of Afro-Malagasy and another of Neotropical taxa nested within (Chaowasku *et al.* 2012a). When the leaf material was subsequently observed, it became clear that this undescribed species is more similar to *Stelechocarpus cauliflorus* (Scheff.) J.Sinclair. Because of the strong resemblance between the petals of *Sageraea* and *Stelechocarpus cauliflorus*, it is not surprising that the latter was originally described as a *Sageraea* species (Scheffer 1885). Presently, the genus *Stelechocarpus* Hook.f. & Thomson contains two species: *Stelechocarpus cauliflorus* and *Stelechocarpus burahol* (Blume) Hook.f. & Thomson (type species); they share “the distinct venation of the leaves” and “the prominent midrib above” (Van Heusden 1995).

It should be noted that the circumscriptions of many genera in Annonaceae based solely on morphology were disputed and have recently been realigned/recircumscribed with the aid of molecular phylogenetics (e.g. Mols *et al.* 2008, Zhou *et al.* 2009, 2010, Surveswaran *et al.* 2010, Saunders *et al.* 2011, Xue *et al.* 2011, 2012, Chaowasku *et al.* 2012a). Molecular phylogenetic analysis is also crucial in identifying undescribed genera (Couvreur *et al.* 2009, Chaowasku *et al.* 2012a). Chaowasku *et al.* (2012a) performed an extensive molecular phylogenetic analysis of genera in the Miliuseae to support the segregation of the genus *Hubera* Chaowasku from *Polyalthia* Blume. Besides establishing *Hubera*, the resolution of the Miliuseae was considerably improved, with *Sageraea lanceolata* Miq., *Stelechocarpus burahol*, and *Stelechocarpus cauliflorus* recovered as a strongly supported clade (parsimony symmetric resampling value 87%; Bayesian posterior probability 1.00). *Stelechocarpus* appeared to be monophyletic, but with only weak to moderate support.

In order to determine the phylogenetic position of the undescribed species morphologically similar to *Stelechocarpus cauliflorus* and *Sageraea*, and assess the morphology-based generic boundary of *Stelechocarpus* and *Sageraea*, this undescribed species as well as three more species of *Sageraea* are added in the present study. Further, the macromorphology and pollen morphology of *Stelechocarpus*, *Sageraea*, and the undescribed species mentioned above are studied in detail to corroborate the resulting phylogeny. The latter is formally described under the appropriate genus elucidated by the combined evidence of macromorphology, pollen morphology, and molecular phylogenetics.

## Materials and methods

The Appendix 1 includes the voucher information for the molecular phylogenetic (with GenBank accession numbers indicated), macromorphological, and pollen morphological studies.

### *Phylogenetic analyses*

Forty-eight accessions comprise the ingroup (Monocarpieae + Miliuseae). Nearly all generic representatives of the Miliuseae were sampled including four species (out of ca.

10 species) of *Sageraea*, as well as *Stelechocarpus burahol*, *Stelechocarpus cauliflorus*, and the undescribed species. Representatives of the genera *Oncodostigma* Diels and *Phoenicanthus* Alston were not included because of the unavailability of (good) leaf material. Outgroups are a species of *Bocageopsis* R.E.Fr. and one of *Oxandra* A.Rich., both from Malmeeae, the second largest tribe of the same subfamily as Miliuseae.

Seven plastid markers (*rbcl* exon, *trnL* intron, *trnL-F* spacer, *matK* exon, *ndhF* exon, *psbA-trnH* spacer, *ycf1* exon) were amplified. In total, 7035 characters, including eight separately coded indels, were included in the analyses. Indel coding follows Simmons & Ochoterena (2000). For a 15-nucleotide stretch in the *psbA-trnH* marker, the reverse complement was present in approximately half of the accessions sequenced, and was converted into the reverse complement, following Pirie *et al.* (2006).

All methods of DNA extraction, amplification, and sequencing performed in Chaowasku *et al.* (2012a) were used in the present study. Sequences were edited using the program Staden version 1.7.0 (<http://staden.sourceforge.net/>) and subsequently manually aligned. Some sequences were obtained from previous studies (Mols *et al.* 2004a, 2004b, Pirie *et al.* 2006, Su *et al.* 2008, Chaowasku *et al.* 2012a). Sequences of the *rbcl* marker were not available for some accessions (36% of all accessions included, see Appendix 1) because of the failures in DNA amplification or unavailability of leaf material. Maximum parsimony analysis was performed in TNT version 1.1 (Goloboff *et al.* 2008). All characters were equally weighted and unordered. Multiple most parsimonious trees were generated by a heuristic search of the combined data, with 6000 replicates of random sequence additions, 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) as 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 additions, saving one tree per replicate. Groups with SR of  $\geq 85\%$ , 70–84%, and  $\leq 69\%$  were considered strongly, moderately, and weakly supported, respectively.

Bayesian analysis was performed in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003). Two independent runs comprising eight Markov chain Monte Carlo (MCMC) chains were simultaneously run; each run was set for  $10^7$  generations. The data matrix was divided into seven partitions [*trnL* intron and *trnL-F* spacer were included in the same partition (= *trnLF*)], including a set of binary indel coding. The most appropriate model of sequence evolution for each partition was selected by Akaike information criterion (AIC) scores, using FindModel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>). The default model as well as the command “coding=variable” were applied for the binary indel partition. The default prior settings were used except for the ratepr (=variable) and brlenspr [=unconstrained:exp(100)]. The latter prior setting was used to prevent 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 0.15. Trees and all parameter values were sampled every 1000<sup>th</sup> generation. Convergence of the runs was checked by both the standard deviation of split frequencies and the values for effective sample

sizes (ESS) using Tracer version 1.5 (Rambaut & Drummond 2009). The 50% majority-rule consensus tree was generated from the two runs combined, with 10% of the first trees removed as burn-in. Groups with posterior probabilities (PP) of  $\geq 0.96$ , 0.91–0.95, and  $\leq 0.9$  were considered strongly, moderately, and weakly supported, respectively.

### *Pollen morphology*

Pollen samples of *Stelechocarpus burahol*, *Stelechocarpus cauliflorus*, the undescribed species, and two species of *Sageraea* were available for this study (Appendix 1). The pollen material for scanning electron microscopy (SEM) was not acetolysed, following Chaowasku *et al.* (2008) and Couvreur *et al.* (2009). The material for transmission electron microscopy (TEM) was prepared according to Van der Ham (1990). Subdivision of the exine into tectum, infratectum, and basal layer (Le Thomas 1980) was applied. Further pollen terminology follows Punt *et al.* (2007).

## Results

### *Phylogenetic analyses*

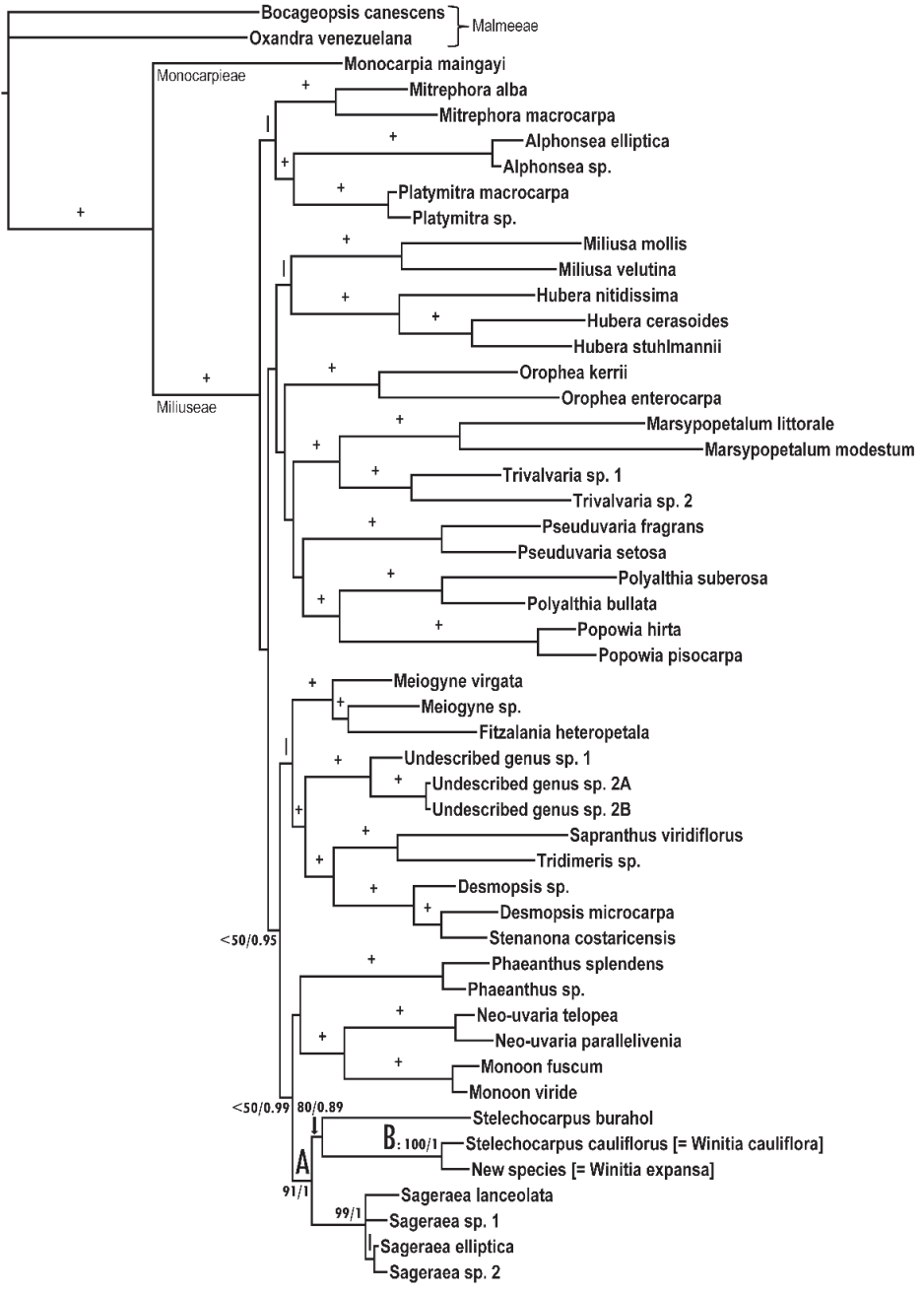
The maximum parsimony analysis resulted in 15 most parsimonious trees with 1718 steps. The consistency and retention indices were 0.77 and 0.71, respectively. For Bayesian analysis, the substitution model was General Time Reversible plus Gamma (GTR + G) for all partitions except for *trnLF* (= *trnL* intron + *trnL-F* spacer) and *psbA-trnH*, which had the Hasegawa-Kishino-Yano plus Gamma (HKY + G) model. The final standard deviation of split frequencies was lower than 0.003 and all ESS values after discarding the burn-in were larger than 600, both indicating convergence of the runs.

The phylogenetic relationships of the genera within Miliuseae (Fig. 1) are similar to those reported in Chaowasku *et al.* (2012a), with *Stelechocarpus burahol*, clade B, and *Sageraea* recovered as a strongly supported monophyletic group (clade A: SR 91%; PP 1). *Sageraea* is monophyletic with strong support (SR 99%; PP 1). The maximally supported clade B (= *Winitia gen. nov.*, see discussion) comprises *Stelechocarpus cauliflorus* (= *Winitia cauliflora comb. nov.*, see discussion) and the undescribed species (= *Winitia expansa sp. nov.*, see Discussion). The sister relationship of *Stelechocarpus burahol* and clade B is weakly to moderately supported (SR 80%; PP 0.89).

### *Macromorphology and pollen morphology*

Important macromorphological and pollen morphological features observed for *Stelechocarpus burahol*, clade B, and *Sageraea* are summarized in Table 1. Figures 2–16 illustrate floral and pollen morphology of the three lineages, whereas Figs. 17–26 contrasts the floral differences between *Stelechocarpus burahol* and clade B.

*Stelechocarpus burahol* can be distinguished from clade B principally by (1) the distribution of male and female flowers in individuals (separated in *Stelechocarpus burahol* vs. mixed in clade B), (2) stigma morphology [bilobed ( $\pm$  heart-shaped) in *Stelechocarpus burahol* vs. multicolumellar in clade B], and (3) pollen features, particularly the infratectum (finely and densely granular in *Stelechocarpus burahol* vs.  $\pm$  colu-



**FIGURE 1.** 50% majority-rule consensus phylogram derived from Bayesian analysis of combined seven plastid markers. Clade support: left of slash – parsimony symmetric resampling values (SR) corresponding to clades recovered in Bayesian tree, right of slash – Bayesian posterior probabilities (PP); + = SR ≥ 85%, PP ≥ 0.99; | = SR 70–80%, PP ≥ 0.96; no symbol and support indicated = SR < 50%, PP < 0.8. (Scale bar: 0.003 substitution per site).

**TABLE 1.** Important macromorphological and pollen morphological features of *Stelechocarpus burahol*, clade B (= *Winitia gen. nov.*), and *Sageraea*.

Character/lineage	<i>Stelechocarpus burahol</i>	Clade B (= <i>Winitia gen. nov.</i> )	<i>Sageraea</i>
- Secondary veins on lower leaf surface	Prominent	Prominent	± Indistinct <sup>a</sup>
- Upper surface of leaf midrib	(Slightly) raised	(Slightly) raised	Slightly sunken <sup>a</sup>
- Petal color	Usually green to yellow/cream <sup>b</sup>	Usually ± rose-colored	White to yellow/cream to reddish(-pink) to purple to brown <sup>a</sup>
- Individual sexuality	Monoecious	Monoecious	Bisexual or monoecious
- Distribution of male and female flowers in individuals	Separated: male flowers on branches; female flowers (on knobs) along trunk	Mixed: (on knobs) along trunk and/or on swollen base of trunk	Mixed (for monoecious species): on branches, sometimes along trunk
- Size of male and female flowers	Different (male flowers smaller for the same individual)	± Equal	± Equal (observed only for <i>Sageraea elliptica</i> )
- Petal texture	Between thick/fleshy and leather-like	Conspicuously thick and fleshy	Conspicuously thick and fleshy
- Number of stamens per male flower	82–97	32–41	8–16 (for monoecious species) <sup>a</sup>
- Stigma shape	Bilobed (± heart-shaped)	Multicolumellar [5–9(–10) columns per stigma]	One (small) orbicular lobe, bilobed, cylindrical or capitate <sup>a</sup>
- Male torus	(Conical-)cylindrical	± Hemispheroid	Nearly flat (observed only for <i>Sageraea elliptica</i> )
- Seed raphe	Slightly grooved on a (slight) ridge	(Slightly) ridged	(Flat to) ± grooved <sup>c</sup>
- Pollen: ornamentation (SEM)	Verrucate-scabrate to rugulate to fossulate(-perforate)	± Scabrate-microgemmate	Microechinate, often also minutely perforate
- Pollen: tectum (TEM)	Considerably thick	Very thin	Thin
- Pollen: infratectum (TEM)	Finely and densely granular	± Columellate/coarsely granular	± Columellate/coarsely granular
- Pollen: basal layer (TEM)	Indistinct	Very distinct	Distinct

<sup>a</sup>(Van Heusden 1997a); <sup>b</sup>(Van Heusden 1995); <sup>c</sup>(Van Setten & Koek-Noorman 1992)

mellate/coarsely granular in clade B), whereas *Sageraea* is chiefly distinguishable from the other two lineages by (1) the ± indistinct secondary veins on the lower leaf surface (prominent in the other two lineages) and (2) the slightly sunken upper surface of the leaf midrib [(slightly) raised in the other two lineages].

## Discussion

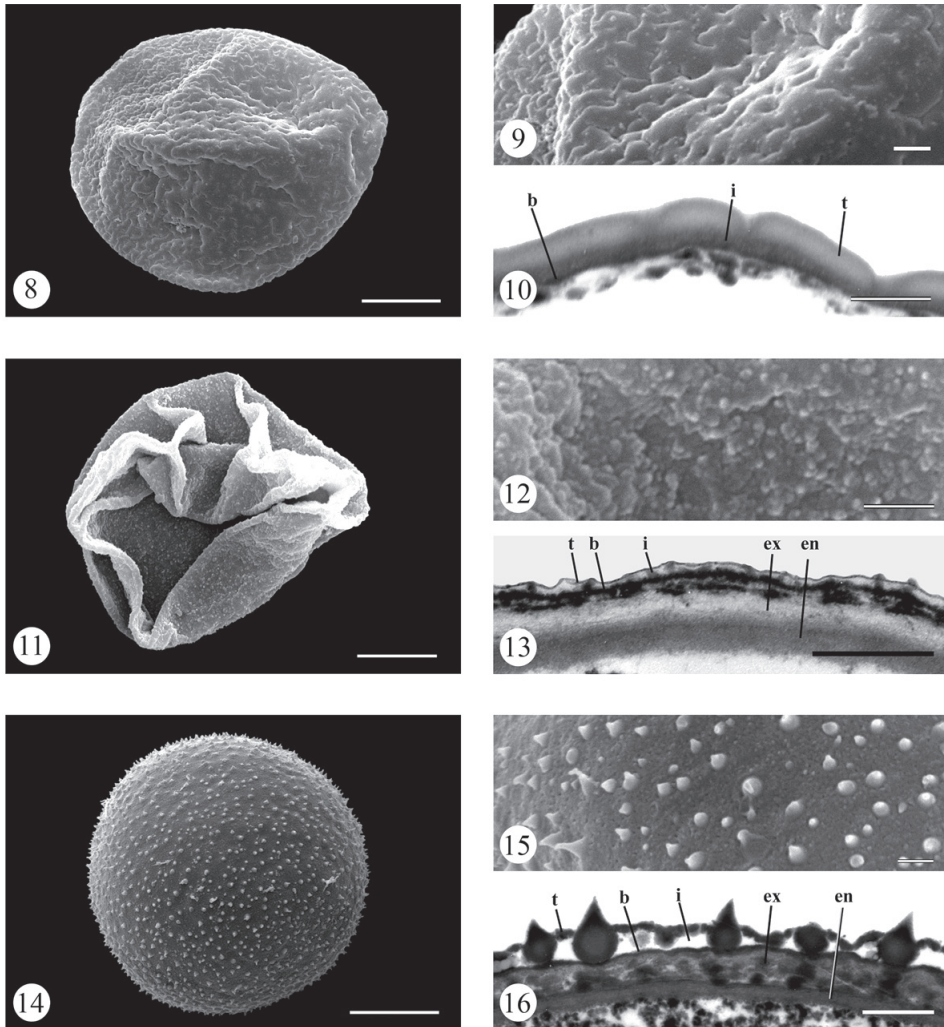
Molecular phylogenetic analyses revealed that the undescribed species is sister to *Stele-*



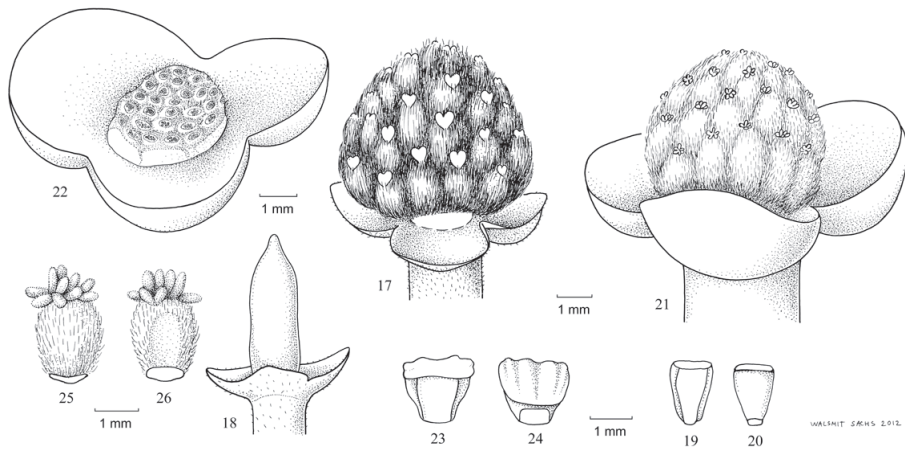
**FIGURES 2–7.** Flowers of *Stelechocarpus burahol* (2, 3), clade B (= *Winitia* gen. nov.), and *Sageraea*. 4, *Winitia cauliflora* comb. nov. (= *Stelechocarpus cauliflorus*). 5, 6, *Winitia expansa* sp. nov. 7, *Sageraea bracteolata*. (Photographs: 2, 3 = ©C. Pradubpet; 4 = ©N. Panitvong; 5, 6 = ©T. Chaowasku; 7 = ©S. Gardner).

*chocarpus cauliflorus* with maximum support. Together, they are only weakly to moderately supported as sister to *Stelechocarpus burahol*, the type species of *Stelechocarpus*. Further, the strongly supported monophyly of the genus *Sageraea* is confirmed for the first time. The three lineages, i.e. *Stelechocarpus burahol*, clade B, and *Sageraea*, comprise clade A, which possesses two diagnostic macromorphological features: the  $\pm$  thick leaves and an ovary with multiple ovules arranged in two rows (Van Heusden 1997a, pers. obs. TC). There are also diagnostic macromorphological traits supporting each of the three lineages in clade A (Table 1), for example, the separated distribution of male and female flowers in individuals of *Stelechocarpus burahol*, the unique stigmas (Figs. 21, 25, 26) of clade B, and the  $\pm$  indistinct secondary veins on the lower leaf surface of *Sageraea*. In Miliuseae, multicolumellar stigmas ( $\geq 5$  columns per stigma: Figs. 21, 25, 26) have not been found elsewhere outside clade B (Van Heusden 1992). This kind of stigma represents an apparent synapomorphy of clade B.

The stamens of clade B (Figs. 5, 23, 24) are somewhat larger than those of *Stelechocarpus burahol* (Figs. 3, 19, 20). They more or less resemble those of *Neo-uvaria*, especially at male anthesis (see Fig. 1B in Chaowasku *et al.* 2011a; this genus is part of a weakly supported clade that is strongly supported as the sister group of clade A in the Bayesian analysis but weakly supported as its sister group in the maximum parsimony analysis, see Fig. 1). The stamens of *Sageraea bracteolata* R.Parker (Fig. 7) are peculiar



**FIGURES 8–16.** Pollen grains of *Stelechocarpus burahol* (8–10; Lörzing 11332), clade B (= *Winitia* gen. nov.; 11–13), and *Sageraea* (14–16): scanning and transmission electron micrographs (SEM, TEM). 8, pollen grain with verrucate-scabrate to rugulate to fossulate(-perforate) ornamentation; 9, detail of 8; 10, pollen wall showing considerably thick tectum, finely and densely granular infratectum, and indistinct basal layer. 11, 12, *Winitia cauliflora* comb. nov. (= *Stelechocarpus cauliflorus*; Maxwell 85-372): 11, collapsed pollen grain with ± scabrate-microgemmate ornamentation; 12, detail of 11. 13, *Winitia expansa* sp. nov. (Chaowasku 93): pollen wall showing very thin tectum with a few short suprategmatal elements, ± columellate/coarsely granular infratectum, and very distinct basal layer. 14–16, *Sageraea elliptica* (Chaowasku 45): 14, pollen grain with microechinate and minutely perforate ornamentation; 15, detail of 14; 16, pollen wall showing thin tectum with several conspicuous suprategmatal elements, ± columellate/coarsely granular infratectum, and distinct basal layer. [Scale bars: ca. 1 µm (9, 10, 12, 13, 15, 16), 5 µm (8, 11), 10 µm (14); b = basal layer; en = endintine; ex = exintine; i = infratectum; t = tectum].



**FIGURES 17–26.** Floral morphology of *Stelechocarpus burahol* (17–20) and clade B (= *Winitia gen. nov.*; 21–26). 17, female flower with petals removed, showing bilobed ( $\pm$  heart-shaped) stigmas; 18, male flower with petals and stamens removed, showing cylindrical torus; 19, stamen, abaxial side; 20, stamen, adaxial side. 21, 22, *Winitia cauliflora comb. nov.* (= *Stelechocarpus cauliflorus*): 21, female flower with petals removed, showing multicolumellar stigmas; 22, male flower with petals and stamens removed, showing  $\pm$  hemispheroid torus. 23–26, *Winitia expansa sp. nov.*: 23, stamen, abaxial side; 24, stamen, adaxial side; 25, carpel, abaxial side, showing multicolumellar stigma; 26, carpel, adaxial side, showing multicolumellar stigma. (17 = *Van Balgooy* 5249; 18–20 = *Pradubpet* 1; 21, 22 = *Van Beusekom & Phengkhai* 1032; 23–26 = *Chaowasku* 93).

in still cohering tightly at anthesis; this feature is also observable in the other species of *Sageraea*: *Sageraea elliptica* (A.DC.) Hook.f. & Thomson (pers. obs. TC), *Sageraea lanceolata* (Van Heusden 1997a), *Sageraea laurina* Dalzell (Yadav & Sardesai 2002: under *Sageraea laurifolia* Blatt., *nom. illeg.*), and thus is likely to represent a synapomorphy of this genus.

Further differences between the three lineages are present in their pollen. *Stelechocarpus burahol* possesses pollen grains with a finely and densely granular infractum (Fig. 10). In Miliuseae, such an infractum has also been observed in the pollen of *Hubera*, though it is less finely and densely granular there than in *Stelechocarpus burahol* (Chaowasku *et al.* 2012a). On the other hand, clade B and *Sageraea* exhibit a  $\pm$  columellate/coarsely granular infractum (Figs. 13, 16). The other exine layers, including the ornamentation, are also considerably different in the three lineages (Figs. 8–16; Table 1).

Macromorphological and pollen morphological data correspond well with the phylogeny of clade A. Based on the results, there are three taxonomic alternatives for delimiting genera in clade A: (1) recognition of the strongly supported clade A (SR 91%; PP 1) as a single genus, *Sageraea*; (2) recognition of two genera: *Sageraea* (strongly supported: SR 99%; PP 1) and *Stelechocarpus* inclusive of clade B (weakly to moderately supported: SR 80%; PP 0.89); and (3) recognition of three genera corresponding to the three lineages of clade A [*Stelechocarpus burahol*, clade B (maximally supported), and *Sageraea* (strongly supported: SR 99%; PP 1)]. As discussed above, members of clade A are recognizable by the  $\pm$  thick leaves and the biserially arranged multiple ovules per ovary; however, the first alternative is not appropriate because there are clear

morphological features distinguishing each of the three lineages in this clade (Table 1), and merging them would result in a highly heterogeneous *Sageraea*. The second option is not appropriate either since, although the two lineages, *Stelechocarpus burahol* and clade B, share the prominent secondary leaf venation (lower side;  $\pm$  indistinct in *Sageraea*) and the (slightly) raised leaf midrib (upper side; slightly sunken in *Sageraea*), the support for their sister relationship is still not strong enough, which implies that the inferred sister group of clade B could change if more molecular data became available. Further, *Stelechocarpus burahol* and clade B differ greatly in a number of morphological features (Table 1), some of which are unique among Miliuseae and therefore undoubtedly apomorphic, e.g. the separated distribution of male and female flowers in individuals of *Stelechocarpus burahol* and the multicolumellar stigmas of clade B. On the basis of the combined evidence of macromorphology, pollen morphology, and molecular phylogenetics discussed above, we believe the third taxonomic choice is the most appropriate. Consequently, clade B is established and described below as a new genus, *Winitia*, whereas the circumscription of *Stelechocarpus* is reduced to include only the type species, *Stelechocarpus burahol*. In addition, the undescribed species is formally described below under the new genus. There are several macromorphological features distinguishing this new species (*Winitia expansa* sp. nov.) from its sister species, *Winitia cauliflora* comb. nov. (see below).

It is worthwhile to mention that the recognition of *Stelechocarpus burahol* and clade B as two distinct genera makes each genus of Miliuseae having two or more species included in the molecular phylogenetic analysis a strongly supported clade (see Fig. 1); *Fitzalania* F.Muell. and *Stenanona* Standl. are considered part of *Meiogyne* Miq. and *Desmopsis* Saff., respectively; the nested position of *Fitzalania* in *Meiogyne* was earlier reported in Thomas *et al.* (2012), while that of *Stenanona* in *Desmopsis* previously reported in Mols *et al.* (2004a), Saunders *et al.* (2011), and Xue *et al.* (2011) is confirmed once again in the present study. Based on these results, each of the two generic pairs should be merged. Moreover, as a consequence of erecting clade B as a new genus, each genus in the Miliuseae having the pollen with a tectate exine possesses only a single type of infratectum, i.e. finely and densely granular or  $\pm$  columellate/coarsely granular (Chaowasku *et al.* unpublished data<sup>1</sup>).

### Establishment of clade B as a new genus

***Winitia*** Chaowasku, gen. nov.

**Type:**–*Winitia expansa* Chaowasku, sp. nov.

**Etymology:**–The genus name is to honour Phraya Winit Wanandorn, who has been praised as the ‘father’ of Thai Botany.

(Small to) medium-sized trees. Young twigs glabrous. Bud scales often observed. Leaves petiolate [petioles (distinctly) grooved on upper surface], usually elliptic, sometimes  $\pm$  (ob)ovate, base (broadly) cuneate or (slightly) obtuse, apex acute or acuminate; upper surface of midrib (slightly) raised, lower surface of midrib raised; secondary veins prominent on lower surface, tertiary veins usually reticulate, sometimes less reticulate/more

<sup>1</sup> = Chapter 2.

percurrent. Flowers separate male and female flowers present in the same individual, flowers of both sexes mixed and clustered (on knobs) along the trunk and/or on the swollen base of the trunk; peduncles of inflorescences inconspicuous, bracts many, each flower pedicellate, pedicel bract(s) not seen (absent?) or one (to few) at the base (or  $\pm$  the midpoint) of pedicels; perianths of male and female flowers same shape and  $\pm$  same size. Sepals (broadly) triangular-ovate,  $\pm$  semicircular, or shortly linguiform;  $\pm$  connate. Petals usually  $\pm$  rose-colored *in vivo*, conspicuously thick and fleshy, inner ones smaller than the outer ones. Outer petals (broadly) elliptic(-ovate). Inner petals (broadly) elliptic, somewhat boat-shaped adaxially. Stamens 32–41 per male flower; connective tissue usually flat-topped and  $\pm$  broadened, seldom reduced. Carpels 26–32 per female flower,  $\pm$  flask-shaped or short-cylindrical, sometimes a bit bending inward; stigmas multicolumellar [5–9(–10) columns per stigma]; ovaries moderately hairy; ovules 5–9(–11) per ovary, lateral, biseriate. Torus  $\pm$  hemispheroid in both male and female flowers. Monocarp(s) 1–4 per fruit, (slightly) subglobose or ellipsoid, subsessile, blackish brown at maturity *in vivo*, surface generally shallowly verruculose. Seeds 2–6(–11) per monocarp, D-shaped, slightly flattened, surface smooth, raphe (slightly) ridged; endosperm ruminations lamelliform, divided into four parts.

**Pollen description:**—SEM (Figs. 11, 12) – Pollen grains released as monads at maturity,  $\pm$  subglobose (collapsed in Fig. 11), apolar, longest axis ca. 18–19  $\mu\text{m}$ , exine ornamentation  $\pm$  scabrate-microgemmate; TEM (Fig. 13) – Exine inaperturate, tectate, ca. 0.24  $\mu\text{m}$  thick, tectum very thin, supratectal elements present, short, infratectum  $\pm$  columellate/coarsely granular, basal layer very distinct. Intine comprising exintinous and endintinous sublayers.

**Distribution:**—Two species occurring in Vietnam, southern Thailand through Peninsular Malaysia, Sumatra?, and Borneo.

**Notes:**—The exine of *Winitia* pollen (Fig. 13) is unique. Its tectum is very thin, while its basal layer is very distinct. There are dark elements inside the basal layer; acetolysis could eventually unravel whether these are part of the exine or of the intine, as intinous structures would be removed by acetolysis.

### Key to the species of *Winitia*

1. Flowers mostly clustered on the swollen base of the trunk, pedicels 12.0–22.0 mm long, inner petals moderately spreading at maturity, adaxial side of both inner and outer petals glaucous, each stigma with 7–9(–10) columns ..... ***W. expansa* sp. nov.**  
- Flowers usually clustered (on knobs) along the trunk, pedicels usually  $\geq$  30.0 mm long, inner petals connivent at maturity, no glaucous appearance observed on adaxial side of either petal whorl, each stigma with 5–7 columns ..... ***W. cauliflora* comb. nov.**

### ***Winitia expansa* Chaowasku, sp. nov. (Figs. 5, 6, 23–28)**

**Type:**—Thailand, Phatthalung Province, Si Ban Phot District, Khao Pu Khao Ya National Park, *Gardner & Tippayasri ST 1183* [holotype L! (barcode L0407137); isotypes BKF, K], in flower.

**Etymology:**—The epithet refers to the moderately spreading inner petals at maturity.

FIGURE 27. Holotype of *Winitia expansa* sp. nov.



**FIGURE 28.** Distribution of *Winitia expansa* sp. nov.

Trees, ca. 11 m tall, ca. 24 cm in diameter. Young twigs glabrous, lenticels observed. Bud scales sometimes observed. Petioles 0.5–0.8 cm long, glabrous, grooved on upper surface. Leaves usually elliptic, sometimes slightly ovate, 11.0–17.7 × 3.5–6.4 cm, base (broadly) cuneate to slightly obtuse, apex acute to acuminate; lamina glabrous both sides; upper surface of midrib (slightly) raised, glabrous, lower surface of midrib raised, glabrous; secondary veins (8–)10–12 pairs per leaf, prominent on lower surface, angle with midrib 45°–52°. Flowers mostly clustered on the swollen base of the trunk, but also randomly observed on trunk knobs above this structure, separate male and female flowers present in the same individual but flowers of both sexes mixed; peduncles of inflorescences inconspicuous, bracts many, pedicels 12.0–22.0 mm long, lenticels observed, pedicel bracts not seen (absent?). Sepals broadly triangular-ovate, 3.8–4.2 × 4.6–5.6 mm, slightly connate at the base; outside (almost) glabrous, inside glabrous, margin sparsely puberulous. Petals (moderately) spreading at maturity, inner ones smaller than the outer ones, adaxial surface of both whorls glaucous *in vivo*. Outer petals broadly elliptic(-ovate), 11.0–12.0 × 9.0–11.0 mm; outside sparsely appressed-puberulous, denser near the margin, inside glabrous, margin puberulous. Inner petals (broadly) elliptic, 9.0–12.0 × 6.0–7.0 mm, somewhat boat-shaped adaxially; outside glabrous except the lower half of the middle part which is sparsely appressed-puberulous, inside glabrous,

margin almost glabrous to sparsely (appressed-)puberulous. Stamens 32–36 per male flower, 1.3–1.6 mm long. Carpels ca. 31 per female flower, 2.5–2.8 mm long; stigmas multicolumellar [7–9(–10) columns per stigma]; ovaries tomentose, with a slight vertical groove; ovules 5 per ovary, lateral, biseriate. Torus ± hemispheroid in both male and female flowers. Monocarps only immature ones observed, ca. 4 per fruit, slightly subglobose. Seeds not observed.

**Distribution:**—Southern Thailand (Fig. 28).

**Habitat and phenology:**—Occurring in understorey of evergreen/deciduous forests; at the base of limestone cliffs. Elevation: ca. 120 m. Flowering: December. Fruiting: September, October.

**Field notes:**—Crown – monopodial, branching horizontal, trunk knobby, swollen at the base. Bark – dark grey-brown, roughened and fissured, inner bark cream to pale orange. Flowers – petals dark pink.

**Notes:**—This species is only known from a few individuals occurring in a restricted area. It principally differs from *Winitia cauliflora* *comb. nov.* in having moderately spreading (connivent in *W. cauliflora*, Fig. 4) inner petals at maturity (Fig. 5), glaucous appearance (no glaucous appearance in *W. cauliflora*, Fig. 4) on adaxial side of the petals (Fig. 5), usually more columns per stigma [7–9(–10) in *W. expansa* (Figs. 25, 26) vs. 5–7 in *W. cauliflora* (Fig. 21)], and generally shorter pedicels (12.0–22.0 mm long in *W. expansa* vs. seldom shorter than 30.0 mm long in *W. cauliflora*). Additionally, the flowers of *W. expansa* are mostly clustered on the swollen base of the trunk, while those of *W. cauliflora* are usually borne (on knobs) along the trunk.

**Additional specimens examined (paratypes):**—*Chaowasku* 57 (L), 58 (L), 93 (BKF, L), 94 (L), all from the type locality (collections *Chaowasku* 58 and 93 are from the same individual as the type collection).

### ***Winitia cauliflora* (Scheff.) Chaowasku, *comb. nov.* (Figs. 4, 21, 22)**

Basionym: *Sageraea cauliflora* Scheff. (Scheffer 1885: 5). Homotypic synonym: *Stelechocarpus cauliflorus* (Scheff.) J.Sinclair (Sinclair 1953: 43).

**Type:**—Indonesia, cultivated at Bogor Bot. Garden, IV-H-58, unknown collector [holotype BO?; isotypes K?, L! (barcodes L0038174, L0038175)], in flower.

**Heterotypic synonyms:**—See Van Heusden (1995: 435).

### **Recircumscription of *Stelechocarpus*.**

***Stelechocarpus*** Hook.f. & Thomson (Hooker & Thomson 1855: 94)

= *Uvaria* L. section *Stelechocarpaceae* Blume (Blume 1830: 13).

**Type:**—*Stelechocarpus burahol* (Blume) Hook.f. & Thomson (Figs. 2, 3, 17–20).

(Small to) medium-sized trees. Young twigs (almost) glabrous. Bud scales not observed. Leaves petiolate [petioles (slightly) grooved on upper surface], usually elliptic, sometimes slightly (ob)ovate, base cuneate, apex acute or (acute-)acuminate; upper surface of midrib (slightly) raised, lower surface of midrib raised; secondary veins prominent on lower surface, tertiary veins usually reticulate, sometimes less reticulate/more percurrent. Flowers male and female flowers separated on the same individual; male

clustered on branches, in axils of fallen leaves; female clustered (on knobs) along the trunk; peduncles of both male and female inflorescences inconspicuous, bracts many, each flower pedicellate (male with shorter pedicels), pedicel bract(s) not seen (absent?) or one to few at (or near) the base of pedicels; perianths of male and female flowers same shape but different size [male smaller than female (for the same individual)]. Sepals (broadly) triangular(-ovate),  $\pm$  connate. Petals usually green to yellow/cream *in vivo*, between thick/fleshy and leather-like, inner ones (slightly) smaller than the outer ones. Outer petals elliptic or triangular-ovate. Inner petals elliptic(-ovate). Stamens 82–97 per male flower, connective tissue flat-topped. Carpels 34–43 per female flower,  $\pm$  flask-shaped or slightly ovoid, a bit bending inward; stigmas bilobed,  $\pm$  heart-shaped; ovaries densely hairy; ovules 4(–6) per ovary, lateral, biseriate. Torus (conical-)cylindrical in male flowers, conical-ovoid in female flowers. Monocarp(s) 1–4 per fruit, subglobose, slightly obovoid, or slightly ellipsoid, subsessile, (light) brown at maturity *in vivo*, surface  $\pm$  shallowly verruculose, sometimes verruculae scale-like. Seeds 2–6 per monocarp, ellipsoid or  $\pm$  D-shaped, slightly flattened, surface smooth, raphe slightly grooved on a (slight) ridge; endosperm ruminations lamelliform, usually divided into four parts.

**Pollen description:**—SEM (Figs. 8, 9) – Pollen grains released as monads at maturity, subglobose, apolar, longest axis ca. 19–20  $\mu\text{m}$ , exine ornamentation verrucate-scabrate to rugulate to fossulate(-perforate); TEM (Fig. 10) – Exine inaperturate, tectate, ca. 0.40  $\mu\text{m}$  thick, tectum considerably thick, suprategal elements absent, infrategum finely and densely granular, basal layer indistinct. Intine not clearly observed.

**Distribution:**—One species occurring in lower Peninsular Malaysia, Sumatra, north-western Borneo, Java, and Bali.

**Notes:**—At first sight, the pollen infrategum of *Stelechocarpus burahol* (Fig. 10) could be considered as part of the basal layer, as observed in the exine of *Mezzettia parviflora* Becc. (Chaowasku *et al.* 2008); however, extensive comparisons with the exine of *Hubera* (Chaowasku *et al.* 2012a) indicate that it is not part of the basal layer but represents a finely and densely granular infrategum.

## Appendix 1 (next page)

**APPENDIX 1.** Voucher information for molecular phylogenetic (including GenBank accessions number), pollen morphological, and macromorphological investigations. (Symbols: # = macromorphologically examined for this study; {...} = pollen samples taken, with applied techniques indicated; \* = sequences newly generated for this study; --- = sequences not available for this study).

Molecular phylogenetic study (GenBank accession numbers)						
Taxon	<i>rbcl</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b><i>Alphonsea elliptica</i></b>						
- Van Balgooy 5141, Bogor Bot. Gard. (L)	AY318966	AY319078	AY518807	JQ690401	JQ690402	JQ690403
<b><i>Alphonsea</i> sp.</b>						
- Keßler PK 3186, Thailand (TISTR, Bangkok)	---	AY319082	AY518808	JQ690404	JQ690405	JQ690406
<b><i>Bocageopsis canescens</i></b>						
- Maas et al. 9243, Brazil (U)	JQ690407	JQ690408	JQ690409	JQ690410	JQ690411	JQ690412
<b><i>Desmopsis microcarpa</i></b>						
- Chatrou et al. 85, Costa Rica (U)	AY319059	AY319173	AY518804	JX544771	AY841461	JX544758
<b><i>Desmopsis</i> sp.</b>						
- Rainer 1593, Mexico (WU)	---	AY841701	KC857552*	KC857553*	KC857554*	KC857555*
<b><i>Fitzalania heteropetala</i></b>						
- Kemp TH 7267, Australia (L)	---	KC857556*	KC857557*	KC857558*	KC857559*	KC857560*
<b><i>Hubera cerasoides</i></b>						
- Chalermglin 440214-4, Thailand (L)	AY319017	AY319131	AY518854	JQ889985	JQ889980	JQ889975
<b><i>Hubera nitidissima</i></b>						
- Ford AF 4967, Australia (L)	---	JQ889988	JQ889989	JQ889986	JQ889981	JQ889976
<b><i>Hubera stuhlmannii</i></b>						
- Luke & Robertson 1424, Kenya (K)	---	AY319149	AY518853	JX544882	JX544862	JX544852
<b><i>Marsypopetalum litorale</i></b>						
- Rastini 153, Bogor Bot. Gard. (L)	AY319026	AY319140	AY518835	JX544827	JX544804	JX544813
<b><i>Marsypopetalum modestum</i></b>						
- Keßler PK 3192, Thailand (L)	AY318980	AY319092	AY518834	KC857561*	KC857562*	KC857563*
<b><i>Meiogyne virgata</i></b>						
- Keßler PK 2751, Borneo (L)	AY318982	AY319094	AY518798	JX544769	JX544784	JX544756

## APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)						
Taxon - Voucher, location (herb.)	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b>Meiogyne</b> sp. - Gardner et al. ST 2014, Thailand (L)	KC857564*	KC857565*	KC857566*	KC857567*	KC857568*	KC857569*
<b>Mililusa mollis</b> - Keßler PK 3207, Thailand (L)	---	AY319102	AY518851	JQ690503	JQ690504	JQ690505
<b>Mililusa velutina</b> - Pholsena & Koonkhunthod 2842, Thailand (L)	AY318993	AY319105	AY518847	JQ690536	JQ690537	JQ690538
<b>Mitrephora alba</b> - Chalermnglin 440304-1, Thailand (TISTR, Bangkok)	AY318994	AY319106	AY518855	JQ889983	JQ889978	JQ889973
<b>Mitrephora macrocarpa</b> - Mols & Bogor Bot. Gard. (L)	---	AY319107	AY518859	JQ889984	JQ889979	JQ889974
<b>Monocarpia maingayi</b> - Kaewruang 1, Thailand (L)	JQ690395	JQ690396	JQ690397	JQ690398	JQ690399	JQ690400
<b>Monoon fuscum</b> - Keßler PK 3222, Thailand (L)	AY318973	AY319085	AY518787	JX544779	JX544792	JX544767
<b>Monoon viride</b> - Chalermnglin 440214-3, Thailand (L)	AY319040	AY319154	AY518784	JX544780	JX544793	JX544768
<b>Neo-uvaria telopea</b> - Chaowasku 77, Thailand (L)	JX544755	JX544783	JX544751	JX544778	JX544791	JX544766
<b>Neo-uvaria parolleivenia</b> - Keßler sub IV-H-73, Bogor Bot. Gard. (L)	AY319000	AY319113	AY518794	KC857570*	KC857571*	KC857572*
<b>Orophea enterocarpa</b> - Chalermnglin 440403, Thailand (TISTR, Bangkok)	AY319006	AY319119	AY518815	JQ690416	JQ690417	JQ690418
<b>Orophea kerrii</b> - Chalermnglin 440416-1, Thailand (L)	AY319008	AY319121	AY518818	JQ690419	JQ690420	JQ690421
<b>Oxandra venezuelana</b> - Chatrou et al. 120, Costa Rica (U)	AY841645	AY841723	JQ690413	JQ690414	AY841495	JQ690415

APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)

Taxon - Voucher, location (herb.)	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b><i>Phaeanthus splendens</i></b> - <i>Keßler B 1564</i> , Borneo (L)	JX544754	AY319126	AY518864	JX544777	JX544790	JX544765
<b><i>Phaeanthus</i> sp.</b> - <i>Takeuchi 18407</i> , Sumatra (L)	---	KC857573*	KC857574*	KC857575*	KC857576*	KC857577*
<b><i>Platymitra macrocarpa</i></b> - <i>Okada 3457</i> , Bogor Bot. Gard. (L)	AY319013	AY319127	AY518812	JQ690422	JQ690423	JQ690424
<b><i>Platymitra</i> sp.</b> - <i>Chaowasku 100</i> , Thailand (L)	---	JQ690425	JQ690426	JQ690427	JQ690428	JQ690429
<b><i>Polyalthia bullata</i></b> - <i>Chaowasku 34</i> , Thailand (L)	---	JX544800	JX544825	JX544839	JX544809	JX544818
<b><i>Polyalthia suberosa</i></b> - <i>Chatrou 480</i> , Utrecht Univ. Bot. Gard. (U)	AY238956	AY231289 AY238949	AY238965	AY841417	AY841502	JX544817
<b><i>Popowia hirta</i></b> - <i>Keßler B 1628</i> , Borneo (L)	AY319042	AY319156	AY518860	JX544830	JX544806	JX544816
<b><i>Popowia pisocarpa</i></b> - <i>Van Balgooy &amp; Van Setten 5683</i> , Bogor Bot. Gard. (L)	AY319044	AY319158	AY518862	KC857578*	KC857579*	KC857580*
<b><i>Pseuduvaria fragrans</i></b> - <i>Chaowasku 27</i> , Thailand (L)	EU522341	EU522231	EU522286	JX544829	EU522176	JX544815
<b><i>Pseuduvaria setosa</i></b> - <i>Chaowasku 66</i> , Thailand (L)	---	KC857581*	KC857582*	KC857583*	KC857584*	KC857585*
<b><i>Sageraea bracteolata</i></b> - <i>Gardner ST 2068</i> , Thailand (L) <sup>#</sup>	---	---	---	---	---	---
<b><i>Sageraea elliptica</i></b> - <i>Chaowasku 45</i> , Thailand (L) <sup>#</sup> (SEM, TEM)	---	KC857586*	KC857587*	KC857588*	KC857589*	KC857590*

## APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)						
Taxon	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b>Sageraea lanceolata</b>						
- Ridsdale DV-M2-1692, Borneo (L)	AY319050	AY319164	AY518799	JX544774	JX544787	JX544762
- Ambriansyah & Arbainsyah AA 1673, Borneo (L) {SEM, TEM}						
<b>Sageraea sp. 1</b>						
- Slik 3868, Borneo (L)	---	KC857591*	KC857592*	KC857593*	KC857594*	KC857595*
<b>Sageraea sp. 2</b>						
- Gardner & Sidsunthorn ST 1006, Thailand (L) <sup>#</sup>	---	KC857596*	KC857597*	KC857598*	KC857599*	KC857600*
<b>Sapranthus viridiflorus</b>						
- Chatrou et al. 55, Costa Rica (U)	AY319051	AY319165	AY743493	AY841422	AY841515	JX544760
<b>Stelechocarpus burahol</b>						
- Mols 13, Bogor Bot. Gard. (L)	AY319053	AY319167	AY518803	JX544775	JX544788	JX544763
- Achmad 1036, Sumatra (L) <sup>#</sup>						
- De Wilde & De Wilde-Duyffes 19866, Sumatra (L) <sup>#</sup>						
- Dransfield 2542, Java (L) <sup>#</sup>						
- Forbes 1604a, Sumatra (L) <sup>#</sup>						
- Kostermans s.n., Java (L) <sup>#</sup>						
- Lörzing 5585, Sumatra (L) <sup>#</sup>						
- Lörzing 11332, Sumatra (L) <sup>#</sup> {SEM, TEM}						
- Pradubpet 1, Thailand (L) <sup>#</sup>						
- S 40553, Borneo (L) <sup>#</sup>						
- Unknown 7692, Bogor Bot. Gard. (L) <sup>#</sup>						
- Van Balgooy 5249, Bali (L) <sup>#</sup>						
- Van Steenis 5706, Sumatra (L) <sup>#</sup>						
<b>Stenanona costaricensis</b>						
- Chatrou et al. 67, Costa Rica (U)	AY319069	AY319183	AY518801	JX544772	AY841516	JX544759
<b>Tridimeris sp.</b>						
- Maas 8646, Missouri Bot. Gard. (U)	JX544753	JX544782	JX544750	JX544773	JX544786	JX544761

## APPENDIX 1 (continued).

Molecular phylogenetic study (GenBank accession numbers)						
Taxon	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>ycf1</i>
<b><i>Trivalvaria</i> sp. 1</b>						
- <i>Chaowasku</i> 35, Thailand (L)	JX544822	JX544794	JX544824	JX544828	JX544805	JX544814
<b><i>Trivalvaria</i> sp. 2</b>						
- <i>Chaowasku</i> 56, Thailand (L)	---	KC857601*	KC857602*	KC857603*	KC857604*	KC857605*
<b><i>Winitia cauliflora</i></b>						
- <i>Unknown</i> s.n., Bogor Bot. Gard. (XV-A-196) (L)	AY319054	AY319168	AY518800	JX544776	JX544789	JX544764
- <i>Chaowasku</i> 71, Thailand (L) <sup>#</sup>						
- <i>Chew</i> CWL 684, Borneo (L) <sup>#</sup>						
- <i>Maxwell</i> 85-372, Thailand (L) <sup>#</sup> {SEM}						
- <i>Ramsri</i> 30, Thailand (L) <sup>#</sup>						
- <i>S</i> 20272, Borneo (L) <sup>#</sup>						
- <i>Van Beusekom</i> & <i>Pheengkrai</i> 1032, Thailand (L) <sup>#</sup>						
<b><i>Winitia expansa</i></b>						
- <i>Chaowasku</i> 93, Thailand (L) <sup>#</sup> {TEM}	---	KC857616*	KC857617*	KC857618*	KC857619*	KC857620*
- <i>Chaowasku</i> 57, Thailand (L) <sup>#</sup>						
- <i>Chaowasku</i> 58, Thailand (L) <sup>#</sup>						
- <i>Chaowasku</i> 94, Thailand (L) <sup>#</sup>						
- <i>Gardner</i> & <i>Tippayasri</i> ST 1183, Thailand (L) <sup>#</sup>						
<b>Undescribed genus sp. 1</b>						
- <i>Chaowasku</i> 111, Thailand (L)	---	KC857611*	KC857612*	KC857613*	KC857614*	KC857615*
<b>Undescribed genus sp. 2A</b>						
- <i>Chaowasku</i> 108, Thailand (L)	JX544752	JX544781	JX544749	JX544770	JX544785	JX544757
<b>Undescribed genus sp. 2B</b>						
- <i>Nakorn-Thiemchan</i> NTC 16, Thailand (L)	---	KC857606*	KC857607*	KC857608*	KC857609*	KC857610*

herb. = herbarium

# Chapter 5

## (2029) Proposal to conserve the name *Meiogyne* against *Fitzalania* (Annonaceae)

TANAWAT CHAOWASKU, GEA ZIJLSTRA & LARS W. CHATROU

Published in *Taxon* 60: 1522–1523. 2011.

(2029) *Meiogyne* Miq. in Ann. Mus. Bot. Lugduno-Batavi 2: 12. 23 Mar. 1865 [Annon.], *nom. cons. prop.*

**Typus:**–*M. virgata* (Blume) Miq. (*Unona virgata* Blume)

(=) *Fitzalania* F.Muell., Fragm. 4: 33. Oct. 1863, *nom. rej. prop.*

**Typus:**–*F. heteropetala* (F.Muell.) F.Muell. (*Uvaria heteropetala* F.Muell.)

*Meiogyne* Miq. is a well-known genus of Asian Annonaceae with 17 species presently recognized (Van Heusden 1994, 1996, Jessup 2007, Turner 2009). The number of species of *Meiogyne*, however, will surely increase as there are several new species to be described (pers. comm. D.M. Johnson). The genus is found from India through Southeast Asia to northern Australia, New Caledonia, and Fiji (Van Heusden 1994, 1996). Several genera (*Ancana* F.Muell., *Chieniodendron* Tsiang & P.T.Li, *Guamia* Merr., *Oncodostigma* Diels, *Polyaulax* Backer) have been synonymized<sup>1</sup> with *Meiogyne* on the basis of gross morphology (Van Heusden 1994). *Meiogyne*, including the synonymized genera, exhibits a corrugated or grooved area at the base of the inner side of the inner petals (Van Heusden 1994, Jessup 2007). Moreover, the apex of those stamens located in the inner whorls (near the carpels) is usually more elongated than those located in the outer whorls (Van Heusden 1992, Jessup 2007). These two important features also occur in *Fitzalania* F.Muell., an Australian endemic genus with two species (Van Heusden 1992, Jessup 2007). However, the color and appearance of the (inner) petals of *Fitzalania* (very dark purple and more or less boat-shaped) are somewhat different from those of *Meiogyne* and hence are the main reasons to still recognize this genus morphologically (Jessup 2007). In addition, one of the two species of *Fitzalania*, *F. bidwillii* (Benth.) Jessup, Kessler & Mols, possesses sepal-like outer petals, resembling those of *Heteropetalum* Benth. (now included in *Guatteria* Ruiz & Pav.), *Marsypopetalum* Scheff. *pro parte*, *Miliusa* Lesch. ex A.DC., *Phaeanthus* Hook.f. & Thomson, *Piptostigma* Oliv., and *Polyalthia* Blume *sensu stricto p.p.*

Recent molecular phylogenetic analyses have shown that *Fitzalania* is nested within *Meiogyne*, a relationship that is strongly supported (Mols *et al.* 2004a). This finding

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<sup>1</sup> The identity of the type of *Oncodostigma leptoneurum* Diels (type species of *Oncodostigma*) is problematic (but the flower shown in the protologue is likely to represent a *Meiogyne* species), and therefore this genus actually has not been formally put into synonymy with *Meiogyne* yet; most species assigned to *Oncodostigma* have been transferred to *Meiogyne* (see also Chatrou *et al.* 2012, Thomas *et al.* 2012).

is also confirmed by the authors (in prep.<sup>2</sup>) as part of the first author's Ph.D. study to understand the phylogenetic relationships of genera in one of the major clades of Annonaceae. The genera *Ancana*, *Guamia*, and *Polyaulax*, which have been included in *Meiogyne* by Van Heusden (1994), are also found to be embedded in *Meiogyne* with strong support. Unfortunately, no suitable material of *Cheni dendron* and *Oncodostigma* is available for DNA extraction. The two species of *Fitzalania* appeared to be sister to each other with maximum support. Therefore, the different color and appearance of the (inner) petals are a synapomorphy of *Fitzalania*. The sepal-like outer petals of *F. bidwillii* is apparently an autapomorphy.

The principle of monophyly is pivotal in the classification of Annonaceae, and several genera have been re-circumscribed or newly described in the recent past. Applying this principle to the situation of *Meiogyne* and *Fitzalania* would result in the transfer of species of *Meiogyne* to *Fitzalania*, according to Art. 11.3 of the International Code of Botanical Nomenclature (McNeill *et al.* 2006), as *Fitzalania* antedates *Meiogyne*.

However, there are good reasons to conserve the name *Meiogyne* against *Fitzalania*. Firstly, the former genus contains many more species. Secondly, *Meiogyne* is better known as it has a considerably larger distribution area covering many more countries. Finally, *Meiogyne* has lent its name to a dimeric sesquiterpenoid, meiogynin A, isolated from the bark of *Meiogyne cylindrocarpa* (Burck) Heusden, which has significant potential as an anti-cancer agent (Litaudon *et al.* 2009, Fotsop *et al.* 2010). Consequently, to maximize the stability of the names, it is appropriate to conserve *Meiogyne* against *Fitzalania*. Whereas at least 17 new combinations would be required if this proposal is not accepted, only two new combinations will be necessary (from *Fitzalania* to *Meiogyne*) if it is accepted.

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<sup>2</sup> = Chapter 2.

# Chapter 6

## A taxonomic revision and pollen morphology of the genus *Dendrokingstonia* (Annonaceae)

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### Abstract

The genus *Dendrokingstonia* (Annonaceae) is taxonomically revised and palynologically studied. Three species are recognized, one of which, *D. gardneri*, is described as new to science. One new combination, *D. acuminata*, is made. The genus occurs from southern Thailand to Peninsular Malaysia and Sumatra. On the basis of macromorphology and pollen characters, it is considered to be related to *Monocarpia*. Both genera show a combination of macromorphological characters that is rare in the family, i.e. considerably enlarged stigmas, leaves with percurrent tertiary veins, a highly reduced number of carpels per flower and relatively large monocarps with a thick, hard wall. Scanning and transmission electron microscopy show that the pollen grains of *Dendrokingstonia* and *Monocarpia* are monosulcate monads with a columellate infratectum and a more or less bulging intine at the sulcus.

**Key words:** new combination, new species, palynology, systematics, taxonomy

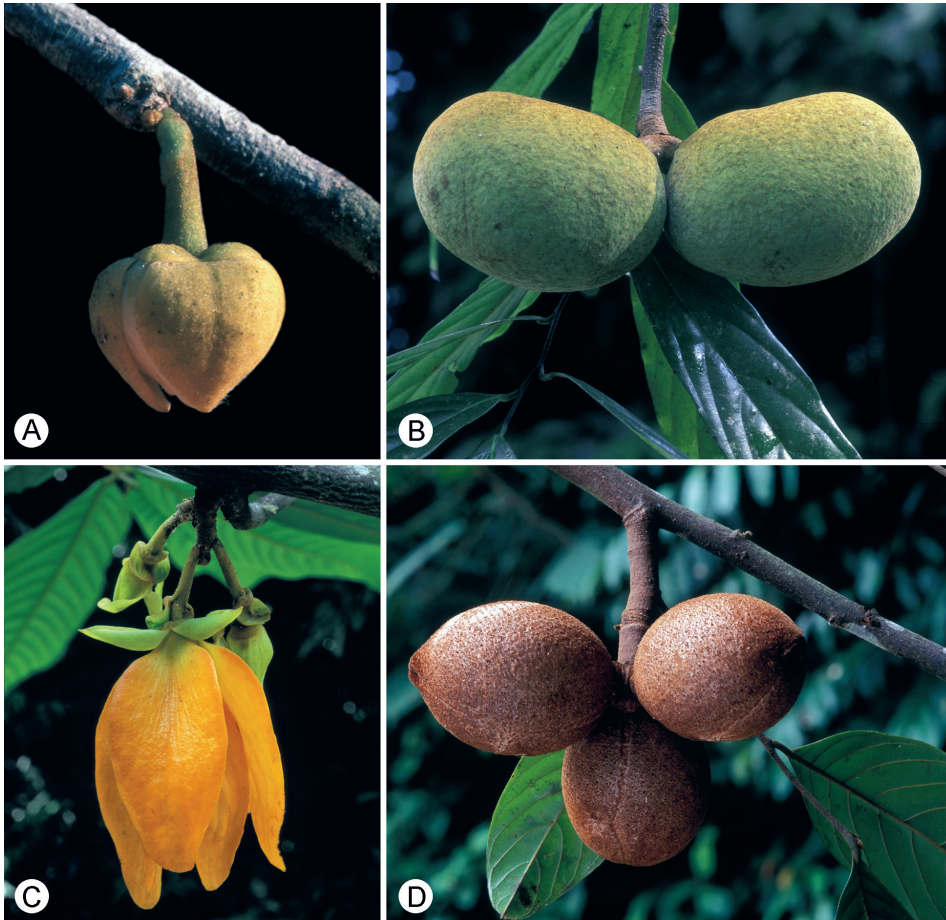
### Introduction

*Dendrokingstonia* is one of the most poorly known genera of Asian Annonaceae. It was described by Hooker & Thomson (1872) as *Kingstonia* Hook.f. & Thomson. Rauschert (1982) found that this name was pre-occupied by *Kingstonia* Gray (Saxifragaceae) and proposed the replacement name *Dendrokingstonia* Rauschert. So far, only a single species, *D. nervosa* (Hook.f. & Thomson) Rauschert, has been recognized. The genus was primarily characterized by the presence of only a single carpel per flower. In contrast, most Annonaceae have a flower with two or more carpels. Recent collections from Thailand with a fruit containing one or sometimes two monocarp(s) (Fig. 1B) appeared to be an undescribed species of *Dendrokingstonia*. Further, material from Sumatra described as *Bergsmia acuminata* Miq. (Flacourtiaceae/Achariaceae) turned out to represent a third *Dendrokingstonia* species. These two species are coherent with the type species in sharing all important diagnostic characters for the genus, i.e. leaves with percurrent tertiary venation, axillary flowers/inflorescences, considerably enlarged stigmas, highly reduced carpel numbers to one or two per flower, huge, thick-walled monocarps and four-part-lamellate ruminations of the endosperm.

In order to investigate the systematic affinity of *Dendrokingstonia*, several pollen morphological characters were included in the present revision. The pollen of one species of *Dendrokingstonia* had been studied earlier by Walker (1971a) using light microscopy (LM). In the present study, the pollen of all three species is investigated using scanning (SEM) and transmission (TEM) electron microscopy.

### Material and methods

The indumentum terminology used follows Hewson (1988). Pollen samples were taken from dried herbarium specimens. Following Chaowasku *et al.* (2008) and Couvreur *et al.* (2009), the pollen of *Dendrokingstonia* was not acetolysed for SEM; however, material used for comparison (*Monocarpia euneura* Miq.) concerns an acetolysed sample. For



**FIGURE 1.** Flowers and fruits of *Dendrokingstonia* and *Monocarpia*. A, B, *Dendrokingstonia gardneri*: A, flower; B, fruit with two monocarps. C, D, *Monocarpia marginalis* (Scheff.) J.Sinclair: C, flowers; D, fruit with three monocarps. [All photographs: Simon Gardner.]

TEM, all material was prepared according to the techniques described by Van der Ham (1990). The subdivision of the exine into tectum, infratectum and basal layer, as described by Le Thomas (1980), is used. The terminology of pollen features follows Punt *et al.* (2007).

*Voucher specimens for pollen morphological study:*

*D. acuminata*: Boschproefstation b.b. 2734 (L; SEM and TEM);  
*D. gardneri*: FRI 32134 (Wong Khoon Meng) (L; TEM), Kerr 19102 (K; SEM);  
*D. nervosa*: Rogstad 961 (L; SEM and TEM);  
*M. euneura*: Leighton 160 (L; SEM), Sidiyasa 737 (L; TEM).

*Specimens used in morphological study:*

*D. acuminata*: Boschproefstation b.b. 2734 (L); b.b. 3068 (L); Forbes 2654 (L); Lörzing 5523 (L); 12332 (L); 16531 (L); Teysmann HB 4559 (L, U).  
*D. gardneri*: FRI 6820 (Y.C. Chan) (L); 32134 (Wong Khoon Meng) (L); Gardner & Sidisunthorn ST 2214 (L); ST 2214a (L); ST 2575 (L); ST 2575a (L); Kerr 19102 (K).  
*D. nervosa*: FRI 0608 (T.C. Whitmore) (L); 2446 (K.M. Kochummen) (L); 16131 (K.M. Kochummen) (L); 17154 (Low Hoy Shing) (L); 26628 (K.M. Kochummen) (L); Goodenough 1329 (K); Rogstad 961 (L); Singapore Field 40149 (J. Sinclair) (L); Wray 3376 (K).

## Taxonomic treatment

***Dendrokingstonia*** Rauschert, Taxon 31: 555 (1982). = *Kingstonia* Hook.f. & Thomson, Fl. Brit. India [J.D. Hooker] 1: 93 (1872), *nom. illeg.*

**Type species:**—*Dendrokingstonia nervosa* (Hook.f. & Thomson) Rauschert.

(Small- or) medium-sized trees, (6–)11–24 m tall. Young twigs glabrous or hairy. Leaves petiolate, (narrowly) elliptic or oblong, base wedge-shaped, obtuse, rounded or subcordate, apex obtuse, (sub)acute or caudate-acuminate, midrib sunken on upper surface, raised on lower surface, secondary veins prominent on lower surface, tertiary veins somewhat percurrent. Flowers bisexual, solitary or in fascicles, axillary (usually in axils of the fallen leaves), peduncles absent or inconspicuous, pedicels 3–8 mm long, distinctly thickened in fruit, bracts (and bracteoles) observed. Sepals (broadly) ovate (-triangular), free or connate, slightly overlapping at the base when free, persistent in fruit. Outer petals heart-shaped or (narrowly) ovate(-oblong). Inner petals ovate(-oblong) or narrowly oblong. Stamens 9–11, 14 or 50–56 per flower, connective tissue flat-topped. Carpels 1 or 2 per flower, stigmas (irregularly) peltate, ovaries flattened-ovoid or (ellipsoid-)cylindrical, ovules 3–10 per ovary, lateral, uni- or biseriate. Torus flat or elevated, enlarged in fruit (= carpophores) when elevated. Monocarps 1 or 2 per fruit, sessile on unenlarged torus or on carpophores, subglobose or (irregularly) cylindrical, surface often wrinkled when dry, sometimes (coarsely) constricted (when dry), indumentum dense and short, monocarp wall rather thick, hard when dry. Seeds 2 to 9 per monocarp, in one or two rows, (flattened-)ellipsoid or (flattened-)D-shaped, raphe nearly flat or ridged,

endosperm ruminations lamelliform, divided into four equal parts (cross-section).

**Distribution:**–Southern Thailand, Peninsular Malaysia to Sumatra (Indonesia) (Fig. 5).

### Key to the species

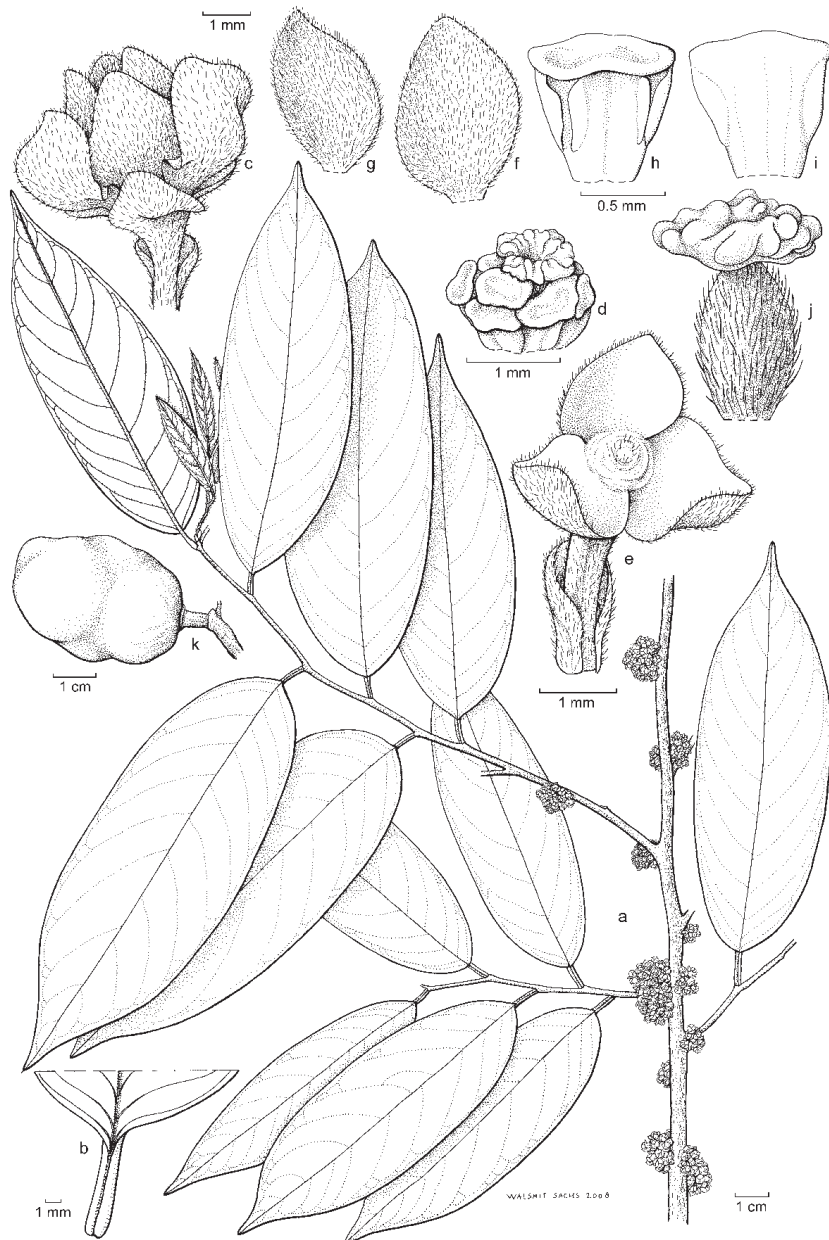
1. Petioles 3–4 mm long, leaf base wedge-shaped, flowers solitary, sepals connate, torus elevated, enlarged in fruit (= carpophores), carpels 2 per flower, stamens 50–56 per flower ..... **2. *D. gardneri*, sp. nov.**
- Petioles 4–11 mm long, leaf base usually obtuse or rounded, flowers in fascicles, sepals free, slightly overlapping at the base, torus flat, not enlarged in fruit, carpels 1 (occasionally 2 in *D. acuminata*) per flower, stamens 9–11 or 14 per flower..... 2
2. Upper surface of the petioles grooved, flower buds globose, bracteoles generally placed at the midpoint of the pedicels, each fascicle usually with  $\geq 15$  flowers, pedicels 3–5(–6) mm long, outer petals and inner petals ovate(-oblong), outer petals 3.5–4 mm long, inner petals 2.9–3.5 mm long ..... **1. *D. acuminata*, comb. nov.**
- Upper surface of the petioles not (or hardly) grooved, flower buds ellipsoid, bracteoles immediately placed below the sepals, each fascicle usually with  $< 15$  flowers, pedicels (5.5–)6–8 mm long, outer petals narrowly ovate, ca. 8 mm long, inner petals narrowly oblong, ca. 7.1 mm long ..... **3. *D. nervosa***

#### **1. *Dendrokingstonia acuminata* (Miq.) Chaowasku, comb. nov. (Figs. 2, 5)**

= *Bergsmia acuminata* Miq., Fl. Ned. Ind., Eerste Bijv. 389 (Miquel, 1861).

**Type:**–Indonesia, Sumatra, Lampong, in fruit, *Teysmann HB 4559* (holotype L0189162!; isotype U!).

Trees 18–20 m tall. Young twigs glabrous to sparsely puberulous. Bud scales observed only at the base of new sprouting shoots, several, ovate to hemispherical, sometimes cup-shaped, basal ones smaller and smaller, respectively. Petioles 5–9 mm long, glabrous to sparsely (appressed-)puberulous, grooved above. Leaves elliptic, 6.5–16 × 2.8–5.7 cm, base usually obtuse, sometimes wedge-shaped or rounded, apex subacute to caudate-acuminate, lamina glabrous above, (almost) glabrous below, upper surface of midrib sunken, (almost) glabrous, raised on lower surface, glabrous to sparsely appressed-pilose, secondary veins 8–13 pairs per leaf, sometimes slightly impressed above, prominent below, angle with midrib 23°–45°. Flowers in fascicles, each fascicle usually with  $\geq 15$  flowers, axillary (mostly in axils of the fallen leaves), peduncles inconspicuous, pedicels 3–5(–6) mm long, (appressed-)tomentose, 6–9 × 3–4 mm in fruit, bracts several (to many), hemispherical-ovate, bracteoles usually 1 per flower, same shape as the bracts, usually placed at the midpoint of the pedicels. Sepals slightly ovate, slightly overlapping at the base, 1.7–1.9 × 1.6–2.1 mm, outside (appressed-)puberulous, denser near the base, margin puberulous, inside glabrous, persistent in fruit. Outer petals ovate(-oblong), 3.5–4 × 2.3–2.7 mm, outside (densely) (appressed-)tomentose, margin tomentose-cobwebbed, inside appressed-puberulous to tomentose, (much) sparser near the base. Inner petals same shape as the outer ones, 2.9–3.5 × 1.5–2.2 mm, indumentum on outside and margin same as the outer ones, inside (appressed-)villous, (much) sparser near the base. Stamens 9–11 per flower, 0.9–1 mm long. Carpels 1 (occasionally 2) per flower, 1.3–1.4 mm long, stigmas (irregularly) peltate with crenate margin, ca. 1 mm in diameter,



**FIGURE 2.** *Dendrokingstonia acuminata*: a, habit with flowers; b, upper leaf surface, showing grooved petiole; c, mature flower, side view; d, stamens surrounding single carpel; e, flower with petals, stamens and carpel detached, top view, showing flat torus and sepals slightly overlapping at the base; f, inside of outer petal; g, inside of inner petal; h, stamen, abaxial view; i, stamen, adaxial view; j, carpel; k, monocarp. [a–j, Lörzing 12332; k, *Boschproefstation* b.b. 3068.]

ovaries more or less ellipsoid-cylindrical, (appressed-)villous, slightly flattened on one side, ovules 3–6 per ovary, lateral, uni- or biseriate. Torus flat, not enlarged in fruit. Monocarps 1 per fruit, sessile on unenlarged torus, subglobose to slightly cylindrical, sometimes somewhat constricted at the middle of the monocarps (when dry), 3.8–5 × 2.8–3.3 cm, surface (a bit) wrinkled when dry, indumentum densely (appressed-)tomentose, monocarp wall ca. 2 mm thick. Seeds 2–6 per monocarp, in one or two rows, more or less ellipsoid to D-shaped, 2.6–2.7 × 1.4–1.5 cm, raphe ridged.

**Distribution:**—Endemic to Sumatra (Indonesia) (Fig. 5).

**Habitat, ecology and phenology:**—Occurring in primary forests on slopes. Elevation: 50–500 m. Flowering specimens: collected in November and February. Fruiting specimens: collected in May.

**Field notes:**—Branches many. Wood hard. Leaves faintly bluish, somewhat glossy, rather stiff (red–brown when young). Flowers fragrant, petals yellow. Fruits pale green with short brown hairs.

**Vernacular names:**—Bakbahan, Benitan teloer.

**Notes:**—Van Setten & Koek-Noorman (1992) studied and illustrated the fruit and seed morphology of *Lörzing 16531* under *Dendrokingstonia nervosa*.

According to Webber & Woodrow (2006), the genus *Bergsmia* Blume is now included in *Ryparosa* Blume (Achariaceae).

Based on macromorphology, *D. acuminata* is close to *D. nervosa*. It differs mainly in having (many) more flowers per fascicle, globose floral buds (ellipsoid in *D. nervosa*), usually (slightly) shorter pedicels and shorter petals (both whorls). Vegetatively, these two species are much alike, except for the petioles, which are grooved on the upper surface in *D. acuminata* (Fig. 2b), and are (hardly or) not grooved in *D. nervosa*.

## 2. *Dendrokingstonia gardneri* Chaowasku, sp. nov. (Figs. 1A, B, 3, 5)

**Type:**—Thailand, Trang, Khao Banthat Wildlife Sanctuary, Ton Plew Waterfall, x.2006, in fruit, *Gardner & Sidisunthorn ST 2575a* (holotype L0298137!; isotypes BKF, K).

*Dendrokingstoniae nervosae* et *D. acuminatae aliquantum diversa, floribus solitariis* (vs. *fasciculatis*), *toro elevato* (vs. *plano*), *sepalis connatis* (vs. *liberis*), *staminum multum plurimorum differt. Praeterea in D. gardneri petioli plerumque breviores, folii basis semper cuneata, versus obtusa ad rotundata in alioribus.*

Trees (6–)11–15(–20) m tall, (8–)17 cm in diameter, ca. 45 cm in girth. Young twigs sparsely appressed-puberulous. Petioles 3–4 mm long, almost glabrous to sparsely (appressed-) puberulous, slightly grooved above. Leaves (narrowly) elliptic to slightly oblong, 7.3–18.2 × 2.2–6 cm, base wedge-shaped, apex obtuse, subacute to caudate-acuminate, lamina glabrous above, (almost) glabrous below, domatia sometimes observed at axils of the secondary veins, each with a small hole or slightly sunken area, usually with few hairs, upper surface of midrib (slightly) sunken, glabrous, raised on lower surface, glabrous to very sparsely appressed-puberulous, secondary veins 6–11 pairs per leaf, prominent below, angle with midrib 26°–37°. Flowers solitary, axillary (in axils of the fallen leaves), peduncles absent, pedicels 4–5.2 mm long, (sparsely) (appressed-)tomentose, 7–8(–10) × 4–6 mm in fruit, bracts 2 or 3 per flower, hemispherical, ovate to triangular, one or two near the base, another a bit higher, slightly larger. Sepals broadly ovate(-triangular),



**FIGURE 3.** *Dendrokingstonia gardneri*: a, habit with fruit; b, flower with petals, stamens and carpels detached, side view, showing elevated torus; c, same as (b), but in bottom view, showing connate sepals; d, inside of outer petal; e, inside of inner petal, showing slightly saccate base; f, stamen, abaxial view; g, stamen, adaxial view; h, carpel. [a, Gardner & Sidisunthorn ST 2214a; b, c, h, FRI 32134 (Wong Khoon Meng); d–g, Kerr 19102.]

connate (generally from the midpoint of their length through the base), 1.7–2 × 2.1–3 mm, outside and margin puberulous, inside glabrous, usually persistent in fruit. Outer petals ovate to slightly heart-shaped, 5.1–6.2 × 4.1–5.5 mm, outside and margin densely tomentose-cobwebbed, inside generally sparser, especially towards the base, base sometimes slightly saccate. Inner petals ovate, 5–6.1 × 3.1–3.5 mm, indumentum same as the outer ones, base slightly saccate. Stamens 50–56 per flower, 1–1.3 mm long. Carpels 2 per flower, 1.8–2.1 mm long, stigmas irregularly peltate, 0.8–1.2 mm in diameter, very sparsely puberulous at the base, ovaries flattened-ovoid, grooved on one side, densely (appressed-)tomentose, ovules 8–10 per ovary, lateral, biseriate. Torus elevated, truncated cone-shaped, 1.1–2 mm long, apex slightly sunken, base tomentose around the torus, which is enlarged in fruit (= carpophores). Monocarps 1 to 2 per fruit, sessile on carpophores, irregularly (slightly) cylindrical, 5.9–6.7 × 4.5–5 cm, surface often coarsely constricted (when dry), indumentum densely tomentose, monocarp wall 2–4 mm thick, carpophores woody, 3–6 × 7–10 mm for each monocarp. Seeds ca. 9 per monocarp, in two rows, flattened-ellipsoid to flattened-D-shaped, 3.2–3.6 × 2.3–2.4 cm, raphe nearly flat to slightly ridged.

**Distribution:**—Southern Thailand and northern Peninsular Malaysia (Fig. 5).

**Habitat, ecology and phenology:**—Occurring in undulating forests or in shady understorey of (primary) lowland (moist) evergreen forests; at base of limestone cliffs or on limestone tufa next to permanent waterfalls. Elevation: under 50 m to 130 m. Flowering specimens: collected in February and April. Fruiting specimens: collected in October, January and March.

**Field notes:**—Bole straight, monopodial. Bark smooth or rugged, dark brown, greyish-green, pale grey or midgrey, slash inner bark fibrous, yellow or brown, slash wood white or pale yellowish-white. Leaves glossy dark green above, paler green below, young leaves whitish. Flowers downturned, sepals yellow in open flowers, petals cream or dull brownish-yellow. Fruits pale brownish-yellow outside, pale yellow inside.

**Etymology:**—Named after the chief collector (Simon Gardner) who kindly sent the interesting specimens to L for identification.

**Notes:**—*Dendrokingstonia gardneri* is considerably different from the other two species in having solitary flowers (in fascicles in the other two species) with an elevated torus (Fig. 3b) that is enlarged in fruit (= carpophores, Fig. 3a) (flat and not enlarged in fruit in the others), connate sepals (Fig. 3c) (free in the others), thicker petals and many more stamens (50–56 vs. 9–11 or 14). The petioles are generally shorter and the leaf base is always wedge-shaped in *D. gardneri*, whereas it is usually obtuse or rounded in *D. nervosa* and *D. acuminata*.

The four-part-lamellate ruminations of the endosperm of *D. gardneri* are less pronounced than those of the other two species. They are more or less lamellate towards the raphe with some flattened pegs in the middle of the sides, as illustrated in figure 3 of Van Setten & Koek-Noorman (1992).

**3. *Dendrokingstonia nervosa* (Hook.f. & Thomson) Rauschert (Figs. 4, 5). Taxon 31: 555 (1982).**

= *Kingstonia nervosa* Hook.f. & Thomson<sup>1</sup>, Fl. Brit. India [J.D. Hooker] 1: 93 (1872).  
= *Bocagea nervosa* (Hook.f. & Thomson) Pierre, Fl. Forest. Cochinch. sub t. 16 (Pierre, 1881).

**Type:**—Peninsular Malaysia, Malacca, ix.1865–66, in flower, *Maingay 1054* (also under herbarium of the late A.C. Maingay, M.D. No. 22, distributed at the Royal Gardens Kew. 1871.) [lectotype K! (photograph), selected here].

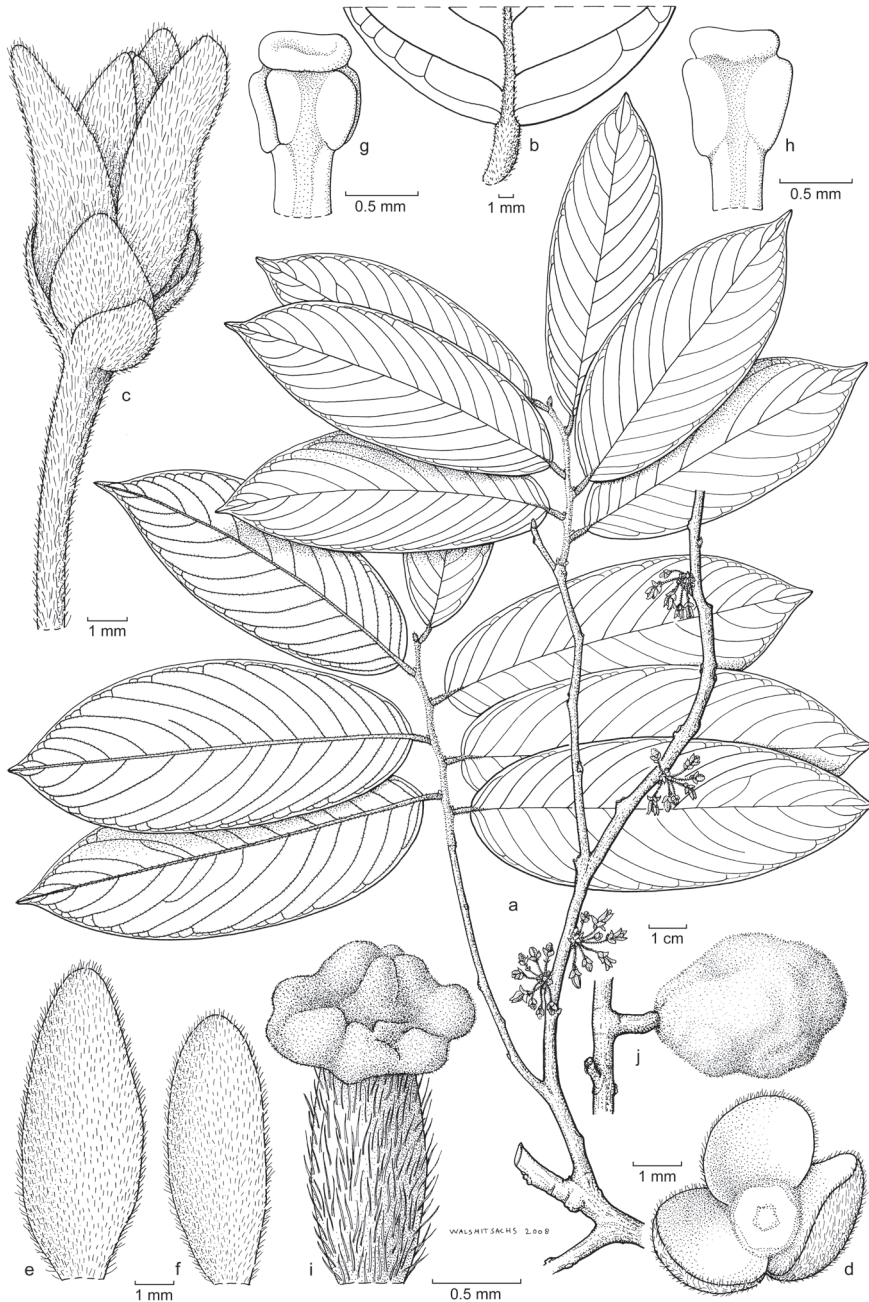
Trees 12–24 m tall, 60–90 cm in girth. Young twigs sparsely puberulous to sparsely tomentose, isolated hairs occasionally stellate. Petioles 4–11 mm long, sparsely (appressed-)puberulous to sparsely tomentose, isolated hairs sometimes stellate, not (or hardly) grooved above. Leaves elliptic to slightly oblong, 8.5–22.4 × 3–8 cm, base obtuse to rounded, sometimes subcordate, apex acute to slightly caudate-acuminate, lamina glabrous both sides except the secondary veins on lower surface which is almost glabrous to appressed-pilose, upper surface of midrib sunken, less so near the petioles, sparsely pilose near the petioles, sparser to glabrous towards another end, raised on lower surface, (sparsely) appressed-pilose, sometimes also with shorter erect hairs, secondary veins 12–14 pairs per leaf, prominent below, angle with midrib 30°–43°. Flowers in fascicles, each fascicle usually with < 15 flowers, axillary (in axils of the fallen leaves), peduncles inconspicuous, pedicels (5.5–)6–8 mm long, (appressed-)tomentose, 1.1–1.4 × 0.4–0.6 cm in fruit, bracts several, hemispherical to ovate, bracteoles 1 or 2 per flower, same shape as the bracts, immediately placed below the sepals. Sepals slightly ovate, slightly overlapping at the base, ca. 2.8 × 2.4 mm, outside (appressed-)tomentose, margin tomentose, inside glabrous, persistent in fruit. Outer petals narrowly ovate, ca. 8 × 2.9 mm, outside and margin densely (appressed-)tomentose, inside shortly cobwebbed. Inner petals narrowly oblong, ca. 7.1 × 2.1 mm, indumentum a bit denser than the outer ones. Stamens 14 per flower, 1.1–1.3 mm long. Carpels 1 per flower, ca. 1.9 mm long, stigmas irregularly peltate, ca. 1 mm in diameter, ovaries more or less cylindrical, slightly grooved on one side, (appressed-)villous, ovules 6 or 7 per ovary, lateral, biseriate. Torus flat, not enlarged in fruit. Monocarps 1 per fruit, sessile on unenlarged torus, subglobose to slightly cylindrical, 4–5.1 × 3.3–3.9 cm, surface (a bit) wrinkled when dry, indumentum densely tomentose, monocarp wall 4–5 mm thick. Seeds 5 or 6 per monocarp, in two rows, more or less flattened-D-shaped, 2.6–3.1 × 1.2 cm, raphe almost flat to slightly ridged.

**Distribution:**—Peninsular Malaysia (Fig. 5).

**Habitat, ecology and phenology:**—Occurring in logged forests, disturbed forests or dense (primary) forests; on (steep) hillsides, on slight well-drained slopes with little humus accumulation or on flat lands; over granites or on sandy soils. Elevation: up to 300 m. Flowering specimens: collected in February. Fruiting specimens: collected in June and July.

**Field notes:**—Crown dense, rounded or bushy, sympodial or monopodial. Bark smooth or finely fissured and cracked, dark grey–green or black with grey patches, slash inner bark pale brown or orange brown, slash wood pale yellow or pale brown near white. New leaves pale pink. Mature petals yellow, with odor of ripe peaches, inner petals

<sup>1</sup> Incorrectly indicated as “nom. illeg.” in the original publication of this chapter.



**FIGURE 4.** *Dendrokingstonia nervosa*: a, habit with flowers; b, lower leaf surface; c, slightly submature flower; d, flower with petals, stamens and carpel detached, top view, showing flat torus and sepals slightly overlapping at the base; e, inside of outer petal; f, inside of inner petal; g, stamen, abaxial view; h, stamen, adaxial view; i, carpel; j, monocarp. [a–i, Rogstad 961; j, FRI 0608 (T.C. Whitmore).]



**FIGURE 5.** Distribution of *Dendrokingstonia*: *D. acuminata* (filled triangles); *D. gardneri* (filled squares); *D. nervosa* (filled circles).

overlapping in bud, stigmas turning black before pollen is released. Fruits (pale) brown, covered with fine hairs, shallowly ridged, mesocarp white, seeds pink, shiny.

Despite the noticeable differences between *D. acuminata* and *D. nervosa*, on the one hand, and *D. gardneri*, on the other, the three species are uniform in sharing a combination of several important characters (see Discussion and Conclusions).

### Pollen description

Pollen grains (Figs. 7A–F, 8A–F) bilateral, monosulcate, medium-sized monads; long axis ca. 28–40  $\mu\text{m}$ . Exine 0.8–1.6  $\mu\text{m}$  thick, tectate; tectum 0.4–0.6  $\mu\text{m}$  thick; infratectum columellate, up to  $\pm$  as thick as the tectum; basal layer  $\pm$  distinct, thinner than the tectum. Exine ornamentation psilate/perforate (*D. acuminata*, *D. gardneri*) or scabrate/verrucate/rugulate (*D. nervosa*). Intine moderately (*D. acuminata*, *D. nervosa*) or strongly (*D. gardneri*) bulging outwards from the sulcus, consisting of an exintinous and an endintinous sublayers.

### Discussion

#### Systematic position of *Dendrokingstonia*

*Dendrokingstonia* exhibits a combination of several characteristics that is rather uncom-

mon in Annonaceae, i.e. leaves with percurrent tertiary veins, a highly reduced number of carpels per flower, considerably enlarged stigmas (Figs. 2j, 3h, 4i) and relatively large monocarps with a thick, hard wall (Fig. 1B).

Van Heusden (1992) found that the carpels of *Dendrokingstonia* resemble those of *Monocarpia* Miq. and *Piptostigma* Oliv., two ‘short branch clade’<sup>2</sup> (SBC) genera outside the ‘miliusoid clade’<sup>3</sup> (Richardson *et al.* 2004, Mols *et al.* 2004b; see Fig. 6). The SBC minus the miliusoid clade is referred to in the present article as the ‘non-miliusoid grade’ (NMG) (Fig. 6). On account of the carpel characters, *Dendrokingstonia* might be provisionally placed in the NMG, and is especially close to the small South-East Asian genus *Monocarpia* (Fig. 1C, D; Mols & Keßler 2000b), as both genera possess all four characters mentioned above. However, the position and appearance of the flowers/inflorescences of the two genera differ: terminal, conspicuous in *Monocarpia* (pers. obs. TC; Fig. 1C) vs. axillary, inconspicuous in *Dendrokingstonia* (Fig. 1A). Their endosperm ruminations are also different: spiniform in *Monocarpia* vs. lamellate in four parts in *Dendrokingstonia* (Van Setten & Koek-Noorman 1992).

The genus *Platymitra* Boerl., which belongs in the miliusoid clade of the SBC (Richardson *et al.* 2004, Mols *et al.* 2004b), also possesses a reduced number of carpels per flower and large, hard-walled monocarps. However, it lacks the percurrent tertiary veins of the leaves and the elaborated stigmas (Keßler 1988b).

It is important to note that most genera in the SBC, including the earlier mentioned genera (*Monocarpia*, *Piptostigma*, *Platymitra*) have spiniform ruminations of the endosperm (Van Setten & Koek-Noorman 1992). This type of endosperm ruminations is considered to be one of the synapomorphic characters of the SBC. The four-parted lamelliform ruminations of the endosperm appear to be a derived character. They only occur in certain genera in the NMG (Chatrou 1998) and the miliusoid clade (Mols *et al.* 2004a).

Several carpel characters of *Dendrokingstonia* also occur outside the SBC, in the more distant ‘ambavioid clade’ [the ambavioid clade is redefined here; it comprises *Ambavia* Le Thomas, *Cleistopholis* Pierre ex Engl., *Mezzettia* Becc. and *Tetrameranthus* R.E.Fr., as originally circumscribed by Doyle & Le Thomas (1996) and Le Thomas & Doyle (1996); the ambavioid clade in a redefined sense is sister to the canangoid clade (see Surveswaran *et al.* 2010); both clades differ in chromosome numbers (Okada & Ueda 1984, Morawetz & Le Thomas 1988), floral morphology (pers. obs. TC; see also Van Heusden 1992 for more details on each genus) and, probably, the branching architecture (Johnson 2003; pers. obs. TC)] and ‘canangoid clade’ (see Pirie *et al.* 2006: figure 2 for the phylogenetic position of both clades in Annonaceae). The reduction in carpel numbers to one per flower and the huge, stony-walled monocarps are found in *Mezzettia* Becc. and *Cyathocalyx* Champ. ex Hook.f. & Thomson *sensu stricto* (Van der Heijden & Keßler 1990, Surveswaran *et al.* 2010). *Cyathocalyx* s.s. also has greatly enlarged stigmas (Van Heusden 1992).

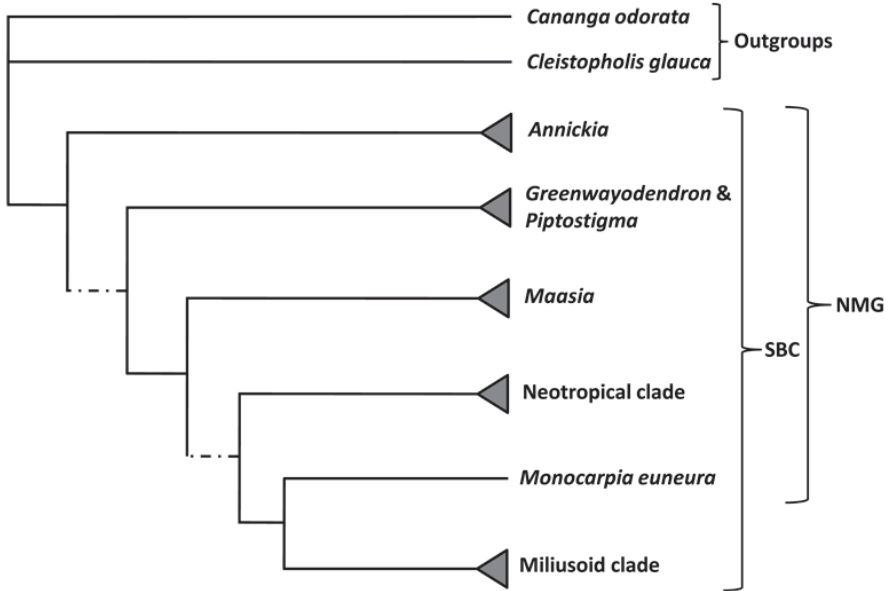
The occurrence of the characters mentioned above is summarized in Table 1.

### Pollen morphology

Walker (1971a) described the pollen of *D. nervosa* (as *Kingstonia nervosa*), using LM, as

<sup>2</sup> Now recognized as subfamily Malmeoideae (see Chatrou *et al.* 2012).

<sup>3</sup> Equivalent to the recircumscribed Miliuseae of Chatrou *et al.* (2012).

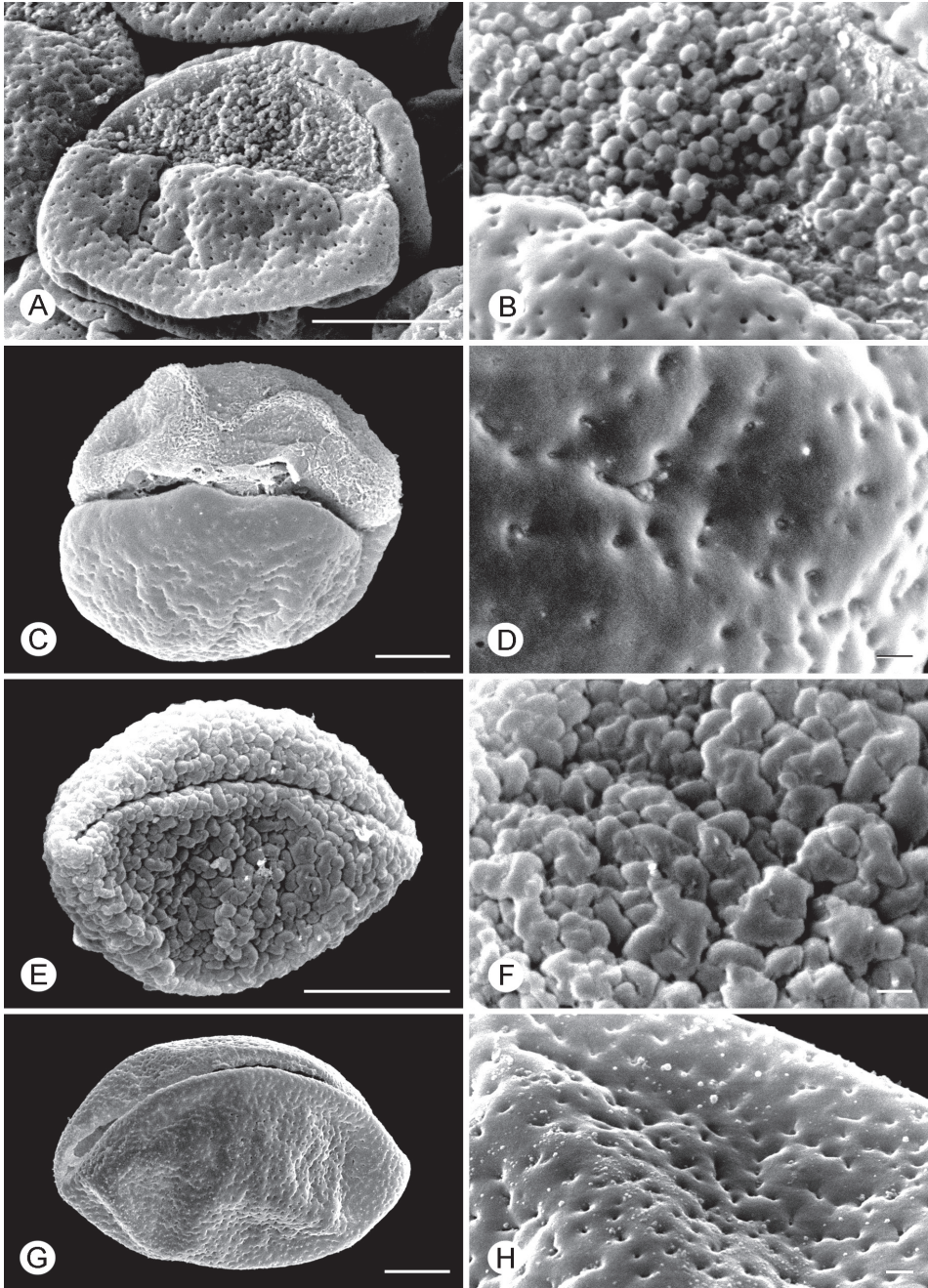


**FIGURE 6.** Simplified cladogram adapted from figure 4 of Pirie *et al.* (2006). Triangles signify at least two terminal taxa. Branches with Bayesian posterior probabilities lower than 0.95 are shown as broken lines. NMG, ‘non-miliusoid grade’; SBC, ‘short branch clade’.

**TABLE 1.** Phylogenetic placement and occurrence of important characters found in *Dendrokingstonia* and other (related) genera.

Character	Genus				
	<i>Dendrokingstonia</i>	<i>Monocarpia</i>	<i>Platymitra</i>	<i>Cyathocalyx s.s.</i>	<i>Mezzettia</i>
- Tertiary venation of leaves	Percurrent	Percurrent	Reticulate	Percurrent/intermediate	Reticulate
- Flower/inflorescence position	Axillary	Terminal	Axillary	Terminal	Axillary
- Enlarged stigmas, ± peltate	+	+	-	+	-
- Reduced number of carpels to 1–4 per flower	+	+	+	+	+
- Relatively large monocarps with hard and thick wall	+	+	+	+	+
- Ruminations of endosperm	Four-part-lamelate	Spiniform	Spiniform	Intermediate	Irregular
- Major pollen types	Monosulcate	Monosulcate	Cryptoaperturate	Inaperturate	Inaperturate
- Phylogenetic position	SBC-NMG?	SBC-NMG	SBC-miliusoid	Canangoid	Ambavioid

+, present; -, absent; NMG, ‘non-miliusoid grade’; SBC, ‘short branch clade’



**FIGURE 7.** Pollen of *Dendrokingstonia* and *Monocarpia* (scanning electron microscopy). A, B, *D. acuminata*: A, pollen grain, collapsed, showing psilate/perforate exine (below) and granular sulcus membrane (above); B, detail of (A), showing exine ornamentation. C, D, *D. gardneri*: C, pollen grain, showing psilate/perforate exine (below) and bulging intine (above); D, detail of (C), showing exine ornamentation. E, F, *D. nervosa*: E, pollen

...continued on page 103

boat-shaped, monosulcate, medium-sized monads with a well-developed columellate infratectum. He did not describe the exine ornamentation, but his LM illustrations show an exine with verrucate to scabrate elements. We obtained the same results for *D. nervosa*. The exines of the pollen of *D. acuminata* and *D. gardneri* are much like that of *D. nervosa*, except for their ornamentation, which is psilate/perforate.

Within the SBC, the combination of a monosulcate aperture, a columellate infratectum and a more or less bulging intine is known only from several genera in the NMG: *Bocageopsis* R.E.Fr., *Malmea* R.E.Fr., *Unonopsis* R.E.Fr. (Waha 1985), *Maasia* Mols, Kessler & Rogstad [under '*Polyalthia*' *sumatrana* (Miq.) Kurz; Waha & Hesse 1988], *Monocarpia* (Figs. 7G–H, 8G–H) and *Mwasumbia* Couvreur & D.M.Johnson (Couvreur *et al.* 2009). The NMG is largely African-American; *Maasia* and *Monocarpia* are the only Asian genera. So far, the pollen of all genera studied in the NMG is monosulcate. It is expected that other genera in the NMG that have not been palynologically investigated also possess monosulcate pollen. The pollen morphology has shifted from monosulcate to cryptoaperturate/disulcate in the miliusoid clade (Mols *et al.* 2004a, Chaowasku *et al.* 2008).

*Dendrokingstonia* is unlikely to be part of the ambavioid, canangoid or miliusoid clades because all members of these clades do not exhibit the combination of pollen characters mentioned above (Le Thomas 1980, Hesse & Waha 1984, Mols *et al.* 2004a, Wang 2004, Chaowasku *et al.* 2008).

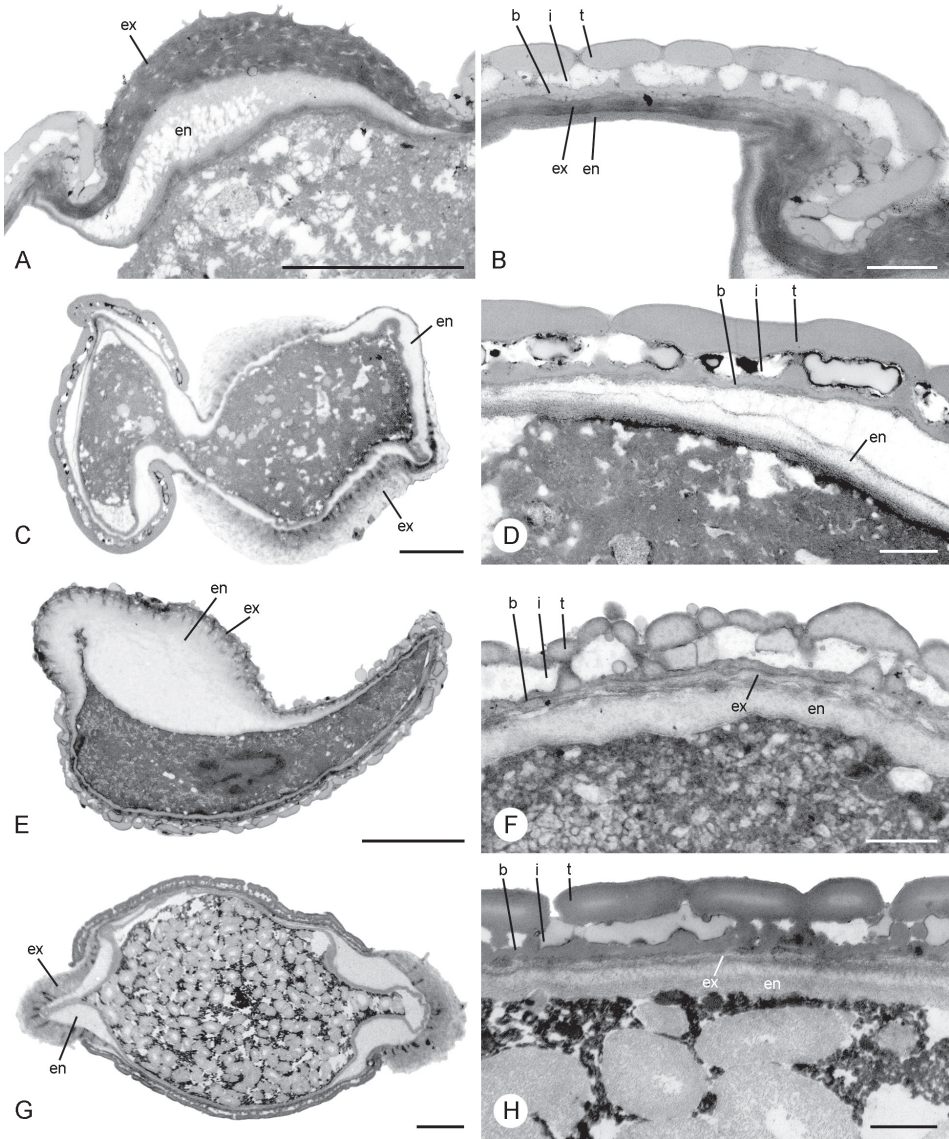
## Conclusions

Macromorphological, pollen morphological and geographical evidence indicates that the systematic affinity of *Dendrokingstonia* is with the NMG, the basal part of the SBC. *Dendrokingstonia* might be closely related to *Monocarpia*, because both genera share several crucial characters (Table 1).

*Dendrokingstonia* consists of three species (*D. acuminata*, *D. gardneri* and *D. nervosa*) and is presently circumscribed by having the following characters:

1. Leaves with percurrent tertiary venation.
2. Axillary flowers/inflorescences.
3. Considerably enlarged stigmas.
4. Carpel numbers reduced to one or two per flower.
5. Massive monocarps with a thick, hard wall.
6. Four-lamellate ruminations of the endosperm.
7. Monosulcate pollen grains, with a columellate infratectum and a more or less bulging intine at the sulcus.

← grain, collapsed, showing scabrate/verrucate exine with invaginated sulcus; F, detail of (E), showing ornamentation. G, H, *Monocarpia euneura*: G, pollen grain, collapsed, showing psilate/perforate exine with invaginated sulcus; H, detail of (G), showing ornamentation. Scale bars: 10 µm (A, C, E, G); 1 µm (B, D, F, H).



**FIGURE 8.** Pollen of *Dendrokingstonia* and *Monocarpia* (transmission electron microscopy). A, B, D. *acuminata*: A, cross-section of sulcus with intine moderately bulging outwards (exintine dark, endintine light); B, cross-section of exine near sulcus (right), showing tectum, columellate infratectum and basal layer. C, D. *D. gardneri*: C, cross-section of pollen grain, showing exine (left) and intine (right) strongly bulging outwards; D, cross-section of exine, showing tectum, columellate infratectum and basal layer. E, F. *D. nervosa*: E, cross-section of pollen grain, showing exine (below) and intine (above) moderately bulging outwards; F, cross-section of exine, showing tectum, columellate infratectum and basal layer. G, H. *Monocarpia euneura*: G, pollen grain, showing two cross-sections of the long sulcus with intine moderately bulging outwards; H, cross-section of exine, showing tectum, columellate infratectum and basal layer. Scale bars : 5  $\mu\text{m}$  (A, C, E, G); 1  $\mu\text{m}$  (B, D, F, H); b, basal layer; en, endintine; ex, exintine; i, infratectum; t, tectum.

# Chapter 7

## Seven new species of *Miliusa* (Annonaceae) from Thailand

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### Abstract

Seven new species of the genus *Miliusa* are described from Thailand (*M. fragrans*, *M. hirsuta*, *M. intermedia*, *M. nakhonsiana*, *M. sessilis*, *M. thailandica*, and *M. umpangensis*). A key to the 19 species of *Miliusa* in Thailand is provided. In addition, the complete taxonomic nomenclature of all known species of *Miliusa* in Thailand is given, with several new proposed synonyms. The new as well as the known species of *Miliusa* in Thailand are classified into four morphological groups on the basis of a combination of flower and/or inflorescence position and inner petal morphology proposed earlier.

### Introduction

*Miliusa* Lesch. ex A.DC. is an Asian palaeotropical genus with ca. 50 species occurring from the Indian subcontinent through Indochina, Peninsular Malaysia, the Southeast Asian islands to New Guinea and northern Australia (Mols & Keßler 2003, Chaowasku & Keßler 2006). The genus consists of shrubs or small to large trees inhabiting evergreen or drier (e.g. deciduous or Dipterocarp forests) habitats (Mols & Keßler 2003). According to the results of molecular phylogenetic analyses (Chatrou *et al.* 2012), *Miliusa* belongs to the tribe Miliuseae of the subfamily Malmeoideae. The tribe Miliuseae consists of predominantly Asian-Australasian genera, with a clade of four neotropical genera nested within (see phylogenetic trees in Saunders *et al.* 2011, Xue *et al.* 2011, Chaowasku *et al.* 2013b). In addition, *Hubera* Chaowasku, a newly described genus of the Miliuseae and a sister clade of *Miliusa*, has been found to occur in Madagascar and eastern Africa, apart from Asia, Australia and Melanesia (Chaowasku *et al.* 2012a). All members of palynologically investigated Miliuseae thus far possess cryptoaperturate or disulculate pollen (Waha & Hesse 1988, Waha & Morawetz 1988, Chaowasku *et al.* 2008, 2012a), which is considered one of the synapomorphies of this tribe.

*Miliusa* is circumscribed by the following characters: 1) equally-sized sepals and outer petals, both of which are much smaller than the inner petals, 2) a densely hairy torus, 3) miliusoid stamens, i.e. stamens without conspicuously dilated connective tissue covering the thecae, and 4) four-part-lamellate ruminations of the endosperm (Chaowasku & Keßler 2006). The morphology of the inner petals of *Miliusa* is very diverse. In combination with the flower and/or inflorescence position, four informal groups can be categorized (Chaowasku & Keßler 2006, Chaowasku *et al.* 2008):

### 1. The *Miliusa mollis* group

Flowers and/or inflorescences axillary; inner petals completely open at anthesis, with crescent-shaped to semicircular glandular structures inside at (or  $\pm$  near) base (Figs. 1A, 2A), base not saccate. Included Thai species: *M. amplexicaulis* Ridl., *M. fragrans* sp. nov., *M. fusca* Pierre, *M. intermedia* sp. nov., *M. mollis* Pierre, *M. nakhonsiana* sp. nov. and *M. sessilis* sp. nov.

### 2. The *Miliusa horsfieldii* group (former *Saccopetalum* Benn. species)

Flowers and/or inflorescences terminal (developing to internodal; sometimes appearing as ‘axillary’); inner petals completely open at anthesis, with narrow, longitudinal (linguiform to linear) glandular structures inside along midrib (Fig. 1B; sometimes less noticeable), base (moderately to) conspicuously saccate. Included Thai species: *M. horsfieldii* (Benn.) Baill. ex Pierre and *M. sclerocarpa* (A.DC.) Kurz.

### 3. The *Miliusa velutina* group

Flowers and/or inflorescences terminal (developing to internodal; sometimes appearing as ‘axillary’); inner petals completely open at anthesis, without pronounced glandular structures inside (Fig. 1D), base usually not to slightly saccate, rarely moderately saccate. Included Thai species: *M. parviflora* Ridl. and *M. velutina* (DC.) Hook.f. & Thomson.

### 4. The *Miliusa campanulata* group [*Miliusa sensu stricto sensu* Chaowasku *et al.* (2008)]

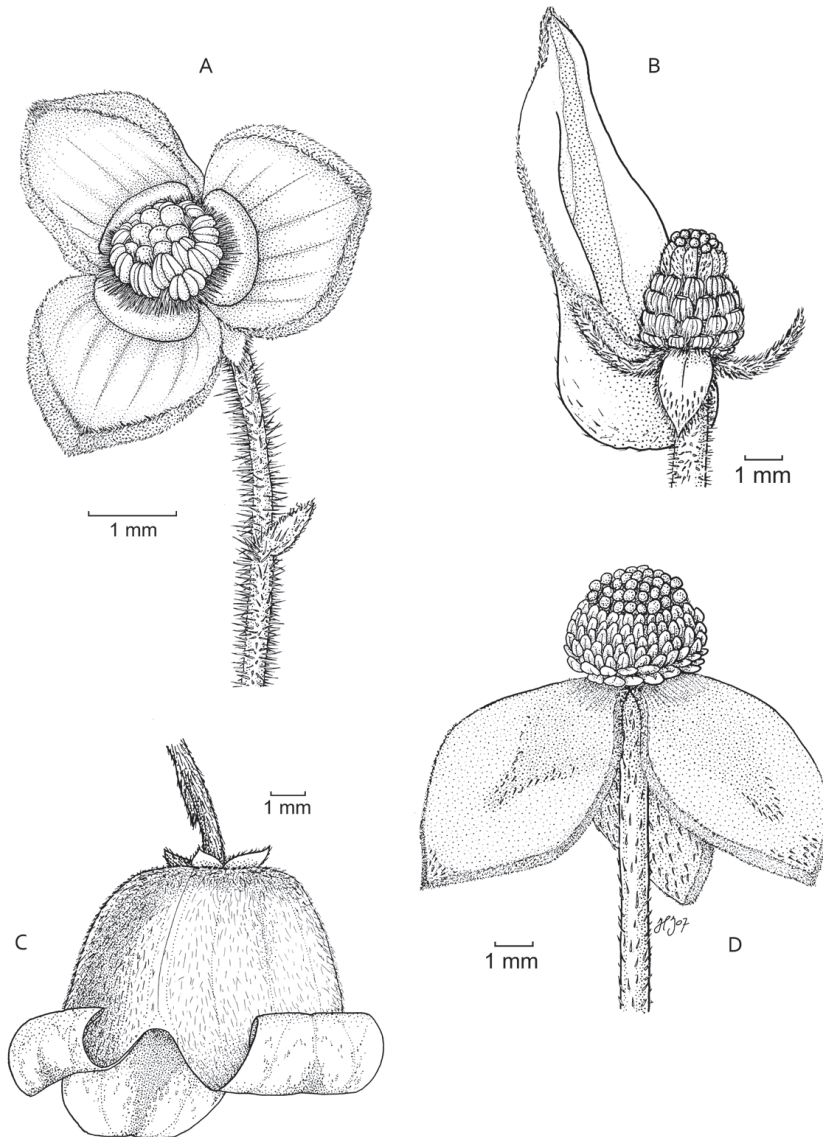
Flowers and/or inflorescences axillary in most species; inner petals usually tightly appressed from the base to  $\pm$  the midpoint at anthesis (Figs. 1C, 2B–C), without pronounced glandular structures inside in most species, but (both sides) with a ( $\pm$  reticulate) discoloration [generally much more observable on the basal half; observed in living plants (Fig. 2B–D), often also when dry; probably representing or being a part of diffuse glandular tissue] in most species, species having such discoloration often also exhibiting  $\pm$  transparent, window-like structures at base (observed only in living plants, Fig. 2D), base usually slightly to moderately saccate, rarely not saccate. Included Thai species: *M. campanulata* Pierre, *M. cuneata* Craib, *M. filipes* Ridl., *M. hirsuta* sp. nov., *M. longipes* King, *M. thailandica* sp. nov., *M. thorelii* Finet & Gagnep. and *M. umpangensis* sp. nov.

A taxonomic revision of *Miliusa* has been conducted for the species that occur in the Austro-Malesian area (Mols & Keßler 2003), leaving the majority of continental Asian species poorly understood, though several new species have recently been proposed, especially from the Indian subcontinent (Narayanan *et al.* 2010, 2012, Chaowasku 2013).

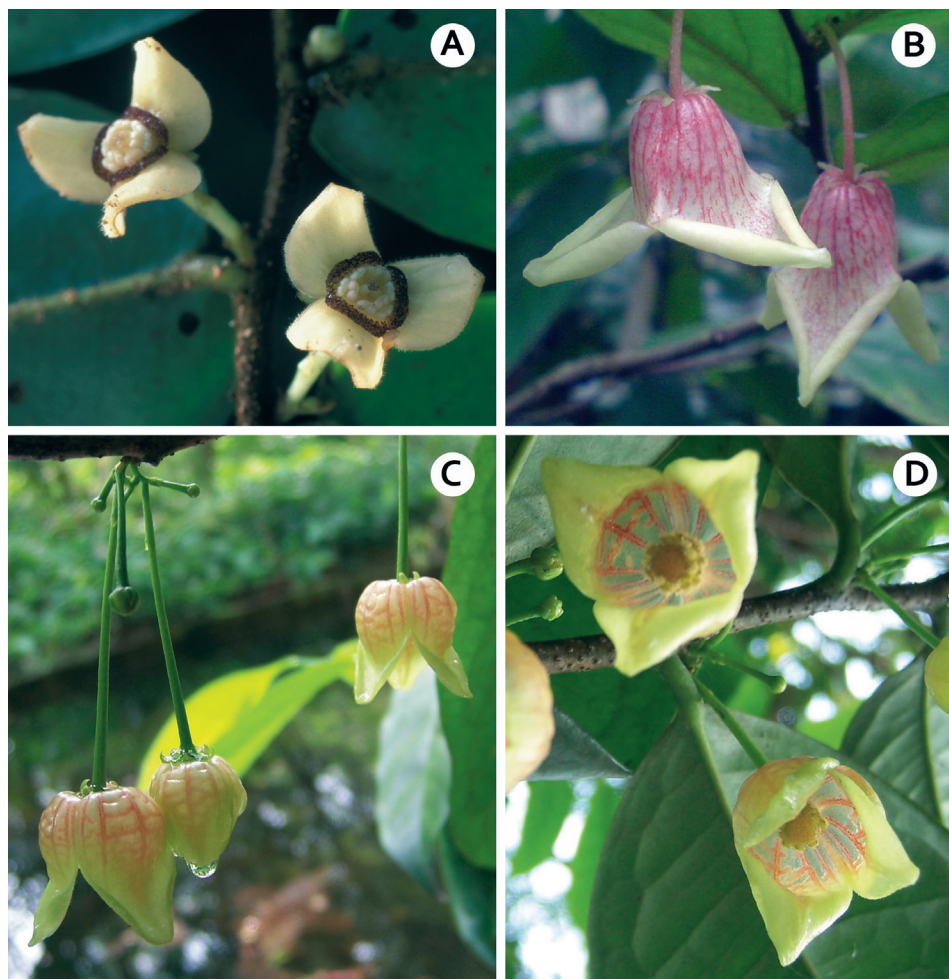
During a study of the phylogeny and taxonomy of the genus as a part of the first author’s PhD study, we found seven undescribed species of *Miliusa* occurring in Thailand, belonging to group 1 or 4. Several of these species are only known from the type or a few specimens collected from the same locality; however, in comparisons with their congeners, there are enough (two or more) morphological differences to convince us that they are new to science. The aim of the present study is to describe these seven species. In addition, a key to the 19 species of *Miliusa* in Thailand is constructed and a complete taxonomic nomenclature of all known species of *Miliusa* in Thailand is provided.

## Material and methods

Specimens of the following herbaria were available for this study: A, AAU, ABD, B, BK, BKF, BM, BO, BR, C, CAL, DD, E, G, HITBC, K, KYO, L, NSW, NY, P, SING, TCD, U, UC, US, WU,



**FIGURE 1.** Morphology of the inner petals of *Milium* groups 1, 2, 3 and 4. (A) group 1: inner petals with crescent-shaped to semicircular glandular structures inside at base (*Milium mollis* Pierre: *Chaowasku* 20), (B) group 2: inner petals with narrow, longitudinal glandular structures inside along midrib (*Milium vidalii* J.Sinclair: *Curran s.n.*, Forestry Bureau no. 6403), (D) group 3: inner petals without pronounced glandular structures inside (*Milium macropoda* Miq.: *Ambriansyah and Arifin B 1515*), (C) group 4: inner petals tightly appressed from the base to  $\pm$  the midpoint at anthesis (*Milium thorelii* Finet & Gagnep.: *Kerr 1162*).



**FIGURE 2.** Flowers of (A) *Miliusa intermedia* sp. nov., (B) *Miliusa thailandica* sp. nov., and (C)–(D) *Miliusa campanulata* Pierre. Photographs: (A) Simon Gardner, (B) Kithisak Aongyong, (C)–(D) Charndanai Pradubpet.

Z. For the species descriptions, when only a single measurement/observation was made, the word ‘circa (ca.)’ was added. The indumentum terminology follows Hewson (1988). The term ‘velvety’ is equivalent to densely hairy/with dense hairs, whereas ‘puberulous’ is equivalent to sparsely hairy/with sparse hairs. Specimens of the following species of *Miliusa* already known from Thailand were studied: *M. amplexicaulis*, *M. campanulata*, *M. cuneata*, *M. filipes*, *M. fusca*, *M. horsfieldii*, *M. longipes*, *M. mollis*, *M. parviflora*, *M. sclerocarpa*, *M. thorelii*, *M. velutina* (Appendix 1). Specimens of these species collected outside Thailand, including those cited in Mols & Keßler (2003), were also examined for comparison.

## Taxonomy

### Key to the species of *Milium* in Thailand

1. Flowers bisexual; inner petals completely open and reflexed at anthesis; stamens (80–)100–200 per flower..... *M. velutina*  
 – Flowers unisexual or bisexual; inner petals completely open or tightly appressed from the base to ± the midpoint, but not reflexed at anthesis; stamens ≤ 80 per bisexual flower ..... 2
2. Inner petals with crescent-shaped to semi-circular glandular structures inside at base ..... 3  
 – Inner petals without crescent-shaped to semi-circular glandular structures inside at base ..... 9
3. Leaf base cuneate, obtuse or (slightly) rounded, generally slightly unequal, not clasping the twigs ..... 4  
 – Leaf base (sub)cordate, slightly to notably unequal, not or (slightly to distinctly) clasping the twigs ..... 5
4. Plants (semi-)deciduous; bud scales present; flowers and/or inflorescences borne on new sprouting shoots; inner petals densely hairy inside (mostly near margin and on the apical half), glandular structures inside at base ± semi-circular; stamens ca. 16 per flower; stipe 1.5–2.0 mm long ..... **1. *M. fragrans* sp. nov.**  
 – Plants evergreen; bud scales absent; flowers and/or inflorescences not borne on new sprouting shoots; inner petals glabrous inside, glandular structures inside at base ± crescent-shaped; stamens 6–13 per flower; stipe 3.0–5.5 mm long ..... *M. fusca*
5. Leaves 9.3–26.0 × 4.0–11.0 cm; inner petals 4.0–7.0 × 4.0–7.0 mm... *M. amplexicaulis*  
 – Leaves 1.8–11.0 × 0.9–3.5 cm; inner petals ≤ 3.5 mm long and wide ..... 6
6. Lower leaf surface densely hairy ..... *M. mollis*  
 – Lower leaf surface (almost) glabrous ..... 7
7. Carpels ≥ 10 per flower; stipe 5.0–7.0 mm long ..... **4. *M. nakhonsiana* sp. nov.**  
 – Carpels 3–4 per flower; stipe nearly absent to 1.5 mm long ..... 8
8. Leaves usually narrowly elliptic-oblong, base always distinctly clasping the twigs; monocarps globose, nearly sessile ..... **5. *M. sessilis* sp. nov.**  
 – Leaves elliptic(-ovate), base often only slightly clasping the twigs; monocarps subglobose, stipe 1.0–1.5 mm long ..... **3. *M. intermedia* sp. nov.**
9. Flowers and/or inflorescences terminal (developing to internodal; sometimes appearing as ‘axillary’); inner petals completely open at anthesis; ovules ≥ 3 per ovary, biseriate ..... 10  
 – Flowers and/or inflorescences axillary; inner petals usually tightly appressed from the base to ± the midpoint at anthesis; ovule(s) 1–2 per ovary, uniseriate when there are two ovules per ovary ..... 12
10. Inner petals 3.0–5.0 × 2.0–4.0 mm, without narrow, longitudinal glandular structures inside along midrib, base not saccate; stamens 30–40 per flower ..... *M. parviflora*  
 – Inner petals 17.0–40.0 × 5.0–15.0 mm, with narrow, longitudinal glandular structures inside along midrib, base (moderately to) conspicuously saccate; stamens ≥ 40 per flower ..... 11

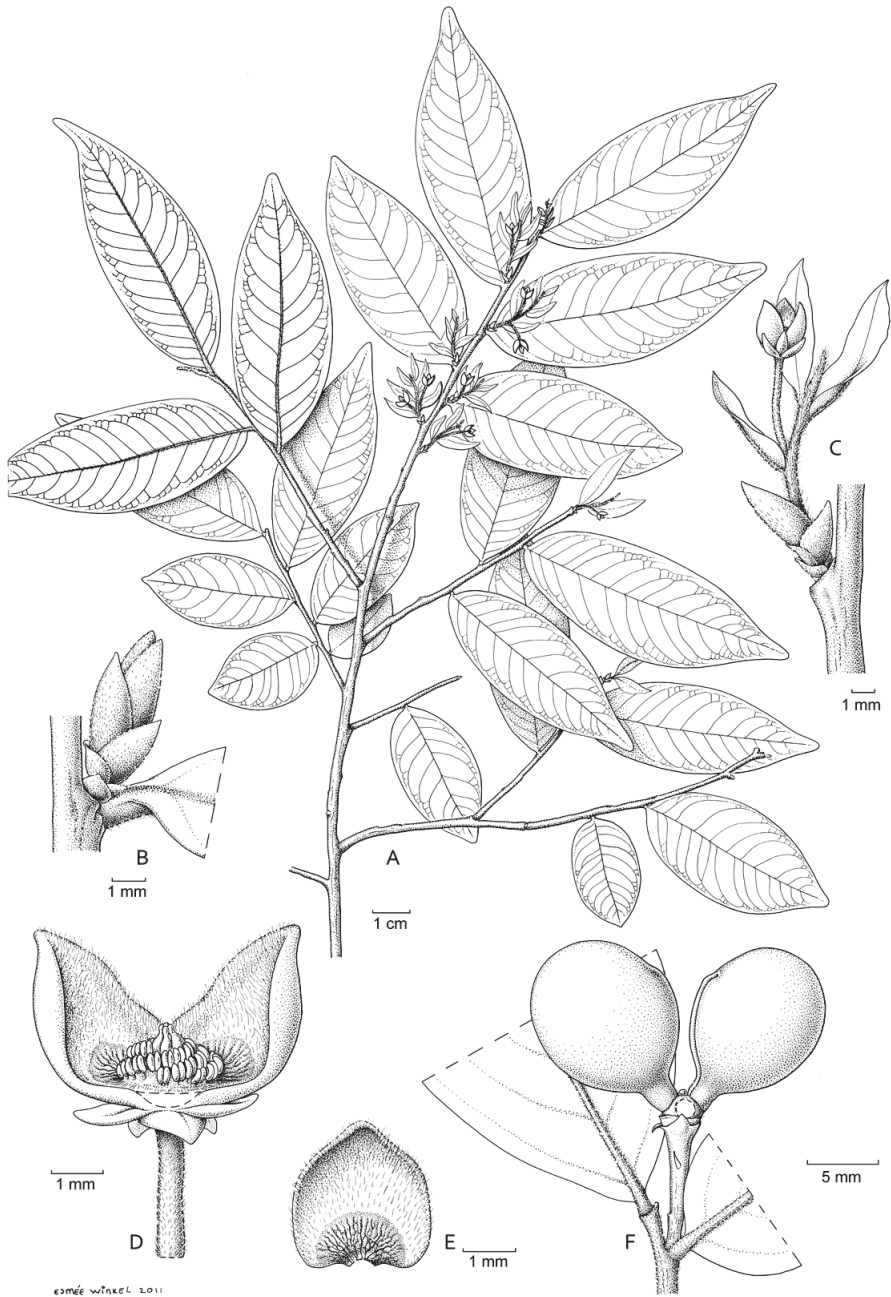
11. Young twigs and lower leaf surface glabrous to sparsely hairy; carpels 7–11 per flower ..... *M. sclerocarpa*  
 – Young twigs and lower leaf surface (sparsely to) densely hairy; carpels  $\geq$  17 per flower ..... *M. horsfieldii*
12. Flowers unisexual or bisexual with 2–8 stamens per flower ..... *M. thorelii*  
 – Flowers bisexual with  $>$  15 stamens per flower ..... 13
13. Inner petals with  $\pm$  warty glandular structures inside the apical part (ca. 1/3 of the inner petal length) ..... **7. *M. umpangensis* sp. nov.**  
 – Inner petals without  $\pm$  warty glandular structures inside the apical half ..... 14
14. Leaf base slightly subcordate to cordate, slightly to moderately unequal; sepals and outer petals linguiform ..... **6. *M. thailandica* sp. nov.**  
 – Leaf base cuneate or obtuse, equal; sepals and outer petals (narrowly to broadly) triangular ..... 15
15. Pedicels 0.4–0.5 cm long ..... **2. *M. hirsuta* sp. nov.**  
 – Pedicels  $\geq$  (0.8–)1.5 cm long ..... 16
16. Secondary veins  $\pm$  indistinct on the lower leaf surface; outer petals 4.0–6.0 mm long; stamens 40–80 per flower ..... *M. cuneata*  
 – Secondary veins prominent on the lower leaf surface; outer petals  $\leq$  3.0 mm long; stamens  $\leq$  27(–36) ..... 17
17. Young twigs moderately hairy; ovaries glabrous ..... *M. filipes*  
 – Young twigs (almost) glabrous; ovaries sparsely hairy ..... 18
18. Discoloration on the basal half of the inner petals (much clearer visible inside) not (or very slightly) reticulate (much clearer observed in living plants), window-like structures at base indistinct (in living plants; sometimes absent); stipe (18.0–)20.0–35.0(–43.0) mm long ..... *M. longipes*  
 – Discoloration on the basal half of the inner petals (both sides) remarkably reticulate (much clearer observed in living plants), window-like structures at base conspicuous (in living plants); stipe (8.0–)10.0–15.0(–19.0) mm long ..... *M. campanulata*

**1. *Miliusa fragrans* Chaowasku & Kessler sp. nov. (Figs. 3–4)**

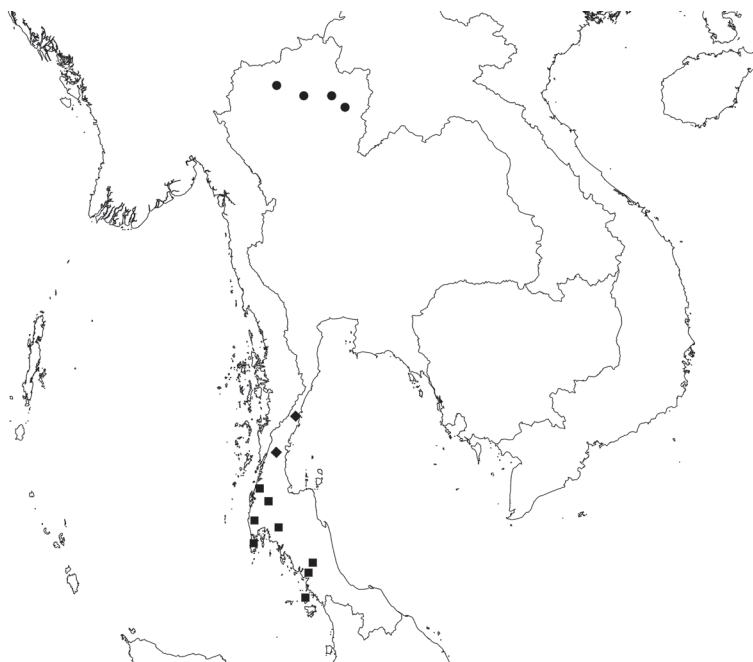
**Type:**–Thailand, Chiang Mai, Doi Chiang Dao, Feb 1958, *Bunchuai* 709 [holotype: BKF! (BKF16717)], in flower.

**Etymology:**–The epithet refers to the fragrant flowers of this species.

Shrubs or trees, (semi-)deciduous, 4–15 m tall, 20–30 cm in diameter. Bud scales 4–5, caducous, ovate-triangular, smaller towards the basal one. Young twigs (appressed-) velvety. Petioles up to 1.5 mm long, appressed-puberulous to velvety. Leaves usually elliptic, sometimes slightly (ob)ovate, 2.7–9.0  $\times$  1.7–3.2 cm; base cuneate, obtuse to rounded, usually slightly unequal; apex acute to slightly acuminate; lamina glabrous above, (almost) glabrous below; midrib nearly flat to slightly sunken above, almost glabrous, raised below, (appressed-)puberulous; secondary veins 12–15 pairs, angle with midrib 37°–46°. Flower solitary, axillary, borne on new sprouting shoots; pedicels 0.4–1.5 cm long, usually appressed-puberulous at base, bracts 2, (narrowly) triangular, one near sepals, another one near the midpoint of pedicels, the latter usually narrower and longer. Sepals triangular, ca. 1.2  $\times$  1.0 mm, slightly connate at base, persistent in fruit; margin puberulous, outside glabrous. Outer petals ovate-triangular, ca. 1.9  $\times$  1.1



**FIGURE 3.** *Milusa fragrans* sp. nov. (A) habit, (B) bud scales, (C) new axillary shoot with a flower, (D) flower with one inner petal removed, (E) inside (adaxial surface) of an inner petal, (F) fruiting twig. (A), (C)–(E) Bunchuai 709, (B) Chaowasku 38, (F) Nakorn-Thiemchan NTC 12.



**FIGURE 4.** Distribution of *Miliusa fragrans* sp. nov. (filled circle), *Miliusa sessilis* sp. nov. (filled diamond) and *Miliusa thailandica* sp. nov. (filled square).

mm, often persistent in fruit; both sides glabrous, margin velvety. Inner petals ovate-triangular, 3.0–3.4 × 2.5–3.1 mm; margin puberulous, outside glabrous, inside curly-velvety (indumentum mostly near margin and on the apical half); glandular structures present inside at base, ± semi-circular, slightly thickened, slightly irregularly warty; base not saccate. Torus ovoid-ellipsoid. Stamens ca. 16, ca. 0.8 mm long. Carpels 2–5, ca. 0.9 mm long; stigmas ellipsoid; ovaries glabrous; ovule 1, subbasal. Monocarp(s) 1–2(–3), subglobose-ellipsoid, ca. 1.0 × 0.8 cm; surface (nearly smooth to) slightly verruculose, glabrous; apex not apiculate; stipe 1.5–2.0 mm long, glabrous. Seed 1, subglobose-ellipsoid, ca. 0.9 × 0.7 cm.

**Distribution, habitat and phenology:**—Thailand (Chiang Mai, Lampang, Nan, Phayao Provinces), occurring in evergreen or evergreen-deciduous forests (with bamboo); on karst; on slopes of hills, near waterfalls and/or in shaded places. Elevation: 350–1000 m a.s.l. Flowering: February, fruiting: July–September.

**Similar species:**—*Miliusa fragrans* belongs to group 1. At present it is the only known species from this group that is (semi-)deciduous and possesses conspicuous bud scales (Fig. 3B). The other species are evergreen and lack bud scales. The (semi-)deciduous habit and bud scales are common in the species of groups 2 and 3.

*Miliusa fragrans* is morphologically close to *M. fusca*. The former chiefly differs in having flowers and/or inflorescences that are borne on new sprouting shoots (not borne on new sprouting shoots in *M. fusca*), inner petals that are densely hairy (glabrous in *M. fusca*) inside (mostly near margin and on the apical half) and exhibit ± semi-circular

(± crescent-shaped in *M. fusca*) glandular structures inside at base, ca. 16 (6–13 in *M. fusca*) stamens per flower, and a stipe that is 1.5–2.0 (3.0–5.5 in *M. fusca*) mm long.

*Milium fragrans* is only found in northern Thailand (Fig. 4). It is allopatric to all other species of group 1.

**Field notes:**—Bark grey(–brown), thin, finely cracked. Leaves dark green and glossy above, slightly paler beneath; midrib and petioles yellow–green. Flowers greenish yellow, scented. Fruits bright light green, ripening notably dark purple; fruiting pedicels brown–green. Vernacular name: Chan-Ti-Ma-Doi (Thai).

**Additional specimens examined (paratypes):**—*Bygrave 117* (L): Lampang, Wang Nua; *Chaowasku 38* (L), *92* (L), *Nakorn-Thiemchan NTC 1* (L), *NTC 12* (L): Chiang Mai, Chiang Dao, Chiang Dao Wildlife Sanctuary; *Kerr 3614* (BM, K): Pang Pue; *Kerr 5240* (K): Chiang Dao, Mè Poi; *Larsen et al. 43575* (BKF): Nan, Tham Pa Tok; *Maxwell 98-758* (BKF): Payao (= Phayao), Bong, Doi Pah Chang Wildlife Sanctuary.

## 2. *Milium hirsuta* Chaowasku & Kessler *sp. nov.* (Figs. 5, 10)

**Type:**—Thailand, Prachuab Khiri Khan, Bang Saphan, Feb 1970, *Van Beusekom and Santisuk 2815* [holotype: BKF! (BKF65232), isotypes: AAU!, E!, L!], in flower.

**Etymology:**—The epithet refers to the densely hairy young twigs and lower leaf surface of this species.

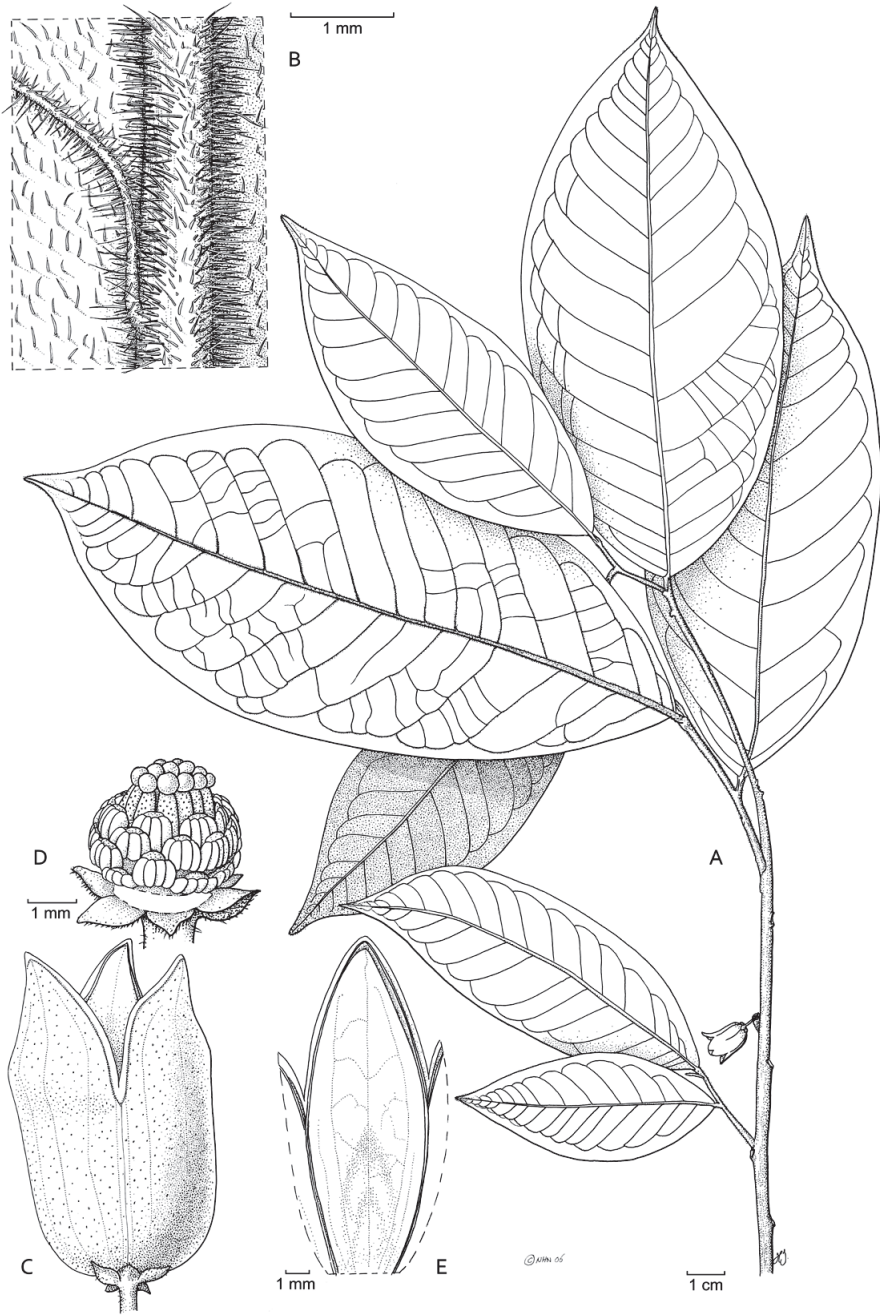
Shrubs, evergreen. Young twigs velvety. Petioles ca. 6.0 mm long, velvety, grooved above. Leaves elliptic, 11.0–19.0 × 4.1–8.1 cm; base cuneate; apex acute-acuminate; lamina glabrous above, velvety below; midrib (slightly) sunken above, puberulous, raised below, velvety; secondary veins ca. 16 pairs, prominent below, raised, angle with midrib 48°–57°. Flowers in 2- to 4-flowered inflorescences, axillary; peduncles 0.1–0.2 cm long, puberulous-velvety, bract not seen (absent?); pedicels 0.4–0.5 cm long, puberulous-velvety, bract(s) 1–2 for each flower, triangular. Sepals triangular, 1.5–1.6 × 0.9–1.0 mm; margin and outside puberulous-velvety. Outer petals triangular, 1.8–2.0 × 1.1–1.2 mm; margin and outside puberulous-velvety. Inner petals elliptic-oblong, ca. 14.0 × 7.0 mm, tightly appressed from the base to ± the midpoint at anthesis; apex acute-obtuse; both sides glabrous, margin (almost) glabrous; some discoloration visible inside at base (in dried material); base slightly saccate. Torus slightly ovoid. Stamens ca. 48, ca. 1.3 mm long. Carpels ca. 11, ca. 1.7 mm long; stigmas globose-capitate; ovaries puberulous; ovules 2, lateral, uniseriate. Monocarps unknown.

**Distribution, habitat and phenology:**—Thailand (Prachuab Khiri Khan Province), occurring in secondary forests among evergreen groves. Flowering: February.

**Similar species:**—*Milium hirsuta* belongs to group 4. It mostly resembles *M. thorelii*. The new species principally differs in having dense hairs on young twigs and the lower leaf surface while those of *M. thorelii* are usually (almost) glabrous. Additionally, *M. hirsuta* has 2-ovuled ovaries whereas they are 1-ovuled in *M. thorelii*. The type specimens are the only available collection for this species.

*Milium hirsuta* is possibly sympatric to *M. thorelii*. The latter has been extensively collected from northern Thailand, but rarely collected from southern Thailand.

**Field notes:**—Flowers campanulate, pale green, purple inside at base. Vernacular name: Ra-Khang-Bai-Khon (Thai).



**FIGURE 5.** *Miliusa hirsuta* sp. nov. (A) habit, (B) lower (abaxial) leaf surface, (C) flower, (D) flower with inner petals removed, (E) inside (adaxial surface) of an inner petal. (A)–(E) Van Beusekom and Santisuk 2815.

**3. *Milium intermedia* Chaowasku & Kessler sp. nov. (Figs. 2A, 6, 10)**

**Type:**—Thailand, Peninsular, Takuapah-Surat Thani Rd, 20–60 km from Takuapah, 1972, Larsen et al. 30964 (holotype: AAU!, isotypes: BKF!, L!), in flower.

**Etymology:**—See below.

Shrubs or trees, evergreen, 2–8 m tall, 2–10 cm in diameter. Young twigs puberulous-velvety. Petioles up to 1.0 mm long, velvety. Leaves elliptic(-ovate), 3.0–9.0 × 1.1–3.5 cm, first leaves of newly developed twigs usually notably smaller, (broadly) ovate; base (sub) cordate, moderately unequal, often slightly clasping the twigs; apex acute-acuminate; lamina glabrous above, almost glabrous below; midrib flat to slightly sunken above, puberulous, raised below, (puberulous-)velvety; secondary veins 12–16 pairs, angle with midrib 46°–62°. Flower solitary, axillary; pedicels 0.4–0.8 cm long, puberulous, bracts 2–3, triangular. Sepals triangular, ca. 1.2 × 1.2 mm, connate at base, persistent in fruit; margin puberulous, outside almost glabrous. Outer petals triangular, ca. 1.3 × 1.0 mm, persistent in fruit; outside and margin puberulous. Inner petals broadly ovate, 2.2–2.5 × 2.7–3.3 mm, both sides of margin somewhat recurved at anthesis; outside puberulous, margin curly-puberulous, inside almost glabrous; glandular structures present inside at base, between crescent-shaped and semicircular, thickened, slightly finely warty; base not saccate. Torus subglobose. Stamens 9–13, ca. 0.6 mm long. Carpels 3–4, ca. 0.7 mm long; stigmas globose; ovaries glabrous; ovule 1, basal. Monocarp 1, subglobose, ca. 0.8 × 0.6 cm, often with 1–2 underdeveloped monocarp(s); surface slightly verruculose, glabrous; apex not apiculate; stipe 1.0–1.5 mm long, glabrous. Seed 1, subglobose, ca. 0.7 × 0.5 cm.

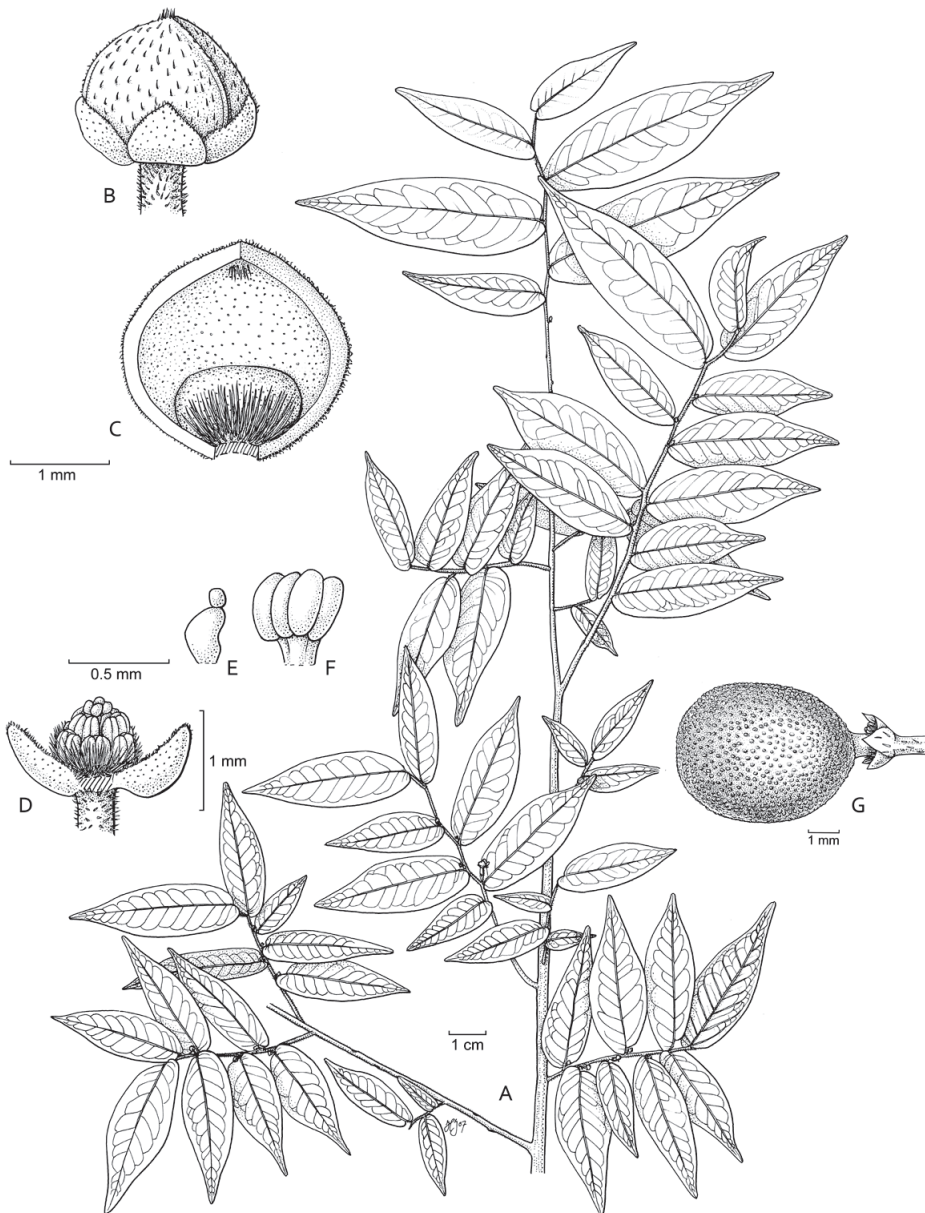
**Distribution, habitat and phenology:**—Thailand (Surat Thani Province), occurring in shady understorey of relatively undisturbed evergreen forests; on rugged limestone terrain. Elevation: 100–250 m a.s.l. Flowering: June, fruiting: June, November.

**Similar species:**—*Milium intermedia* belongs to group 1. Its affinity seems to lie between *M. mollis* and *M. sessilis* sp. nov., hence its epithet. *Milium intermedia* mainly differs from *M. mollis* in having a (slightly) more unequal leaf base and shorter stipe (1.0–1.5 mm long in *M. intermedia* vs. 3.0–4.0 mm long in *M. mollis*). Another obvious character is the lower leaf surface that is almost glabrous, but densely hairy in *M. mollis*. The new species chiefly differs from *M. sessilis* in having elliptic(-ovate) leaves (Fig. 6A) while they are usually narrowly elliptic-oblong in *M. sessilis* (Fig. 8A). The leaf base of *M. sessilis* is notably asymmetrical and distinctly clasping the twigs (Fig. 8A) whereas that of *M. intermedia* is much less so (Fig. 6A). Table 1 compares important morphological characters of *M. intermedia* and several morphologically similar species in group 1.

*Milium intermedia* is allopatric to all other species of group 1. During a recent expedition to the localities of this species, we found a substantial number (no less than 500) of individuals of various size restricted to shaded areas among rugged limestone terrain (pers. obs. TC).

**Field notes:**—Bark dark brown, smooth. Leaves grey–green below, bright greenish–yellowish in young leaves. Flowers cream, stamens cream with dark brown ring around them. Fruits pale green (immature). Vernacular name: Jing-Jaab-Tai (Thai).

**Additional specimens examined (paratypes):**—*Chaowasku* 26 (BKF, L), *Gardner and Sidisunthorn* ST 0757 (BKF, L): Surat Thani, Phanom, Klong Phanom National Park.



**FIGURE 6.** *Miliusa intermedia* sp. nov. (A) habit, (B) flower bud, (C) inside (adaxial surface) of an inner petal, (D) flower with petals removed, (E) carpel, (F) stamen, abaxial side, (G) fruit. (A)–(F) Gardner and Sidsuthorn ST 0757, (G) Chaowasku 26.

**TABLE 1.** Comparison of four morphologically similar species in group 1 (= *Milium mollis* group).

Character/species	<i>M. intermedia</i>	<i>M. mollis</i>	<i>M. nakhonsiana</i>	<i>M. sessilis</i>
- Lower leaf surface	Almost glabrous	Velvety	(Almost) glabrous	(Almost) glabrous
- Leaf shape	Elliptic(-ovate)	Elliptic to ovate	Usually (narrowly) elliptic-oblong	Usually narrowly elliptic-oblong
- Leaf base (degree of asymmetry/clasping in parentheses)	Often slightly clasping the twigs (++)	Sometimes slightly clasping the twigs (+)	Always distinctly clasping the twigs (++++)	Always distinctly clasping the twigs (++++)
- Size of inner petals (mm)	2.2–2.5 × 2.7–3.3	3.0–3.3 × 3.2–3.5	Ca. 3.0 × 3.5	2.3–2.5 × 2.6–3.7
- Number of stamens/flower	9–13	12–18	Unknown	Ca. 13
- Number of carpels/flower	3–4	4–8	≥ 10	Ca. 4
- Length of stipe (mm)	1.0–1.5	3.0–4.0	5.0–7.0	Almost absent

#### 4. *Milium nakhonsiana* Chaowasku & Kessler *sp. nov.* (Figs. 7, 10)

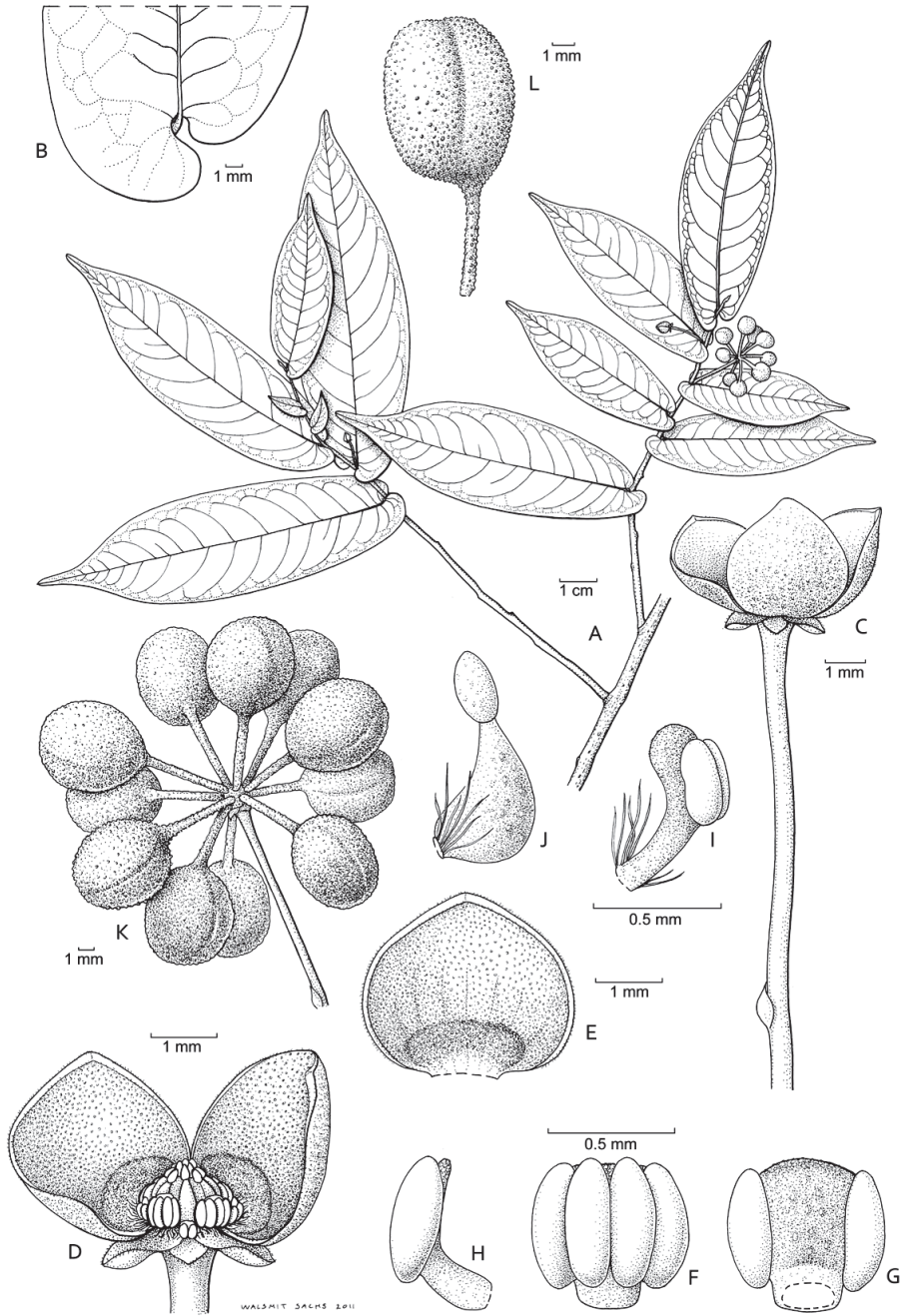
**Type:**—Thailand, Nakhon Si Thammarat, Nopphitum, Tham Lot cave, Feb 2005, *Williams 1472* [holotype: L! (barcode L0298136), isotypes: A, BKF], in flower and fruit.

**Etymology:**—Named after the Thai Province (Nakhon Si Thammarat, often called Nakhon or Nakhon Si for short) where this species is endemic.

Shrubs, evergreen, ca. 2 m tall. Young twigs (appressed-)puberulous. Leaves (almost) sessile, usually (narrowly) elliptic-oblong, sometimes slightly obovate, 5.5–10.1 × 1.8–3.1 cm; base cordate, notably unequal, always distinctly clasping the twigs; apex caudate-acuminate; lamina glabrous above, (almost) glabrous below; midrib (slightly) sunken above, glabrous, raised below, almost glabrous; secondary veins 12–14 pairs, angle with midrib 40°–54°. Flowers in 2-flowered inflorescences, axillary; peduncles inconspicuous, bracts ca. 2, ovate-semicircular; pedicels 1.2–1.4 cm long, almost glabrous, bract(s) 1–2 for each flower, (slightly) triangular. Sepals and outer petals ovate-triangular, not clearly observed due to limited material. Inner petals broadly ovate, ca. 3.0 × 3.5 mm; both sides glabrous, margin puberulous; glandular structures observed inside at base, between crescent-shaped and semicircular, thickened, slightly finely warty; base not saccate. Torus, stamens and carpels not observed, but carpels ≥ 10 (deduced from the number of monocarps). Monocarps ca. 10, (sub)globose, 0.6–0.7 × 0.6 cm; surface (slightly) verruculose, glabrous; apex not apiculate; stipe 5.0–7.0 mm long, glabrous. Seed 1, (sub)globose, 0.5–0.6 × 0.5 cm.

**Distribution, habitat and phenology:**—Thailand (Nakhon Si Thammarat Province), occurring at base of limestone mountains. Elevation: ca. 80 m a.s.l. Flowering and fruiting: February.

**Similar species:**—*Milium nakhonsiana* belongs to group 1 and is allopatric to all other species of group 1. It most resembles *M. sessilis sp. nov.* The main differences are the greater number of carpels per flower (≥ 10 in *M. nakhonsiana* vs. ca. 4 in *M. sessilis*) and the longer stipe [5.0–7.0 mm long in *M. nakhonsiana* (Fig. 7K) vs. nearly absent in *M. sessilis*, Fig. 8E]. The description was based on the holotype only; however, during



**FIGURE 7.** *Miliusa nakhonsiana* sp. nov. (A) habit, (B) lower (abaxial) leaf surface, (C) flower, (D) flower with one inner petal removed, (E) inside (adaxial surface) of an inner petal, (F) stamen, abaxial side, (G) stamen, adaxial side, (H) stamen, side view, (I) stamen with (just?) two smaller pollen sacs, side view, (J) carpel, (K) fruit, (L) monocarp. (A)–(L) Williams 1472.

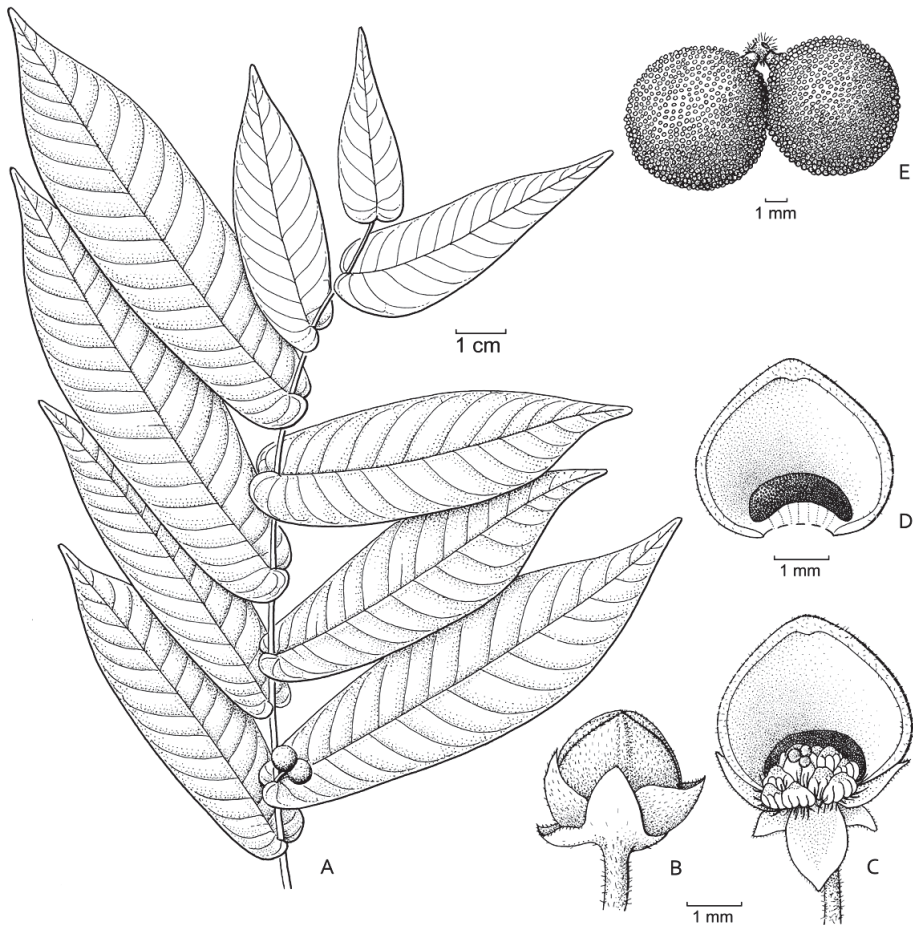
a recent expedition (pers. obs. TC), several more individuals of this species were also found at the type locality. Table 1 compares important morphological characters of *M. nakhonsiana* and several morphologically similar species in group 1.

**Field notes:**—Fruits purple–black. Vernacular name: Bai-Biaw-Lek (Thai).

**5. *Milium sessilis* Chaowasku & Kessler sp. nov. (Figs. 4, 8)**

**Type:**—Thailand, Prachuab Khiri Khan, Bang Saphan, Feb 1970, *Van Beusekom and Santisuk 2807* (holotype: L!, isotypes<sup>1</sup>: AAU!, BKF, E!), in flower and fruit.

**Etymology:**—The epithet refers to the nearly sessile monocarps (and also sessile leaves).



**FIGURE 8.** *Milium sessilis* sp. nov. (A) habit, (B) flower bud, (C) flower with two inner petals removed, (D) inside (adaxial surface) of an inner petal, (E) fruit. (A)–(E) *Van Beusekom and Santisuk 2807*.

<sup>1</sup> L! was incorrectly indicated as one of the isotypes in the original publication of this chapter.

(Understorey) shrubs, evergreen, 1–2 m tall. Young twigs puberulous(-velvety). Leaves sessile, usually narrowly elliptic-oblong, sometimes slightly obovate-oblong, 4.5–11.0 × 1.2–2.9 cm; base cordate, notably unequal, always distinctly clasping the twigs; apex (slightly) acuminate; lamina glabrous above, (almost) glabrous below; midrib (slightly) sunken above, almost glabrous, raised below, puberulous; secondary veins 11–16 pairs, angle with midrib 54°–63°. Flower(s) solitary or in 2-flowered inflorescences, axillary; peduncles up to 0.2 cm long, puberulous(-velvety), bracts ca. 3, triangular; pedicels 0.6–1.3 cm long, puberulous, bract(s) 1–2 per flower, whether solitary or in 2-flowered inflorescences, triangular. Sepals triangular, ca. 1.5 × 1.5 mm, connate at base; outside (almost) glabrous, margin puberulous. Outer petals triangular, ca. 1.9 × 1.3 mm; outside and margin puberulous. Inner petals broadly ovate-triangular, 2.3–2.5 × 2.6–3.7 mm; outside puberulous, margin curly-puberulous, inside glabrous; glandular structures clearly seen inside at base, ± crescent-shaped, thickened, finely warty; base not saccate. Torus hemispheroid. Stamens ca. 13, ca. 0.7 mm long. Carpels ca. 4, ca. 0.9 mm long; stigmas globose to capitate; ovaries glabrous; ovule 1, basal. Monocarps ca. 3, nearly sessile, globose, 0.6–0.7 × 0.6–0.7 cm; surface slightly verruculose, glabrous; apex not apiculate. Seed 1, globose, ca. 0.5 × 0.5 cm.

**Distribution, habitat and phenology:**—Thailand (Chumphon, Prachuab Khiri Khan Provinces), occurring in moist evergreen forests or secondary forests among evergreen groves; in (very) shady areas at base of (small) limestone hills. Elevation: ca. 100 m a.s.l. Flowering and fruiting: February.

**Similar species:**—*Miliusa sessilis* has the shortest stipe (nearly absent, Fig. 8E) among group 1 to which it belongs, and it is allopatric to all other species of group 1. *Miliusa sessilis* and the morphologically most similar species, *M. nakhonsiana*, possess the most asymmetrical leaf base (among all species of *Miliusa* examined) that distinctly clasps the twigs (Fig. 8A: *M. sessilis*, Fig. 7A: *M. nakhonsiana*). Table 1 compares leaf base (including the degree of asymmetry/clasping) and other important morphological characters of *M. sessilis* and several morphologically similar species in group 1.

**Field notes:**—Fruits dark red. Vernacular name: Bai-Biaw-Dam-Kwan (Thai).

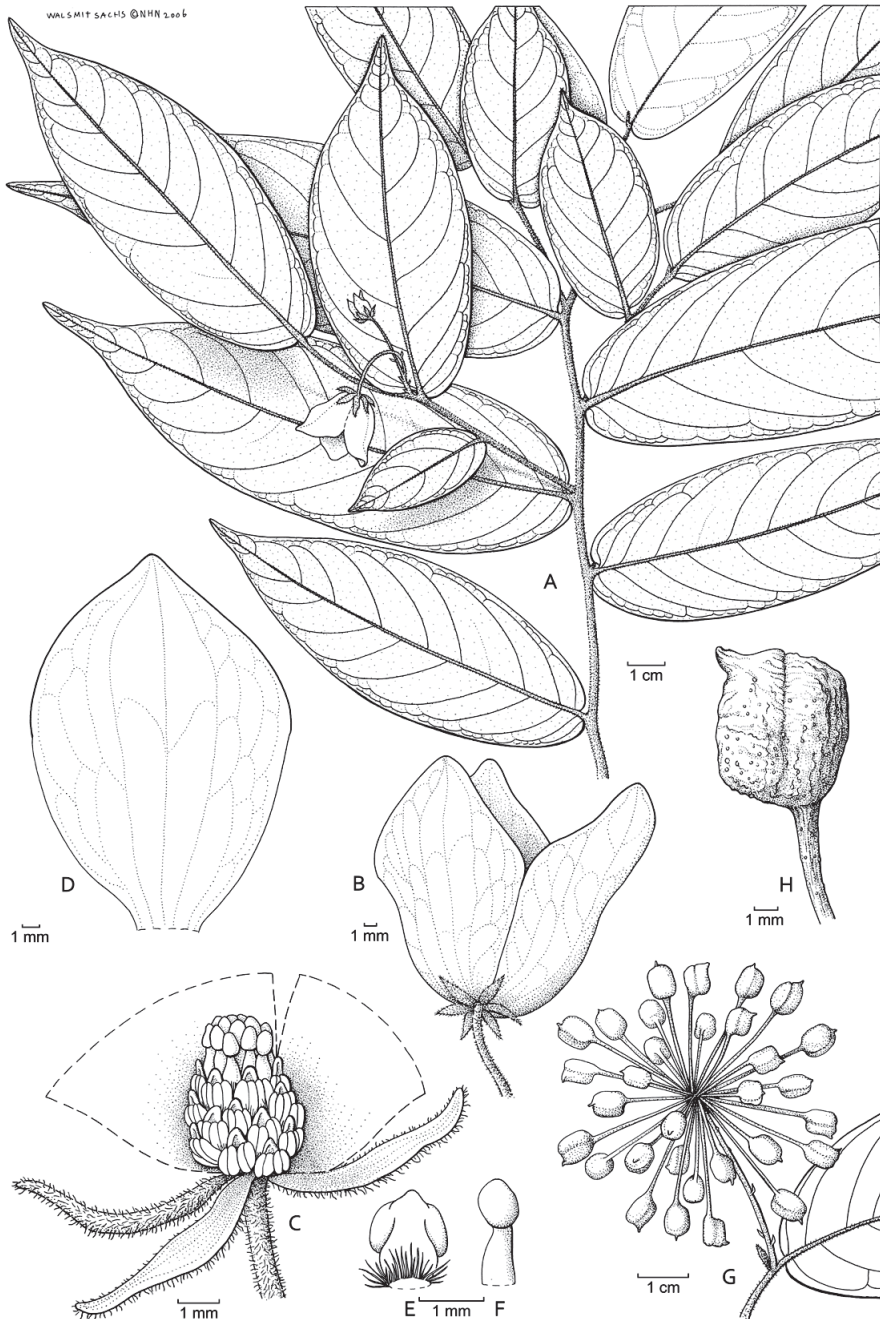
**Additional specimens examined (paratypes):**—*Chaowasku 44* (L): Chumphon, Sawi, Ban Tham Suai Huai Thab Thong; *Koonkhunthod et al. 311* (BKF): Chumphon, Thung Tako, Ban Khao Talu.

## 6. *Miliusa thailandica* Chaowasku & Kessler sp. nov. (Figs. 2B, 4, 9)

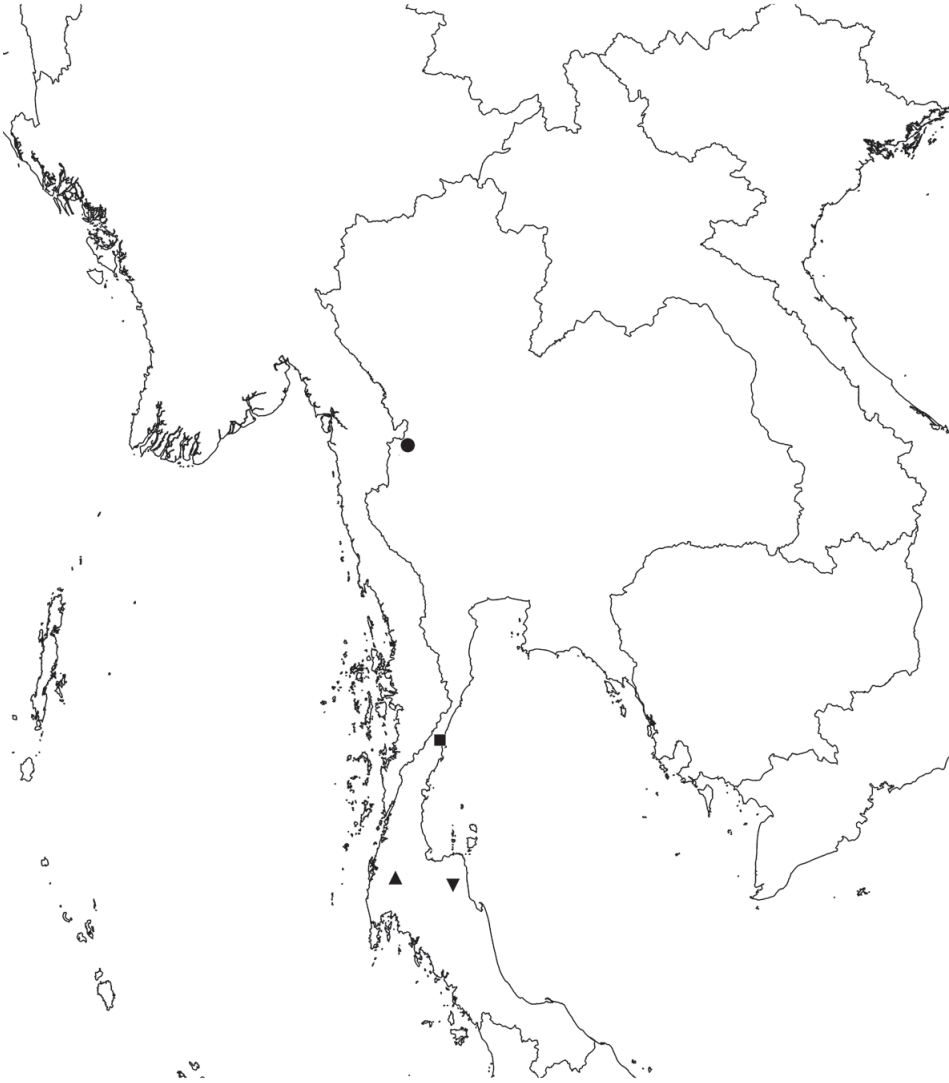
**Type:**—Thailand, Ranong, Hard Hin Dam (near the sea), Apr 1974, *Larsen and Larsen 33368* (holotype: LI, isotypes: AAU!, BKF), in flower.

**Etymology:**—Named after Thailand, where this species is likely endemic.

Shrubs or trees, evergreen, 2–6 m tall, 12–17 cm in diameter. Young twigs (puberulous-)velvety. Petioles up to 2.0 mm long, puberulous(-velvety). Leaves elliptic to obovate, 5.0–20.0 × 2.5–7.3 cm; base slightly subcordate to cordate, slightly to moderately unequal; apex acute to caudate-acuminate; lamina glabrous above, glabrous to puberulous below; midrib slightly sunken above, puberulous, raised below, almost glabrous to appressed-puberulous; secondary veins 8–14 pairs, angle with midrib 40°–60°. Flower(s) solitary or in 2- to 4-flowered inflorescences, axillary; peduncles up to 0.5 cm long,



**FIGURE 9.** *Milusa thailandica* sp. nov. (A) habit, (B) flower, (C) flower with stamens and carpels exposed, (D) inside (adaxial surface) of an inner petal, (E) stamen, adaxial side, (F) carpel, (G) fruiting twig, (H) monocarp. (A)–(F) Geesink and Santisuk 5036, (G), (H) Fukuoka and Na Nakhon T-36095.



**FIGURE 10.** Distribution of *Miliusa hirsuta* sp. nov. (filled square), *Miliusa intermedia* sp. nov. (filled upright triangle), *Miliusa nakhonsiana* sp. nov. (filled inverted triangle), and *Miliusa umpangensis* sp. nov. (filled circle).

puberulous, bracts 3–4, triangular to semicircular; pedicels 1.6–5.0 cm long, almost glabrous to puberulous, bract(s) (1–)2–3 for solitary and each flower of multi-flowered inflorescences, triangular. Sepals linguiform, 3.0–5.3 × 0.8–1.5 mm, persistent in fruit; outside and margin puberulous, inside almost glabrous to curly-puberulous. Outer petals linguiform, ca. 6.0 × 1.5 mm; outside and margin puberulous, inside almost glabrous to curly-puberulous. Inner petals elliptic, 18.0–26.0 × 11.0–15.0 mm, tightly appressed from the base to ± the midpoint at anthesis; apex acute-obtuse; margin almost glabrous to puberulous, both sides glabrous; some discoloration often observed

inside near/at base (in dried material); base moderately saccate. Torus slightly cylindrical. Stamens 27–28, 1.6–1.8 mm long. Carpels 15–50, ca. 2.0 mm long; stigmas capitate to slightly ovoid; ovaries glabrous; ovule(s) 1(–2), basi-lateral. Monocarps 7–50, ± subglobose when containing 1 seed, more irregular when containing 2 seeds, 0.7–1.1 × 0.6–0.9 cm; surface verruculose, glabrous; apex usually (notably) apiculate; stipe 15.0–35.0 mm long, glabrous, sometimes attached obliquely to monocarps. Seed(s) 1 (rarely 2), (sub)globose, 0.6–0.8 × 0.5–0.6 cm.

**Distribution, habitat and phenology:**—Thailand (Phang Nga, Phuket, Ranong, Satun, Trang Provinces), occurring in (dense) evergreen forests; on granitic rocks or limestone hills; on hillsides, under considerably dense canopy, along waterfalls, by streams and/or near the sea. Elevation: up to 440 m a.s.l. Flowering: February–May, fruiting: June–December.

**Similar species:**—*Miliusa thailandica* belongs to group 4. It is distinguishable from the other species of this group occurring in Thailand by the slightly to moderately unequal leaf base (Fig. 9A), a character that is common in species of group 1. Additionally, *M. thailandica* exhibits the largest inner petals among species of group 4 occurring in Thailand.

Among group 4, *M. thailandica* is possibly sympatric to *M. longipes*, although the latter is generally found in the eastern part of southern Thailand (pers. obs. TC) whereas the former is usually found in the western part of southern Thailand (Fig. 4).

**Field notes:**—Bole grey. Flowers pendulous with a penetrating (pleasant or unpleasant) smell, white to creamy yellow, nerves red, only at base. Fruits green ripening red. Vernacular name: Ra-Khang-Siam (Thai).

**Additional specimens examined (paratypes):**—*Bunkert 94* (BKF), *Collins 2455* (K), *Geesink et al. 7196* (BKF, K, L), *Middleton et al. 324* (E), *Nimanong and Pochanart 1605* (BKF), *Phusomsaeng and Pinnin 354* (C, KYO, L), *Pochanart 221* (BKF, KYO): Trang, Khao Chong; *Chaowasku 70* (L): Phuket, Thalang, Khao Phra Taew Non-hunting Area; *Fukuoka and Na Nakhon T-36095* (BKF, KYO, L): Phang Nga, Kuraburi, Klong Saeng Wildlife Sanctuary; *Gardner et al. ST 1116* (L): Surat Thani, Phanom, Klong Phanom National Park; *Geesink and Santisuk 5036* (AAU, C, E, KYO, L): Pangnga (= Phang Nga), Khlong Nang Yon; *Geesink and Santisuk 5200* (L): Pangnga, Takuapa, Kao Lak; *Geesink et al. 7356* (AAU, L): Satun, Koh Talutao; *Kerr 17276* (BM), *Shimizu et al. T-7848* (KYO), *T-7849* (KYO): Phang Nga; *Kerr 19123* (BM): Trang, Palien; *Larsen et al. 31157* (AAU): Peninsular; *Larsen et al. 31174* (AAU, K): Peninsular, Phang Nga, Khao Nang Hong; *Niyomdham et al. 254* (BKF, C, E, K, KYO, L, P): Puket (= Phuket), Kao Pa Ra; *Rogstad 800* (L): Trang, Khao Chong Botanic Garden.

### 7. *Miliusa umpangensis* Chaowasku & Kessler *sp. nov.* (Figs. 10, 11)

**Type:**—Thailand, Tak, Umpang, Dec 2009, *Chaowasku 88* (holotype: L!, isotypes: BKF!), in flower.

**Etymology:**—Named after Umpang, the mountainous district where this species was found and is likely to be endemic.

Trees, evergreen, 2–4 m tall. Young twigs (appressed-)puberulous. Petioles ca. 2.0 mm long, appressed-puberulous, usually grooved above. Leaves elliptic, 4.6–14.0 × 2.0–4.8



**FIGURE 11.** *Miliusa umpangensis* sp. nov. (A) habit, (B) flowering twig, (C) flower with one inner petal removed, (D) outside (abaxial surface) of an inner petal, (E) inside (adaxial surface) of an inner petal, (F) stamen, abaxial side, (G) stamen, adaxial side, (H) stamen, side view, (I) carpel, (J) fruiting twig, (K) monocarp. (A) *Chaowasku* 88, (B)–(I) *Chaowasku* 87, (J), (K) *Chaowasku* 89.

cm; base cuneate; apex usually caudate-acuminate, rarely acute; lamina glabrous above, almost glabrous below; midrib sunken above, glabrous to (appressed-)puberulous, raised below, appressed-puberulous; secondary veins 11–14 pairs, prominent below, raised, angle with midrib 48°–61°. Flower solitary, axillary; pedicels 1.3–1.9 cm long, almost glabrous, bracts 3, triangular, placed at base of the pedicels. Sepals ovate-triangular, ca. 1.5 × 1.1 mm, persistent in fruit; both sides glabrous, margin puberulous. Outer petals ovate, ca. 1.7 × 1.2 mm; both sides glabrous, margin puberulous. Inner petals ovate, 9.0–10.0 × 6.0 mm, tightly appressed at the basal part (ca. 2/3 of the inner petal length) at anthesis, the apical part (ca. 1/3 of the inner petal length) considerably recurved at anthesis; both sides glabrous, margin puberulous; inner side of the apical part (ca. 1/3 of the inner petal length) with ± warty glandular structures; base slightly saccate. Torus slightly ovoid. Stamens ca. 20, ca. 1.0 mm long. Carpels ca. 12, ca. 1.8 mm long; stigmas subglobose-ellipsoid; ovaries appressed-velvety; ovule 1, lateral. Monocarps 2–7, subglobose-ellipsoid, ca. 0.9 × 0.7 cm; surface slightly verruculose, often with larger warts in some areas, almost glabrous; apex not apiculate; stipe ca. 5.0 mm long, almost glabrous, obliquely attached to monocarps. Seed 1, subglobose-ellipsoid, ca. 0.8 × 0.6 cm.

**Distribution, habitat and phenology:**—Thailand (Tak Province), occurring in evergreen forests; ca. 100 m from a stream. Elevation: not less than 1000 m a.s.l. Flowering and fruiting: December.

**Similar species:**—*Milium umpangensis* is remarkable among the species of group 4 observed thus far because it is the only one to possess inner petals with ± warty glandular structures inside the apical part (ca. 1/3 of the inner petal length) (Fig. 11B, D). Other morphological characters of *M. umpangensis* seem similar to those of *M. filipes*, except the ovaries which are densely hairy in the new species while glabrous in *M. filipes*. *Milium umpangensis* is allopatric to all other species of group 4.

**Field notes:**—Flowers purplish–brown in general, the apical half somewhat purplish–greenish, fragrant like *Pandanus amaryllifolius* Roxb. leaves but fainter. Fruits half purplish half whitish green, ripening dark purple. Vernacular name: Ra-Khang-Hom (Thai).

**Additional specimens examined (paratypes):**—*Chaowasku* 87 (BKF, L), 89 (BKF, L): both collected from the same locality as the type.

### Taxonomy of species previously known from Thailand

Our study of the genus *Milium* in Thailand revealed that several taxa described from material collected from or outside Thailand are conspecific to species described earlier. Therefore, they are herein formally reduced into synonymy (*syn. nov.*). In addition, the complete taxonomic nomenclature (including lectotypifications, basionyms and synonyms) of all known species of *Milium* in Thailand is provided.

#### 1. *Milium amplexicaulis* Ridl. (1910, p. 13)

**Type:**—Peninsular Malaysia, Langkawi, Apr 1896, *Curtis 3205* [lectotype selected by Turner (2012, p. 237): SING (barcode SING0045995), isotypes: K!, SING, UC!]<sup>2</sup>, in flower.

<sup>2</sup> Incorrectly indicated as “(holotype: K!, isotypes: SING, UC!)” in the original publication of this chapter.

**Distribution:**—Southern Thailand to Peninsular Malaysia.

**2. *Miliusa campanulata* Pierre (1881, t. 41) (Fig. 2C, D)**

**Type:**—Cambodia, in Montibus Knang Repeu, May 1870, *Pierre 602b* [lectotype selected here: P! (barcode P00160858), isotypes: B!, C!, L!], in flower.

**Distribution:**—Eastern/northeastern Thailand to Cambodia.

**3. *Miliusa cuneata* Craib (1912, p. 145)**

**Type:**—Thailand, Chiang Mai, Doi Sutep, May 1911, *Kerr 1837* [holotype: K!, isotypes: ABD!, B!, BK! (photograph), BM!, CAL!, E!, K!, TCD! (photograph)], in flower.

**Taxonomic synonym:**—*Miliusa elongata* Craib (1925, p. 12) *syn. nov.*

**Type:**—Thailand, Nakawn Sawan, Klawng Kung, Jun 1922, *Kerr 6050* [holotype: K!, isotypes: ABD!, B!, BK! (photograph), BM!, E!, TCD! (photograph), UC!], in flower.

**Distribution:**—China (Yunnan Province), Laos (Sainyabuli Province) to Thailand (Chiang Mai, Chiang Rai, Kamphaeng Phet, Kanchanaburi, Lampang, Nan, Phayao Provinces).

**4. *Miliusa filipes* Ridl. (1920, p. 81)**

**Type:**—Thailand, western coast and islands of Peninsular Siam [now Thailand], Tasan, *Kloss 6968* (holotype: K!), in flower.

**Distribution:**—Thailand (Chumphon Province).

**5. *Miliusa fusca* Pierre (1881, t. 42)**

**Type:**—Cambodia, Samrongtong, crescit in Montibus Kereev, Apr 1870, *Pierre 737a* [lectotype selected here: P! (barcode P00160866)], in flower and fruit.

**Taxonomic synonym:**—*Miliusa mollis* Pierre var. *sparsior* Craib (1931, p. 59) *syn. nov.*

**Type:**—Thailand, Loei, Wang Sapung, Mar 1924, *Kerr 8630* [holotype: K, isotypes: AAU!, BK! (photograph), BM!, C!, E!, L!, UC!], in fruit.

**Distribution:**—Thailand (Loei, Nakhon Ratchasima Provinces) to Cambodia (Kampong Thom, Koh Kong Provinces).

**6. *Miliusa horsfieldii* (Benn.) Baill. ex Pierre (1881, t. 38)**

Basionym: *Saccopetalum horsfieldii* Benn. (1840, p. 165).

**Type:**—Indonesia, Java, *Horsfield s.n.* [holotype: BM! (barcode BM000554019), isotypes: BM!, K!], in flower.

**Taxonomic synonyms:**—*Saccopetalum arboreum* Elmer (1913, p. 1739).

Based on the same type: *Miliusa arborea* (Elmer) J.Sinclair (1955, p. 378).

**Type:**—The Philippines, Islands of Palawan, Province of Palawan, Brooks Point (Addison Peak), Feb 1911, *Elmer 12677* [lectotype selected by Mols & Keßler (2003, p. 440): A! (barcode A00039668), isotypes: B!, BM!, BO!, E!, G!, LI, NSW!, NY!, P!, U!, US!, Z!], in flower.

— *Miliusa tectona* Hutch. ex C.E.Parkinson (1923, p. 75).

Based on the same type: *Saccopetalum tectonum* (Hutch. ex C.E.Parkinson) Chatterjee (1948, p. 59).

**Type:**—India, Andaman Islands, Middle Andaman, May 1915, *Parkinson 545* (holotype: K!, isotype: DD!), in flower and fruit.

— *Saccopetalum lineatum* Craib (1924, p. 82).

Based on the same type: *Miliusa lineata* (Craib) Ast (1938, p. 120).

**Type:**—Thailand, Nakawn Sawawn, Mê Wong, May 1922, *Kerr 6012* [holotype: K!, isotypes: BK! (photograph), BM!, CAL, E!, P!, UC!], in flower.

— *Saccopetalum unguiculatum* C.E.C.Fisch. (1926, p. 454).

Based on the same type: *Miliusa unguiculata* (C.E.C.Fisch.) J.Sinclair (1955, p. 378).

**Type:**—Myanmar, South Tenasserim, Theinkun Chaung, Naungbwa, Feb 1926, *Parkinson 1694* (holotype: K!, isotypes: ABD!, K!), in flower.

— *Alphonsea prolifica* Chun & F.C.How (1958, p. 1).

Based on the same type: *Saccopetalum prolificum* (Chun & F.C.How) Tsiang, in Tsiang and Li (1964, p. 380). — *Miliusa prolifica* (Chun & F.C.How) P.T.Li (1993, p. 315). — *Alphonsea mollis* Merr. & Chun (1935, p. 230) *nom. illeg.*

**Type:**—China, Hainan, Yaichow, Mar-Jul 1933, *How 70951* [holotype: NY! (barcode NY00334809), isotypes: A!, B!, K!, P!], in fruit.

**Distribution:**—Southern China, Laos, Thailand, southern Myanmar, Andaman/Nicobar Islands, Peninsular Malaysia through Southeast Asian islands (except three major islands: Borneo, Luzon, and Mindanao) to southern New Guinea and northeastern Australia.

### 7. *Miliusa longipes* King (1892, p. 124)

**Type:**—Peninsular Malaysia, Perak, Mar 1885, *King's collector 7352* (holotype: CAL, isotypes: BM!, BO!, G!, L!, WU!), in flower.

**Taxonomic synonym:**—*Miliusa smithiae* Craib (1923, p. 44).

**Type:**—Thailand, Khao Ram, Mar 1922, *Smith 684* [holotype: K!, isotypes: BK! (photograph), BM!], in flower.

**Distribution:**—Southern Thailand, Peninsular Malaysia, Singapore, Sumatra to Java.

**Notes:**—During the preparation of this paper, it was decided to combine this species with *M. campanulata* because of the overall similarity, although *M. longipes* generally possesses (much) longer stipe. However, during a recent expedition, several flowering living plants of *M. longipes* were seen and their flowers are quite different from those of *M. campanulata*. The discoloration on the basal half of the inner petals of *M. longipes* is not (or very slightly) reticulate (remarkably reticulate in *M. campanulata*, Fig. 2C) and the window-like structures at base of the inner petals are indistinct (sometimes absent) (conspicuous in *M. campanulata*, Fig. 2D).

Mols and Keßler (2003) cited some specimens that are *M. campanulata* as *M. longipes*. It is now becoming clear that *M. longipes* only occurs in southern Thailand and southwards to Peninsular Malaysia, Singapore, Sumatra, and Java.

### 8. *Miliusa mollis* Pierre (1881, t. 40) (Fig. 1A)

Based on the same type: *Orophea mollis* (Pierre) Bân (1994, p. 10).

**Type:**—Cambodia, Prov. Samrongtong, crescit in Montibus Kereev, Mar 1870, *Pierre 3274* [lectotype selected here: P! (barcode P00160889), isotypes: A!, BM!, L!], in flower.

**Distribution:**—Thailand (Chaiyaphum, Chanthaburi, Khon Kaen, Lopburi, Nakhon Ratchasima, Saraburi Provinces) to Cambodia (Kampong Thom Province).

### 9. *Miliusa parviflora* Ridl. (1911, p. 65)

**Type:**—Peninsular Malaysia. Perlis, Tebing Tinggi, Mar 1910, *Ridley 15340* [lectotype selected by Turner (2012, p. 238): SING!, isotypes: BM!, K!]<sup>3</sup>, in flower and fruit.

**Taxonomic synonyms:**—*Miliusa jainii* Goel & S.C.Sharma (1991, p. 629) *syn. nov.*

**Type:**—India, Andaman Islands, South Andamans, Tarmugli Island, Sep 1987, *Goel 16846* (holotype: CDRI, isotypes: CDRI, L!), in flower and fruit.

– *Miliusa mukerjeeana* Debika Mitra & Chakr. (1994, p. 326) *syn. nov.*

**Type:**—India, South Andaman Island, Alimusjid Hill Jungle, Oct 1895, *King's collector s.n.* (holotype: CAL; isotype: CAL). [The type of *M. mukerjeeana* could not be traced. Study of a drawing and description of this species in the original publication was the ground for synonymization.]

**Distribution:**—Andaman and Nicobar Islands, southern Thailand, Peninsular Malaysia to Sumatra.

#### 10. *Miliusa sclerocarpa* (A.DC.) Kurz (1872, p. 291)

Basionym: *Uvaria sclerocarpa* A.DC. (1832, p. 203).

Based on the same type: *Saccopetalum sclerocarpum* (A.DC.) Hook.f. & Thomson (1872, p. 88).

**Type:**—Myanmar, Moalmyne, 1827, Wallich Numer. List no. 6461 [lectotype selected by Turner (2011b, p. 48): K!, isotypes: BM!, G!], in fruit.

**Taxonomic synonym:**—*Saccopetalum longiflorum* Hook.f. & Thomson (1855, p. 151) *syn. nov.*

Based on the same type: *Miliusa longiflora* (Hook.f. & Thomson) Baill. ex Finet & Gagnep. (1906, p. 153).

**Type:**—Wallich Numer. List no. 6443a [lectotype selected here: K! (photograph), isotypes: BR!, G!], in flower.

**Distribution:**—India (Bihar State), Myanmar (Mon State) to Thailand (Kanchanaburi?, Lampang, Lamphun, Mae Hong Son, Sukhothai Provinces).

**Notes:**—This species was reported to occur in Thailand for the first time under the name *M. longiflora* (Khumchompoo & Thongpukdee 2005), which is considered a synonym of *M. sclerocarpa* in this study. However, specimens collected from south-western Thailand cited in Khumchompoo & Thongpukdee (2005) were not seen by us except one collection: *Van Beusekom and Santisuk 2876*, which has somewhat smaller leaves, denser vegetative indumentum, and more carpels per flower. Hence, this collection probably represents an undescribed species.

#### 11. *Miliusa thorelii* Finet & Gagnep. (1907, p. 89) (Fig. 1C)

**Type:**—Laos, Pak-Lay, 1866–1868, *Thorel 3301* [holotype: P! (barcode P00160898)], in male flower.

**Taxonomic synonym:**—*Miliusa bannaensis* X.L.Hou, in Hou *et al.* (2004, p. 79) *syn. nov.*

**Type:**—China, *Zhu and Wang 2125* (holotype: HITBC!, photograph), in flower.

**Distribution:**—China (Yunnan Province), Laos (Sainyabuli Province) to Thailand (Chanthaburi, Chiang Mai, Chiang Rai, Kamphaeng Phet, Kanchanaburi, Lampang, Mae Hong Son, Nan, Phang Nga, Phrae, Surat Thani, Tak Provinces) and Myanmar (Tanintharyi Division).

<sup>3</sup> Incorrectly indicated as “(holotype: K!, isotypes: BM!, SING!)” in the original publication of this chapter.

**12. *Miliusa velutina* (DC.) Hook.f. & Thomson (1855, p. 151)**

Basionym: *Uvaria velutina* DC., in Dunal (1817, p. 91).

Based on the same type: *Guatteria villosa* G.Don (1831, p. 100) *nom. illeg. superfl.*  
– *Uvaria villosa* Roxb. (1832, p. 664) *nom. illeg. superfl.* – *Miliusa villosa* W.Theob.,  
in Mason (1883, p. 667) *nom. illeg. superfl.*

**Type:**—India Orient., Wallich Numer. List no. 6441a (holotype: G!, isotypes: A!, BM?), in flower.

**Taxonomic synonym:**—*Guatteria velutina* A.DC. (1832, p. 218).

**Type:**—Myanmar, Atran, Apr 1827, Wallich Numer. List no. 6441c [holotype: G, isotypes: BM!, K! (photograph)], in flower.

**Distribution:**—India (Assam, Jharkhand, Orissa, Uttar Pradesh, Uttarakhand, West Bengal States), Nepal (Central, Eastern Regions), Bangladesh (Dhaka Division) through Myanmar (Bago Division, Kachin State, Yangon Division), Thailand (Chachoengsao, Chiang Mai, Kanchanaburi, Lampang, Mae Hong Son, Nakhon Ratchasima, Nong Khai, Phetchaburi, Phrae, Prachuab Khiri Khan, Ratchaburi, Saraburi, Tak Provinces), Laos (Attapeu, Champasak Provinces), Cambodia (Kampong Thom, Kampong Speu, Kratié, Preah Vihear, Pursat, Stung Treng Provinces) to Vietnam (Ninh Thuan, Tay Ninh Provinces).

**Appendix 1**

Studied specimens of the *Miliusa* species previously known from Thailand

***M. amplexicaulis:*** Bunnab 90; Chaowasku 54.

***M. campanulata:*** Chaowasku 23; Collins 1281; Fukuoka T-63705; Geesink et al. 6985; Gentry and Niyomdham 66500; Hardial 587; Kerr 8824, 10816; Larsen 9938; Larsen et al. 566, 3237, 31486, 31957; Maxwell 76-389; Murata et al. T-16310, T-16415; Phusomsaeng et al. 5; Plernchit 435; Pooma 1560; Sangkachand et al. 3141; Santisuk 199; Shimizu et al. T-8849; Smitinand 3820; Takahashi T-63255; Van Beusekom and Charoenpol 1807.

***M. cuneata:*** Bunchuai 1321; Chalermglin 420408; Kerr 1837, 5011, 6050, 10345; Keßler PK 3183, PK 3277; Kostermans 842; Koyama et al. 15600; Larsen et al. 46329; Maxwell 89-613, 90-1098, 95-407, 95-1208, 96-374, 96-600, 96-1430, 97-478, 99-282, 01-351, 02-152; Murata et al. T-15036; Parnell et al. 95-585; Phengklai et al. 2939; Pooma 1198, 1437; Shimizu and Hutoh T-10238; Tagawa et al. T-9895; Van Beusekom and Phengklai 208, 1322; Van Beusekom et al. 3700, 3848; Van de Bult 480, 682; Vidal 5182.

***M. filipes:*** Kloss 6968.

***M. fusca:*** Chaowasku 46; Kerr 8630; Murata et al. T-16322, T-16404; Van Beusekom and Phengklai 3129.

***M. horsfieldii:*** Chaowasku 109; Charoenchai 789, 809; Gardner and Sidisunthorn ST 0263; Kerr 6012; Larsen et al. 31294; Maxwell 02-90.

***M. longipes:*** Iwatsuki et al. T-8453, T-8486; Niyomdham et al. 322.

***M. mollis:*** Chaowasku 20, 49; Geesink et al. 6746, 6937; Kerr 8017; Keßler PK 3207;

Koyama et al. T-33050; Lakshnakara 274; Marcan 1520; Maxwell 75-479; Phengnaren 444; Phuphathanaphong 31; Put 1095, 1870, 2417, 4365; Shimizu et al. T-23919; Smitinand 7455; Smitinand and Phengkklai 8828; Smitinand & Sleumer 1313, 8375; Van Beusekom and Charoenpol 1927.

**M. parviflora:** Chaowasku 98.

**M. sclerocarpa:** Chaowasku 19; Geesink et al. 5970; Maxwell 72-126, 94-284, 94-573, 94-1311.

**M. thorelii:** Geesink et al. 6718, 8228; Kerr 1162, 1834, 5448; Keßler PK 3184, PK 3224; Kostermans 449; Koyama T-61148; Larsen et al. 2559, 2620, 3051, 30956; Maxwell 85-800, 89-427, 89-651, 89-677, 89-801, 91-765, 93-667, 94-492, 95-513, 95-944, 96-1303, 97-584, 98-797; Murata et al. 16898; Sangkachand 828; Smitinand 3983; Smitinand and Cheke 10828; Somkid 394; Sørensen et al. 3077, 4523; Van Beusekom and Phengkklai 1327; Winit 862.

**M. velutina:** Bjørnland and Schumacher 220; Buasawng 2; Cheeranun 5; Geesink et al. 5954; Keith 362; Kerr 1078, 5237, 10629; Keßler PK 3216; Kostermans 420; Maxwell 75-165, 88-732, 98-541; Na Nakhon and Beck 88150; Phusomsaeng 1821; Panatkool 480; Pholsena and Koonkhunthod 2842; Pooma 165, 399; Prasert 1; Put 2790; Santisuk 039, 997; Smitinand and Phengkklai 8647; Soora 3; Umnat 8; Vanpruk 449; Winit 87.

# Chapter 8

## Pollen morphology of *Milium* and relatives (Annonaceae)

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### Abstract

In order to provide additional data for the subdivision of the palaeotropical genus *Milium* (Annonaceae), its pollen was examined using light, scanning and transmission electron microscopy, including 20 of the 30–40<sup>1</sup> species. Further, species of the other genera in the former tribe Miliuseae (*Alphonsea*, *Mezzettia*, *Orophea*, *Platymitra*; *Phoenicanthus* not available) and of the *Polyalthia cerasoides* group, the sister group to *Milium* in a recent molecular analysis, were included. The pollen of *Mezzettia* and *Platymitra* is described for the first time with scanning and transmission electron microscopy. The pollen of all species studied with transmission electron microscopy appeared to possess an inaperturate exine, but apertural areas ('germination zones') were observed in the intine. The pollen morphological variation within *Milium* does not correlate with the macromorphological subdivision of the genus. It appeared to be impossible to define distinct pollen types. The former tribe Miliuseae cannot be characterized or subdivided with the help of pollen characters either. All genera fit in the larger miliusoid clade<sup>2</sup> (25 genera). The pollen of the *Polyalthia cerasoides* group deviates in its finely and densely granular infratectum.

**Key words:** Annonaceae, Miliuseae, *Milium*, miliusoid clade, pollen

### Introduction

The genus *Milium* Lesch. ex A.DC. (Annonaceae), including *Saccopetalum* Benn., comprises 30–40 species, which occur from India and South China to North Australia (Mols & Keßler 2003, 2004, Chaowasku & Keßler 2006). Together with *Alphonsea* Hook.f. & Thomson, *Mezzettia* Becc. (doubtfully), *Mezzettiopsis* Ridl. (now included in *Orophea* Blume; Leonardía & Keßler 2001), *Orophea*, *Phoenicanthus* Alston and *Platymitra* Boerl., it was considered to make up the tribe Miliuseae (Keßler 1993). However, more recent studies, including morphological and molecular analyses (*Phoenicanthus* not available; Mols & Keßler 2003, Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004), indicate that this tribe is polyphyletic. Informally, Mols *et al.* (2004b) distinguished a much larger group (25 genera in total), the 'miliusoid clade', containing *Milium* and related genera. This group

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<sup>1</sup> Now estimated to contain ca. 50 species (see Chapter 7).

<sup>2</sup> Equivalent to the recircumscribed Miliuseae of Chatrou *et al.* (2012).

includes, among others, *Alphonsea*, *Orophea* and *Platymitra*, but excludes *Mezzettia*. The closest relatives of *Miliusa* appeared to be three *Polyalthia* Blume species [*Polyalthia cerasoides* group<sup>3</sup>: *P. cerasoides* (Roxb.) Bedd., *P. pendula* Capuron ex G.E.Schatz & Le Thomas, *P. stuhlmannii* (Engl.) Verdc.], which make up the sister group of *Miliusa*. On the basis of morphological similarities this clade might eventually embrace all African/Malagasy and some of the Asian and Australian *Polyalthia* species (pers. obs. TC; see also Ratnayake *et al.* 2006).

The genus *Miliusa* was found to be monophyletic (Mols *et al.* 2004b), but morphologically it is rather diverse. On the basis of flower position, characters of the inner petals and the number of ovules per carpel it can be subdivided into four groups (Chaowasku *et al.* in prep.<sup>4</sup>): 1. *Miliusa mollis* group, 2. *Miliusa horsfieldii* group (the former genus *Saccopetalum*), 3. *Miliusa velutina* group and 4. *Miliusa s.s.* group<sup>5</sup>.

The pollen morphology of *Miliusa* is poorly known. The pollen of eight species was described using only light microscopy (Agababian 1971, Mitroiu 1970, Walker 1971b): *M. balansae* Finet & Gagnep. (incl. *M. chunii* W.T.Wang), *M. campanulata*<sup>6</sup> Pierre, *M. dioeca* (Roxb.) Chaowasku & Kessler (incl. *M. roxburghiana* Hook.f. & Thomson, *nom. illeg. superfl.*), *M. horsfieldii* (Benn.) Baill. ex Pierre (incl. *Saccopetalum arboreum* Elmer), *M. indica* Lesch. ex A.DC., *M. tomentosa* (Roxb.) Finet & Gagnep., *M. velutina* (DC.) Hook.f. & Thomson and *M. vidalii* J.Sinclair (as *Saccopetalum longipes* S.Vidal), and only one species, *M. indica*, using also scanning and/or transmission electron microscopy (Le Thomas 1980, 1981, Straka & Friedrich 1988). Mols *et al.* (2004a) used data on pollen grain shape and size, ornamentation and aperture type of seven *Miliusa* species, and, among others, the *Polyalthia cerasoides* group and species of *Alphonsea*, *Orophea* and *Platymitra* in a phylogenetic analysis of the miliusoid clade.

The aim of the present study is to describe the pollen of *Miliusa* and to evaluate its taxonomic significance and its affinity with the pollen of the supposed relatives in the former tribe Miliuseae (*Alphonsea*, *Mezzettia*, *Orophea*, *Platymitra*) and in the present miliusoid clade (*Polyalthia cerasoides* group).

## Material and methods

Twenty *Miliusa* species covering all four macromorphological groups distinguished by Chaowasku *et al.* (in prep.) were investigated (see Specimens investigated). Further, pollen data of *Alphonsea*, *Mezzettia*, *Orophea* and *Platymitra* from the former tribe Miliuseae, and of *Polyalthia cerasoides* and *P. pendula* from the present sister group of *Miliusa* were available for comparison. Data of *Polyalthia stuhlmannii* were taken from the literature (Le Thomas 1980, 1988). Data of *Phoenicanthus* are not available.

Almost all samples were obtained from herbarium specimens (3 samples from spirit collections). All material was prepared for light microscopy (LM; acetolysed) and

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<sup>3</sup> Now classified in the genus *Hubera* Chaowasku (see Chapter 3).

<sup>4</sup> = Chapter 7.

<sup>5</sup> Now known as the *Miliusa campanulata* group (see Chapter 7).

<sup>6</sup> The voucher specimen *Petelot 1817* (cited by Walker 1971b) is presently identified as *M. balansae* (see Chaowasku & Keßler 2014).

scanning electron microscopy (SEM; acetolysed, unless indicated otherwise). A selection of species from all genera was prepared for transmission electron microscopy (TEM).

The techniques described by Van der Ham (1990) were applied. For pollen shape and size, ten pollen grains per sample were measured, if possible. The subdivision of the exine into tectum, infratectum and basal layer (ectexinous), as described by Le Thomas (1980), is used. A 'germination zone' is defined as a local thinning of the tubular exintine with an underlying thickening of the homogeneous endintine, while the exine over these structures does not deviate from the rest of the exine (Waha & Hesse 1988; see also Discussion). Terminology follows Punt *et al.* (2007).

## Results

The exine ornamentation of the pollen of *Miliusa* and its relatives appeared hard to classify. Four main types are distinguished:

1. rugulate (Fig. 1B, F): elements (muri) prominent, > 1  $\mu\text{m}$ , irregularly anastomosing;
2. verrucate (Fig. 1G): elements (verrucae) prominent, > 1  $\mu\text{m}$ , circular to irregularly elliptic;
3. scabrate (Fig. 1D, E): elements (scabrae) prominent, < 1  $\mu\text{m}$ , subcircular to irregular;
4. fossulate (Fig. 2D): elements  $\pm$  indistinct, separated by irregular grooves (fossulae).

Intermediates occur between: rugulate and verrucate (variation in degree of anastomosing; Figs. 1H, 2H) and verrucate and scabrate (variation in size of elements; Fig. 2B). Sometimes, the elements and grooves in the fossulate type are so indistinct (LM) that the exine might be described as  $\pm$  psilate. Small tectum perforations are present (TEM), usually hidden between the ornamentation elements (SEM). Different types of ornamentation elements may be found within a single sample, or even in a single pollen grain (e.g. scabrae and verrucae; Fig. 2B). Below, the main ornamentation types per genus are given (see Table 1 for the individual species).

### *Alphonsea*

LM: Pollen grains apolar, subspheroidal monads, 38–45  $\mu\text{m}$  in diameter.

SEM (Fig. 1A, B): Exine ornamentation fossulate or rugulate.

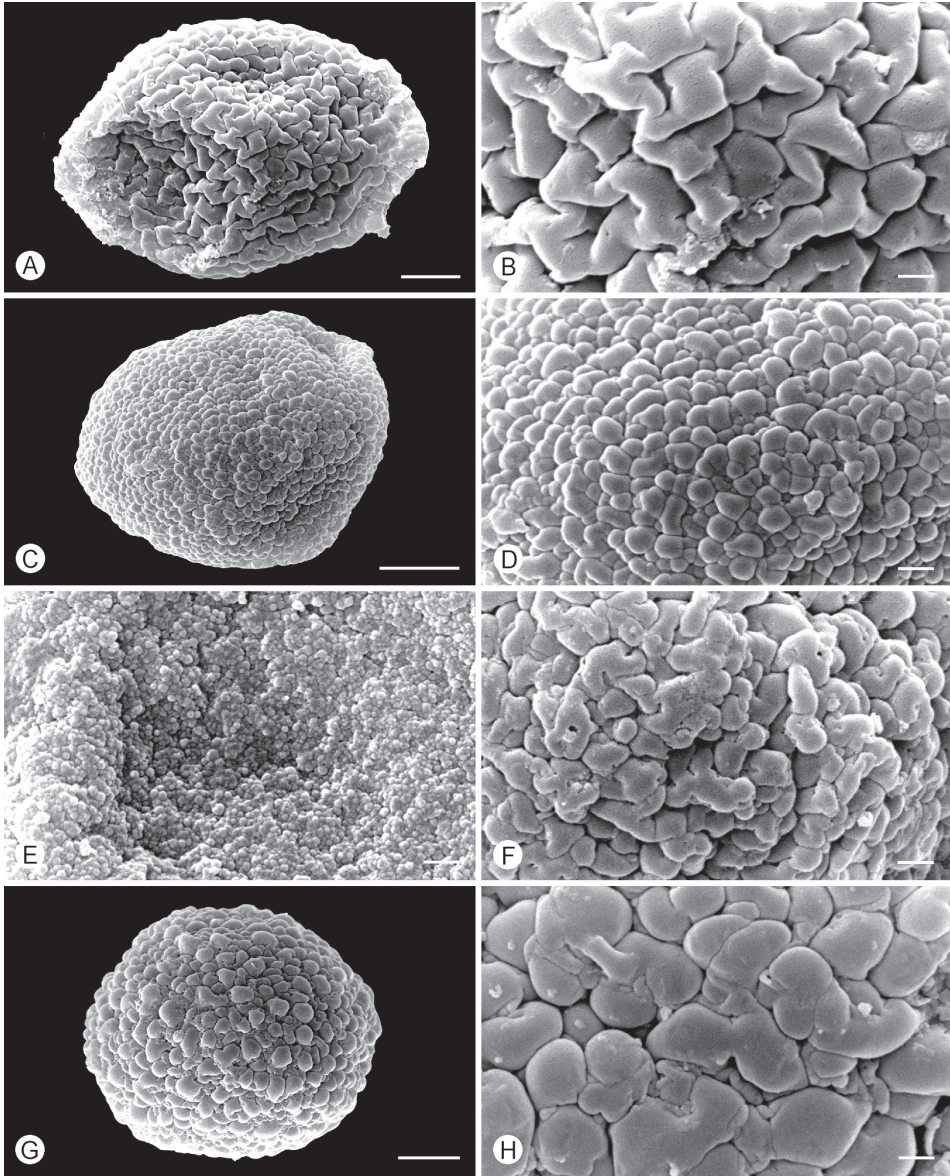
TEM (Fig. 3A, B): Exine inaperturate, tectate. Infratectum coarsely granular, up to as thick as tectum. Basal layer thin, sometimes indistinctly lamellate. Intine consisting of a thick tubular exintine and a thin homogeneous endintine, with one (?) or two germination zones.

### *Mezzettia*

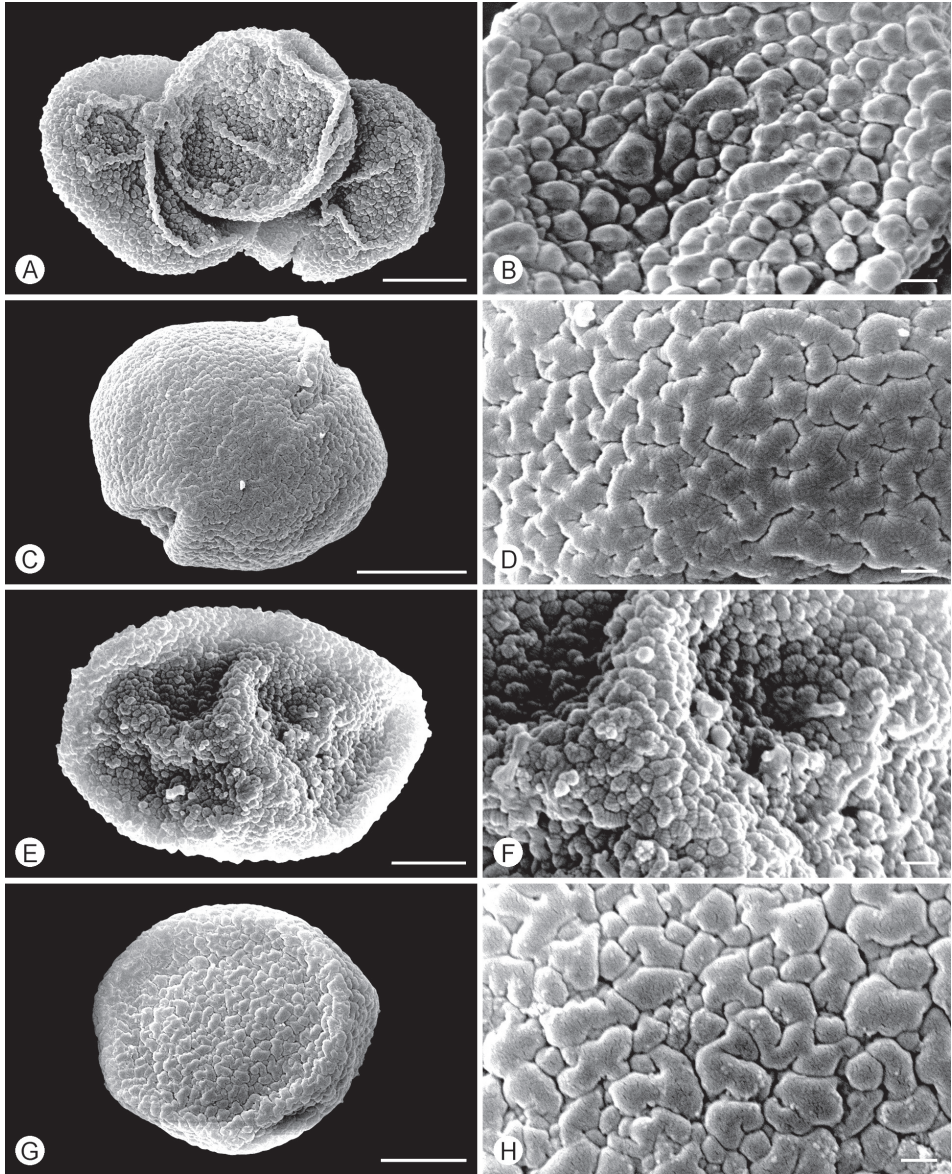
LM: Pollen grains apolar, subspheroidal monads, 34  $\mu\text{m}$  in diameter.

SEM (Fig. 2A, B): Exine ornamentation verrucate-scabrate.

TEM (Fig. 3E): Exine inaperturate. Outer layer consisting of scabrae and verrucae, without differentiation into tectum and infratectum. Basal layer clearly thinner than outer layer, lamellate. Intine consisting of a thin tubular exintine and a thin homogeneous endintine, with one (?) indistinct germination zone.



**FIGURE 1.** Pollen of *Alphonsea* and *Miliusa*, SEM. A, B. *Alphonsea siamensis*: (A) Pollen grain; (B) Detail showing rugulate ornamentation. C, D. *Miliusa brahei*: (C) Pollen grain; (D) Detail showing coarsely scabrate ornamentation. E. *Miliusa traceyi*: Detail showing finely scabrate ornamentation. F. *Miliusa mollis* (Chaowasku 20): Detail showing rugulate ornamentation. G. *Miliusa macropoda*: Pollen grain showing verrucate ornamentation. H. *Miliusa balansae* (Soejarto & Cuong 10642): Detail showing rugulate-verrucate ornamentation. Scale bars – 5  $\mu\text{m}$  (A, C, G), 1  $\mu\text{m}$  (B, D–F, H).



**FIGURE 2.** Pollen of *Mezzettia*, *Orophea*, *Platymitra* and *Polyalthia*, SEM. A, B. *Mezzettia parviflora*: (A) Three pollen grains; (B) Detail showing verrucate-scabrate ornamentation. C, D. *Orophea polycarpa* (*Chaowasku* 21): (C) Pollen grain with two depressed areas indicating two intinous germination zones; (D) Detail showing foliulate ornamentation. E, F. *Platymitra macrocarpa* (*Gardner et al. ST 1648*): (E) Pollen grain; (F) Detail showing coarsely scabrate ornamentation. G, H. *Polyalthia cerasoides* (*Vajravelu* 36762): (G) Pollen grain; (H) Detail showing rugulate-verrucate ornamentation. Scale bars – 5 µm (A, C, E, G), 1 µm (B, D, F, H).

**TABLE 1.** Pollen size ( $\mu\text{m}$ ; LM), exine ornamentation (SEM), infratectum type (TEM) and number of germination zones (TEM) in *Alphonsea*, *Mezzettia*, *Miliusa* (arranged according to macromorphological group: 1–4), *Orophea*, *Platymitra* and *Polyalthia*.

Genus/species	Group	Size	Ornamentation	Infratectum	Germination zones
<b><i>Alphonsea</i></b>					
<i>A. boniana</i>		45	Fossulate	Coarsely granular	2
<i>A. elliptica</i>		38	Fossulate	Coarsely granular	1?
<i>A. javanica</i>		38	Fossulate	Coarsely granular	1?
<i>A. kinabaluensis</i>		43	Fossulate	Coarsely granular	2
<i>A. siamensis</i>		41	Rugulate		
<b><i>Mezzettia</i></b>					
<i>M. parviflora</i>		34	Verrucate-scabrate	Absent	1?
<b><i>Miliusa</i></b>					
<i>M. amplexicaulis</i>	1	45	Rugulate	Coarsely granular	2
<i>M. fusca</i>	1	50	Verrucate		
<i>M. glandulifera</i>	1	47	Rugulate		
<i>M. mollis</i>	1	46	Rugulate	Coarsely granular	2
<i>M. brahei</i>	2	36	Coarsely scabrate		
<i>M. horsfieldii</i>	2	41	Rugulate-verrucate	Coarsely granular	2
<i>M. tomentosa</i>	2	45	Rugulate-verrucate		
<i>M. vidalii</i>	2	40	Rugulate		
<i>M. banghoiensis</i>	3	49	Verrucate		
<i>M. macropoda</i>	3	41	Verrucate		
<i>M. traceyi</i>	3	39	Finely scabrate		
<i>M. velutina</i>	3	42	Rugulate-verrucate	$\pm$ Columellate	2
<i>M. balansae</i>	4	44	Rugulate-verrucate	Coarsely granular	2
<i>M. campanulata</i>	4	43	Verrucate-scabrate	$\pm$ Columellate	2
<i>M. cuneata</i>	4	54	Rugulate-verrucate	Coarsely granular	2
<i>M. dioeca</i>	4	45	Coarsely scabrate		
<i>M. macrocarpa</i>	4	47	Rugulate-verrucate		
<i>M. montana</i>	4	45	Verrucate	$\pm$ Columellate	1?
<i>M. thailandica</i>	4	53	Verrucate-scabrate		
<i>M. thorelii</i>	4	47	Rugulate-verrucate	Coarsely granular	2
<b><i>Orophea</i></b>					
<i>O. brandisii</i>		44	Verrucate	$\pm$ Columellate	?
<i>O. celebica</i>		37	Verrucate	$\pm$ Columellate	2
<i>O. creaghii</i>		33	Fossulate	$\pm$ Columellate	2
<i>O. enneandra</i>		28	Rugulate		
<i>O. enterocarpa</i>		34	Rugulate		

TABLE 1 (continued).

Genus/species	Group	Size	Ornamentation	Infratectum	Germination zones
<i>O. kerrii</i>		35	Fossulate		
<i>O. myriantha</i>		32	Fossulate		
<i>O. polycarpa</i>		37	Fossulate		
<b><i>Platymitra</i></b>					
<i>P. macrocarpa</i>		27	Coarsely scabrate	± Columellate	2
<b><i>Polyalthia</i></b>					
<i>P. cerasoides</i>		42	(Rugulate-)verrucate	Finely, densely granular	1?
<i>P. pendula</i>		43	Verrucate	Finely, densely granular	2
<i>P. stuhlmannii</i> (lit. data)				Finely, densely granular	1?

***Miliusa***

LM: Pollen grains apolar, subspheroidal monads, 36–54 µm in diameter.

SEM (Fig. 1C–H): Exine ornamentation rugulate, verrucate or scabrate.

TEM (Fig. 4): Exine inaperturate, tectate. Infratectum coarsely granular to ± columellate, up to as thick as tectum, but usually thinner. Basal layer thin, ± lamellate, possibly absent in *M. velutina*. Intine consisting of a thick tubular exintine and a thin homogeneous endintine, with one (?) or two germination zones.

***Orophea***

LM: Pollen grains apolar, subspheroidal monads, 28–44 µm in diameter.

SEM (Fig. 2C, D): Exine ornamentation rugulate, verrucate or fossulate.

TEM (Fig. 3C, D): Exine inaperturate, tectate. Infratectum ± columellate, up to as thick as tectum. Basal layer rather thick and distinctly lamellate (*O. brandisii*) or thin and not lamellate (*O. celebica*, *O. creaghii*). Intine consisting of a thick tubular exintine and a thin homogeneous endintine, with two (unknown in *O. brandisii*) germination zones.

***Platymitra***

LM: Pollen grains apolar, subspheroidal monads, 27 µm in diameter.

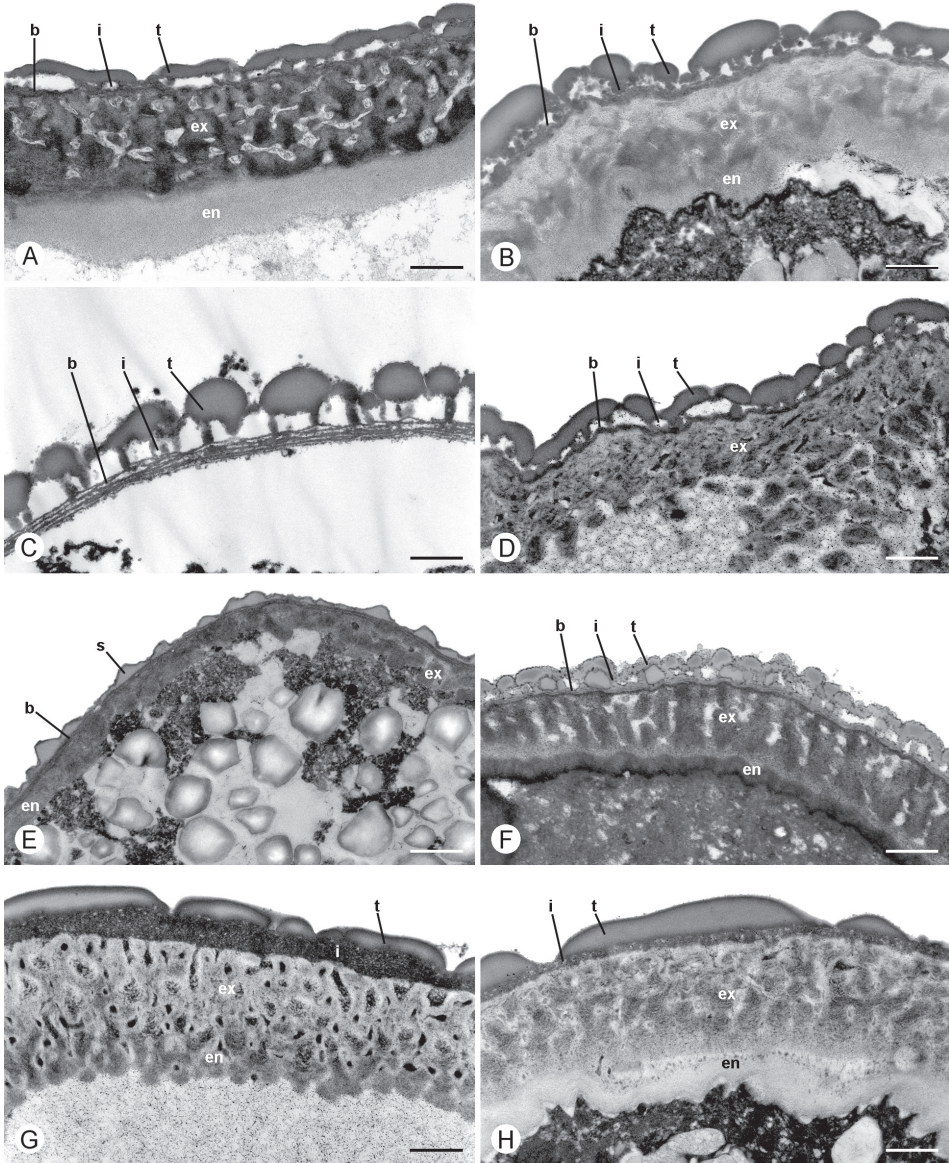
SEM (Fig. 2E, F): Exine ornamentation coarsely scabrate.

TEM (Fig. 3F): Exine inaperturate, tectate. Infratectum ± columellate, up to as thick as tectum. Basal layer rather thick and distinctly lamellate. Intine consisting of a thick tubular exintine and a thin homogeneous endintine, with two germination zones.

***Polyalthia cerasoides* group (*P. cerasoides*, *P. pendula*, *P. stuhlmannii*)**

LM (*P. cerasoides*, *P. pendula*): Pollen grains apolar, subspheroidal monads, 42–43 µm in diameter.

SEM (*P. cerasoides*, *P. pendula*; Fig. 1G, H): Exine ornamentation (rugulate-)verrucate.



**FIGURE 3.** Pollen of *Alphonsea*, *Mezzettia*, *Orophea*, *Platymitra* and *Polyalthia*, TEM. A. *Alphonsea boniana*: Detail of pollen wall with coarsely granular infratectum, tubular exintine and homogeneous endintine. B. *Alphonsea elliptica*: Detail of pollen wall with coarsely granular infratectum, tubular exintine and homogeneous endintine. C. *Orophea brandisii*: Detail of pollen wall with  $\pm$  columellate infratectum and distinctly lamellate basal layer. D. *Orophea creaghii*: Detail of pollen wall with  $\pm$  columellate infratectum and tubular exintine. E. *Mezzettia parviflora*: Detail of pollen wall showing basal layer with scabrae and verrucae, tubular exintine (right) and homogeneous endintine (left). F. *Platymitra macrocarpa*: Detail of pollen wall with  $\pm$  columellate infratectum, lamellate basal layer, tubular exintine and homogeneous endintine. G. *Polyalthia cerasoides*: Detail of pollen wall with finely and densely granular infratectum and tubular exintine. H. *Polyalthia pendula*: Detail of pollen wall with finely and densely granular infratectum and tubular exintine. Scale bars – 1  $\mu$ m (A–H); b=basal layer, en=endintine, ex=exintine, i=infratectum, s=scabra/verruca, t=tectum.

TEM (*P. cerasoides*, *P. pendula*; Fig. 3G, H) (*P. stuhlmannii*; Le Thomas 1980, 1988): Exine inaperturate, tectate. Infratectum finely and densely granular, up to as thick as tectum. Basal layer absent (or very thin?). Intine consisting of a thick tubular exintine and a thin homogeneous endintine, with one (?) or two (*P. pendula*) germination zones.

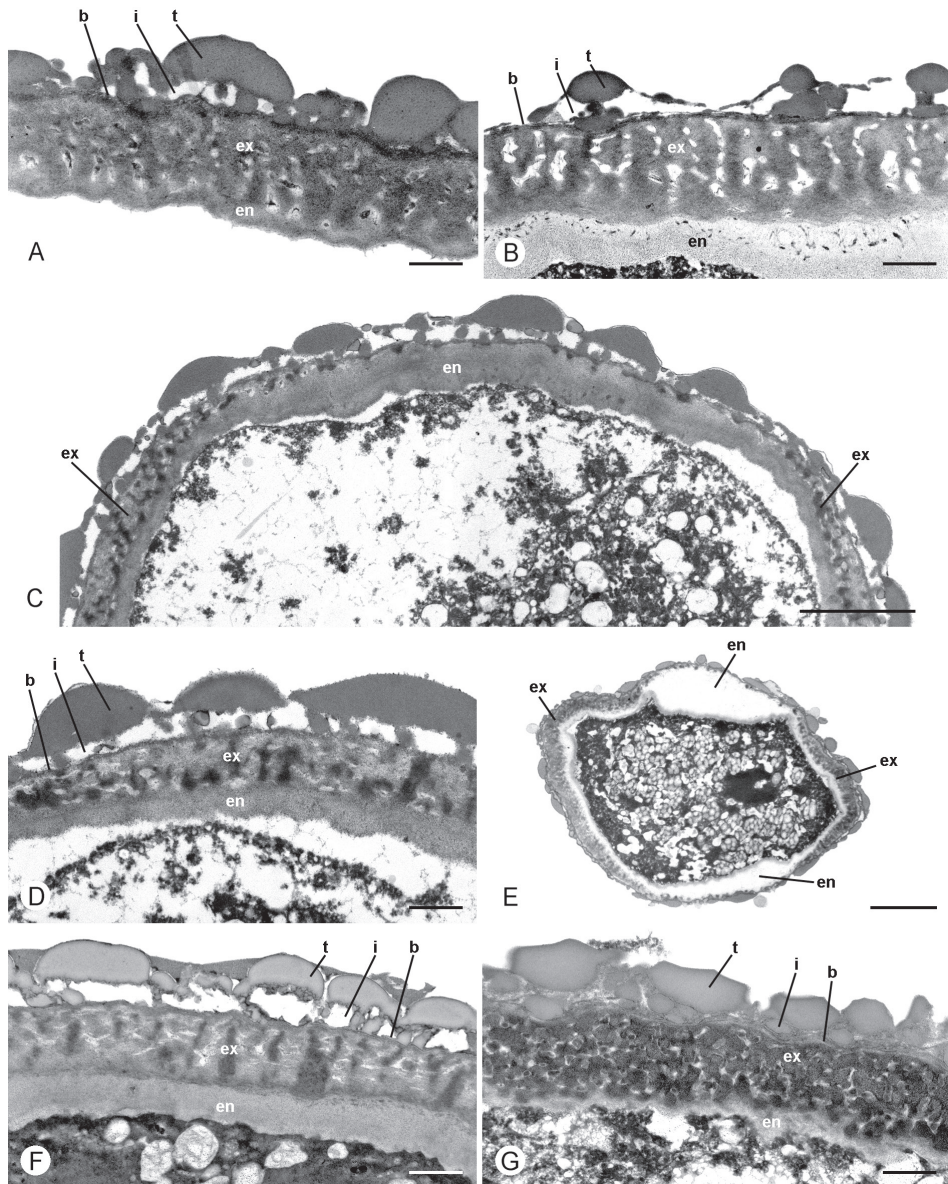
## Discussion

### Apertural system

On the basis of an extensive LM study of exine characters, Walker (1971b) concluded that the pollen of *Alphonsea*, *Mezzettia*, *Miliusa*, *Orophea*, *Platymitra* and *Polyalthia* is inaperturate. As expressed by Le Thomas (1980), apertures of Annonaceae pollen have always posed problems (see also Le Thomas *et al.* 1986 and Le Thomas 1988). Waha & Hesse (1988) proposed to take into account also intine characters in defining apertural areas ('germination zones') in the large and heterogeneous genus *Polyalthia*. They described five pollen types based on various patterns of differential thickening of the tubular exintine and the homogeneous endintine ('exintine lacunaire' and 'endintine compacte', respectively, in Le Thomas 1988). Besides underlining the polyphyletic nature of *Polyalthia* (see also Rogstad & Le Thomas 1989, Schatz & Le Thomas 1990, Mols *et al.* 2004b), this explained the concept of pollen with an inaperturate exine (Walker 1971b) though with (a) particular intine area(s) functioning as (an) aperture(s). Such pollen was also observed in the present study in all TEM samples (Fig. 4C, E); Mols *et al.* (2004a) denoted it as 'inaperturate' (cryptoaperturate) or disulculate. Possibly there are always two germination zones per pollen grain (cf. Fig. 4d, d' of Waha & Hesse 1988). They are difficult to observe with LM or SEM, though sometimes recognizable as depressed areas (Fig. 2C). Only a thorough TEM study may reveal their presence, position, orientation and taxonomic distribution. Similar pollen is known from several Australian endemic genera within the miliusoid clade, being denoted as disulculate (Waha & Morawetz 1988; see also Doyle & Le Thomas 1994, Doyle *et al.* 2000). In contrast to Le Thomas (1980, 1988), Waha & Hesse (1988) and Mols *et al.* (2004a), we do not consider the pollen of *Polyalthia stuhlmannii* to be monosulcate, but inaperturate with one (or two?) germination zone(s). There is still exinous material ('membrane aperturale') present over the zone, while the zone itself is nontubular (see Le Thomas 1988: pl. 6, Fig. 3). As far as known, monosulcate (boat-shaped) pollen with a tubular apertural intine does not occur within the miliusoid clade.

### *Miliusa*

Chaowasku *et al.* (in prep.) distinguished four macromorphological groups within *Miliusa*. The pollen morphology of *Miliusa* is diverse too, but this variation does not reflect the macromorphological subdivision (Table 1). Due to the occurrence of intermediate forms between the three main exine ornamentation types distinguished within *Miliusa*, it was even impossible to define distinct ornamentation types. Neither do pollen shape or size suggest any subdivision, while the TEM data obtain only to a selection of the species (9 of 20). One species (*M. traceyi* Jessup) deviates from all other species of *Miliusa* and other genera described in the present study in having finely scabrate pollen (Fig. 1E). It deviates



**FIGURE 4.** Pollen of *Miliusa*, TEM. A. *Miliusa balansae*: Detail of pollen wall with coarsely granular infractectum, tubular exintine and homogeneous endintine. B. *Miliusa cuneata*: Detail of pollen wall with coarsely granular infractectum, tubular exintine and homogeneous endintine. C, D. *Miliusa horsfieldii* (Mols 12): (C) Detail of pollen wall with coarsely granular infractectum, tubular exintine (left and right) and germination zone (middle) consisting of homogeneous endintine; (D) Detail of pollen wall with coarsely granular infractectum, tubular exintine and homogeneous endintine. E. *Miliusa horsfieldii* (How 71794): Pollen wall with tubular exintine (left and right) and two germination zones (top and bottom) consisting of homogeneous endintine. F. *Miliusa mollis*: Detail of pollen wall with coarsely granular infractectum, tubular exintine and homogeneous endintine. G. *Miliusa thorelii*: Detail of pollen wall with coarsely granular infractectum and tubular exintine. Scale bars – 1  $\mu\text{m}$  (A, B, D, F, G), 5  $\mu\text{m}$  (C), 10  $\mu\text{m}$  (E); b=basal layer, en=endintine, ex=exintine, i=infractectum, t=tectum.

also in its macromorphology and is hard to connect to the other species. It is best placed in morphological group 3 but it has a reduced number of stamens (less than 10), while the other species in group 3<sup>7</sup> never have less than 24 stamens.

### Tribe Miliuseae/miliusoid clade

The former tribe Miliuseae (*Alphonsea*, *Mezzettia*, *Miliusa*, *Orophea*, *Platymitra*) does not offer any useful character for a pollen morphological subdivision either. The fossulate ornamentation type is found only in *Alphonsea* and *Orophea*, but together with types/forms occurring in three of the other four genera as well (not in *Platymitra*). *Mezzettia* is remarkable, because it lacks a distinct infratectal layer. In contrast to the other genera, which have a tectate exine with a  $\pm$  columellate or coarsely to finely and densely granular infratectal layer, the exine of *Mezzettia parviflora* Becc. pollen consists of a basal layer with scabrae and verrucae, without any differentiation into tectum and infratectum (Fig. 3E). This is a rare character in Annonaceae pollen, known only from two mutually unrelated genera in the miliusoid clade (Mols *et al.* 2004b): *Phaeanthus*<sup>8</sup> Hook.f. & Thomson and *Stelechocarpus*<sup>9</sup> Hook.f. & Thomson (pers. obs. TC). In several other miliusoid genera the infratectum is very thin: *Meiogyne* Miq., *Polyalthia borneensis* Merr., *Sapranthus* Seem. (own obs.). In view of the position of *Mezzettia* outside the miliusoid clade (Mols *et al.* 2004a, 2004b), its nonstratified outer exine layer must be considered as autapomorphic, not indicating relationship with *Phaeanthus* and *Stelechocarpus*.

### *Polyalthia cerasoides* group

The pollen of the *Polyalthia cerasoides* group, the sister group of *Miliusa* in the miliusoid clade, resembles that of *Miliusa* very much in size, shape, exine ornamentation and apertural system (Table 1). However, it differs clearly in its infratectal structure. All three species have a finely and densely granular infratectum (Fig. 3G, H). In *Miliusa* it is coarsely granular to  $\pm$  columellate. As far as known a finely and densely granular infratectum does not occur in the miliusoid clade outside the *Polyalthia cerasoides* group, but it was observed in a few more basal Annonaceae: *Piptostigma* Oliv. and *Polyceratocarpus* Engl. & Diels (Le Thomas 1980). In contrast to the inaperturate pollen of the miliusoid genera, these genera have monosulcate pollen. This indicates that the finely and densely granular infratectum evolved independently in the ancestor of the *Polyalthia cerasoides* group.

## Conclusions

The pollen morphology of *Miliusa* is diverse and is not able to demonstrate the monophyly of the genus. Due to the occurrence of intermediate forms between the three main exine ornamentation types, it appeared to be impossible to define distinct ornamentation types within *Miliusa*. The pollen morphological variation does not correlate with

<sup>7</sup> Listed in Table 1.

<sup>8</sup> The exine of *Phaeanthus* has been re-interpreted as being atectate (see Chapter 2).

<sup>9</sup> The basal layer of *Stelechocarpus* has been re-interpreted as consisting of a finely and densely granular infratectum and a basal layer (therefore its exine is tectate; see Chapter 4).

the macromorphological subdivision of *Miliusa*. *Miliusa traceyi* deviates in its pollen and macromorphology. The former tribe Miliuseae (*Alphonsea*, *Mezzettia*, *Miliusa*, *Orophea*, *Platymitra*) cannot be characterized or subdivided with help of pollen characters either (*Phoenicanthus* pollen unknown). *Mezzettia* is most deviating. All five genera fit in the miliusoid clade. Because aperture characters in this clade reside especially in the intine, which can only be visualized with transmission electron microscopy, a lot of additional work is necessary in order to establish more detailed pollen morphological relationships.

## Specimens investigated

\* = SEM unacetolysed

### ***Alphonsea* Hook.f. & Thomson**

- A. boniana* Finet & Gagnep.: Thailand. *Van Beusekom & Smitinand 2116* (L) TEM  
*A. elliptica* Hook.f. & Thomson: Indonesia. *Kostermans 418* (L) TEM  
*A. javanica* Scheff.: Indonesia, Borneo, Sumatra. *Kostermans 9907* (L) TEM  
*A. kinabaluensis* J.Sinclair: Indonesia, Borneo. *SAN 76435* (L) TEM  
*A. siamensis* Kessler: Thailand. *Chaowasku 11\** (L; spirit coll.)

### ***Mezzettia* Becc.**

- M. parviflora* Becc.: Indonesia, Borneo. *SAN 40606* (L) TEM

### ***Miliusa* Lesch. ex A.DC.**

- M. amplexicaulis* Ridl.: Thailand. *Maxwell 87-565\** (BKF) TEM  
*M. balansae* Finet & Gagnep.: Vietnam. *Petelot 6297\** (P), *Soejarto & Cuong 10642\** (L) TEM  
*M. banghoiensis* Jovet-Ast: Vietnam. *Poilane 8540\** (P)  
*M. brahei* (F.Muell.) Jessup: Australia. *Kanis 2134\** (CSIRO)  
*M. campanulata* Pierre: Indonesia, Sumatra. *Forbes 1771\*<sup>10</sup>* (BM); Thailand. *Gentry & Niyomdham 66500\** (U), *Kerr 8824* (BM) TEM  
*M. cuneata* Craib: Thailand. *Geesink et al. 6985<sup>11</sup>* (L) TEM, *Koyama et al. 15600\** (NY)  
*M. dioeca* (Roxb.) Chaowasku & Kessler: India. *Pierre s.n.\** (sheet 1351449) (BO)  
*M. fusca* Pierre: Cambodia. *Monyrak & Meng 79\** (K)  
*M. glandulifera* C.E.C.Fisch.: Myanmar. *Parkinson 1902\** (K)  
*M. horsfieldii* (Benn.) Baill. ex Pierre: Australia. *Hyland 06563\** (L); China. *How 71794* (A) TEM; Indonesia, Java (Bogor). *Mols 12* (L) TEM; Thailand. *Charoenchai 809\** (L)  
*M. macrocarpa* Hook.f. & Thomson: India. *Ribu 728\** (MEL)  
*M. macropoda* Miq.: Indonesia, Borneo. *Kostermans 13973\** (BO)  
*M. mollis* Pierre: Thailand. *Chaowasku 20\** (L; spirit coll.), *Geesink et al. 6937* (L) TEM  
*M. montana* Gardner ex Hook.f. & Thomson: Sri Lanka. *Weerasooriya s.n.* (L) TEM, *Wirawan 621\** (UC)

<sup>10</sup> Re-identified as *M. longipes* King (see Chapter 7); therefore, the actual number of *Miliusa* species palynologically investigated in this study is 21.

<sup>11</sup> Re-identified as *M. campanulata* (see Chapter 7).

- M. thailandica* Chaowasku & Kessler: Thailand. *Geesink & Santisuk 5200\** (L)  
*M. thorelii* Finet & Gagnep.: Myanmar. *Mg Tha Myiang 169\** (K); Thailand. *Maxwell 89-651\** (BKF), *97-584* (L) TEM  
*M. tomentosa* (Roxb.) Finet & Gagnep.: India. *Anglade 23\** (G), *Ritchie 1605/2\** (K)  
*M. traceyi* Jessup: Australia. *Leach & Dunlop 1603\** (NSW)  
*M. velutina* (DC.) Hook.f. & Thomson: India. *Koels 25102* (L) TEM; Thailand. *Pooma 399\** (BKF) TEM  
*M. vidalii* J.Sinclair: The Philippines. *Ramos & Deroy 2-107\** (NSW)

### **Orophea Blume**

- O. brandisii* Hook.f. & Thomson: Thailand. *Keßler PK 3180* (L) TEM  
*O. celebica* (Blume) Miq.: Indonesia, Sulawesi. *Burley et al. 3578* (L) TEM  
*O. creaghii* (Ridl.) Leonardía & Kessler: Indonesia, Java (Bogor). *Mols 19* (L) TEM  
*O. enneandra* Blume: Indonesia, Borneo. *De Vogel 1819* (L)  
*O. enterocarpa* Maingay ex Hook.f. & Thomson: The Philippines. *Argent et al. 25380* (L)  
*O. kerrii* Kessler: Thailand. *Chalermglin 440416-1* (L)  
*O. myriantha* Merr.: Indonesia, Borneo. *Ridsdale & Duwol 2077* (L)  
*O. polycarpa* A.DC.: Thailand. *Chaowasku 21\** (L; spirit coll.), *Geesink et al. 5642* (L)

### **Platymitra Boerl.**

- P. macrocarpa* Boerl.: Indonesia, Borneo. *Kostermans 19274* (L); Thailand, *Gardner et al. ST 1648\** (L) TEM

### **Polyalthia Blume**

- P. cerasoides* (Roxb.) Bedd.: India. *Vajravelu 36762\** (L); Thailand. *Larsen & Larsen 33731* (L) TEM  
*P. pendula* Capuron ex G.E.Schatz & Le Thomas: Madagascar. *Rabehohitra 2386* (K) TEM



# Chapter 9

## Taxonomic novelties and pollen morphological study in the genus *Neo-uvaria* (Annonaceae)

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### Abstract

Two new species of *Neo-uvaria* are described from southern Thailand: *Neo-uvaria sparsistellata* and *N. telopea*. In addition, new combinations are made for two *Mitrephora* species from the Philippines: *N. merrillii* and *N. viridifolia*. The pollen morphology of eight *Neo-uvaria* species and three species of the closely related genus *Encisanthum*<sup>1</sup> is studied, using light, scanning electron and transmission electron microscopy. The systematic affinity of *Neo-uvaria* is discussed on the basis of macromorphology, pollen morphology and molecular phylogenetics. The genus *Encisanthum* appears to be the closest relative of *Neo-uvaria*.

**Key words:** miliusoid clade<sup>2</sup>, palynology, short branch clade<sup>3</sup>, systematics, taxonomy, the Philippines, Thailand

### Introduction

*Neo-uvaria* Airy Shaw is one of the poorly known genera of Asian Annonaceae. It was erected by Airy Shaw (1939) based on *Popowia foetida* Maingay ex Hooker & Thomson (1872: 69) and *Uvaria acuminatissima* Miquel (1865: 6). The main reasons for establishing *Neo-uvaria*, which he thought to be allied to *Uvaria* Linnaeus (1753: 536), were the tree habit and the stellate hairs. *Uvaria* species, in contrast, are usually woody climbers. In a revision of the Malayan Annonaceae, Sinclair (1955) stated that *Neo-uvaria* is likely to be related to *Popowia* Endlicher (1839: 831). The only resemblance between *Uvaria* and *Neo-uvaria* he observed was the stellate indumentum.

Van Heusden (1992) studied the floral morphology of all Annonaceae. She noticed that the petals of *Neo-uvaria* are unusually thick and fleshy. Additionally, the presence of stellate indumentum is also peculiar for *Neo-uvaria* because most annonaceous genera

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<sup>1</sup> Now become *Monoon* Miq. (see Xue *et al.* 2012).

<sup>2</sup> Equivalent to the recircumscribed Miliuseae of Chatrou *et al.* (2012).

<sup>3</sup> Now recognized as subfamily Malmeoideae (see Chatrou *et al.* 2012).

do not possess stellate hairs. Therefore, a genus of tall trees having stellate indumentum and unusually thick and fleshy petals was, according to her, somewhat difficult to place in any group she recognized.

Recent phylogenetic studies using molecular data (Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004) have confirmed that *Neo-uvaria* is unrelated to *Uvaria*, since *Neo-uvaria* was resolved in the ‘miliusoid clade’, which also includes *Popowia*. All members of the ‘short branch clade’ (SBC), to which the miliusoid clade belongs, are shrubs or small to large trees, while the occurrence of climbers is restricted to the ‘long branch clade’<sup>4</sup> (LBC), which includes *Uvaria* and allied genera (Richardson *et al.* 2004).

Recent collections from southern Thailand show the aforementioned features of *Neo-uvaria*: tall trees, stellate indumentum and remarkably thick/fleshy petals (Fig. 1A, B, E). Comparisons with the known *Neo-uvaria* species necessitate the description of two new species: *N. sparsistellata* Chaowasku and *N. telopea* Chaowasku, which represent the first records of *Neo-uvaria* for Thailand.

During the study it became evident that two Philippine species of *Mitrephora* Hooker & Thomson (1855: 112): *M. merrillii* Robinson (1908: 67) and *M. viridifolia* Elmer (1913: 1716), considered by Airy Shaw (1939) and Sinclair (1955) as synonyms of *N. acuminatissima* (Miq.) Airy Shaw (1939: 279), differ considerably from this species. Therefore, two new combinations, *N. merrillii* (C.B.Rob.) Chaowasku and *N. viridifolia* (Elmer) Chaowasku, are proposed here. Further, the lectotypes of *N. foetida* (Maingay ex Hook. f. & Thomson) Airy Shaw, *N. merrillii* and *N. viridifolia* are designated herein.

The total number of *Neo-uvaria* species is still doubtful. Prior to this article, three species were recognized: *N. acuminatissima*, *N. foetida* and *N. parallelevenia* (Boerlage 1899a: 32) Okada & Ueda (1984: 173). Personal observations by the first author suggest that 9–15 species occur. Further taxonomic study is needed to reveal the actual diversity found in this genus.

In the present article, the pollen morphology of eight *Neo-uvaria* species including two unidentifiable (because of the incomplete material, but the first author’s impression is that they are likely to be also new to science) collections from the Philippines (*Neo-uvaria* sp. 1) and Thailand (*Neo-uvaria* sp. 2) and three species of the related genus *Enicosanthum* Beccari (1871: 183) (on the basis of macromorphology and molecular phylogenetics hitherto known) was investigated, using light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) in order to provide additional evidence for elucidating and evaluating the infra- and inter-generic relationships of *Neo-uvaria*.

In addition, the branching architecture of *Neo-uvaria* and some other Annonaceae is discussed as it appears to be one of the good characters for recognizing major clades in Annonaceae.

## Material and methods

Dried herbarium specimens (and associated spirit material) from BKF, BM, E, G, K, KEP, L, NY, P and U herbaria were used for observations and measurements (= types of all seven

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<sup>4</sup> Now recognized as subfamily Annonoideae (see Chatrou *et al.* 2012).

recognized species + *N. acuminatissima*: Elmer 21112, Sinclair et al. 9250; *N. foetida*: Rogstad 950, Soepadmo & Mahmud 1032; *N. parallelivenia*: Keßler sub IV-H-73, Okada 3391; *N. sparsistellata*: Chaowasku 99; *N. telopea*: Gardner & Sidisunthorn ST 1992; *Neo-uvaria* sp. 1: PNH 91908; *Neo-uvaria* sp. 2: Binhasun 1). The indumentum terminology follows Hewson (1988). Material for the pollen morphological study was sampled from dried herbarium specimens (*Enicosanthum* spp., *N. acuminatissima*, *N. foetida*, *N. parallelivenia*, *N. viridifolia*, *Neo-uvaria* sp. 1) or spirit collections (*N. sparsistellata*, *N. telopea*, *Neo-uvaria* sp. 2) (Table 1). The pollen was not acetolysed, following Chaowasku et al. (2008) and Couvreur et al. (2009). The material for TEM was prepared after the techniques described by Van der Ham (1990). The subdivision of the exine into tectum, infratectum and basal layer (Le Thomas 1980) is used. Further pollen terminology follows Punt et al. (2007).

## Taxonomy

***Neo-uvaria*** Airy Shaw (1939: 278).

**Type:**–*Neo-uvaria foetida* (Maingay ex Hook.f. & Thomson) Airy Shaw (1939: 278).

**Basionym:** *Popowia foetida* Maingay ex Hook.f. & Thomson (1872: 69). Lectotype (here designated):–PENINSULAR MALAYSIA. Malacca, 1867, *Maingay 1349A* (K-000190013!), in fruit.

**Observations:**–The inner petals of *Neo-uvaria foetida*, *N. sparsistellata* (Fig. 1E), *N. telopea* (Fig. 1A, B), *N. viridifolia* and *N. sp. 1* are markedly apically thickened while they are not or less so in *N. acuminatissima*, *N. parallelivenia* (see Fig. 20 in Okada & Ueda 1984) and *N. sp. 2*. Furthermore, the inner petals of *N. foetida*, *N. sparsistellata*, *N. telopea*, and *N. viridifolia* in submature stage (i.e. not yet expanded) bear three small openings at the base of two adjacent ones, which can be seen when the outer petals are removed. This feature is also observable in *Goniothalamus* (Blume) Hooker & Thomson (1855: 105), some species of *Friesodielsia* Steenis (1948: 458), *Stelechocarpus cauliflorus* (Scheffer 1885: 5) Sinclair (1953: 43) and certain species of *Trivalvaria* (Miq.) Miquel (1865: 19) (pers. obs. TC; see also Sinclair 1955). Unfortunately, it cannot be verified in the other species of *Neo-uvaria* due to insufficient material.

## Key to *Neo-uvaria* species in Thailand

1. Lower leaf surface sparsely covered with stellate indumentum. Flowers pedicellate, pedicels (in flower and in fruit) (3–)5–6(–8) mm long, sepals ca. 2.1 × 2.5 mm, outer petals 5.2 × 2.8–3.0 mm, inner petals 4.0–5.0 × 2.5 mm, stamens 12–15 per flower. Monocarps 3.1–3.3 × 2–2.5 cm ..... ***N. sparsistellata***
- Lower leaf surface densely covered with stellate indumentum. Flowers (almost) sessile, sepals ca. 7.5 × 6.5 mm, outer petals 11.5–13.5 × 6.5–8.0 mm, inner petals 8.5–10.0 × 7.5–8.5 mm, stamens 16–20(–22) per flower. Monocarps 6.5–7.0 × 5.2–5.5 cm..... ***N. telopea***

***Neo-uvaria sparsistellata* Chaowasku, sp. nov. (Figs. 1E, F; 2; 3)**

*Neo-uvaria viridifolia proxima, praecipue petalis minoribus, staminibus carpellisque pau-*

*cioribus differt.*

**Type:**—THAILAND. Phatthalung Province: Si Ban Phot District, Khao Pu/Khao Ya National Park, headquarters, August 2005, *Gardner et al. ST 1894* (holotype L!, isotypes BKF, K), in flower and fruit (quite young).

Medium-sized trees, ca. 15 m tall, ca. 24 cm in dbh, all parts generally covered with stellate hairs intermixed with simple hairs. Young twigs tomentose. Petioles 2.0–4.5 mm long, densely tomentose. Leaves elliptic, 10.2–32.3 × 3.3–10.1 cm, base (broadly) wedge-shaped, apex generally (narrowly) acuminate, lamina (very) sparsely (appressed-)puberulous above, indumentum mostly on the lower half near the midrib, sparsely puberulous below, upper surface of midrib slightly sunken, (sparsely) (appressed-)tomentose, lower surface of midrib raised, (sparsely) tomentose, secondary veins 15–21 pairs per leaf, angle with midrib 35°–43°. Flowers solitary, axillary, pedicels (in flower and in fruit) (3–)5–6(–8) mm long, densely (appressed-)tomentose, bracts ca. 2 per flower, rather inconspicuous, at the base of the pedicels. Sepals broadly triangular, ca. 2.1 × 2.5 mm, persistent in fruit, outside and margin densely tomentose, inside glabrous. Outer petals elliptic-ovate, 5.2 × 2.8–3.0 mm, outside and margin tomentose, inside glabrous at the base, indumentum of the rest same as outside. Inner petals ovate, 4.0–5.0 × 2.5 mm, apically thickened, indumentum same as outer petals. Stamens 12–15 per flower, ca. 1.4 mm long, connective tissue flat-topped. Carpels 4–6 per flower, stigmas subglobose-ellipsoid, ovaries appressed-tomentose, ovules 1 per ovary, basal. Torus more or less flat, sparsely puberulous, slightly enlarged in fruit. Monocarps 1–3 per fruit, sessile, ellipsoid, 3.1–3.3 × 2.0–2.5 cm, surface (sparsely) tomentose-villous. Seeds 1 per monocarp, ellipsoid, 2.6–2.8 × 1.6–2.1 cm.

**Distribution:**—Peninsular Thailand [Phatthalung Province (Fig. 3)] [only known from two collections (*Chaowasku 99* and *Gardner et al. ST 1894*) collected from the same individual].

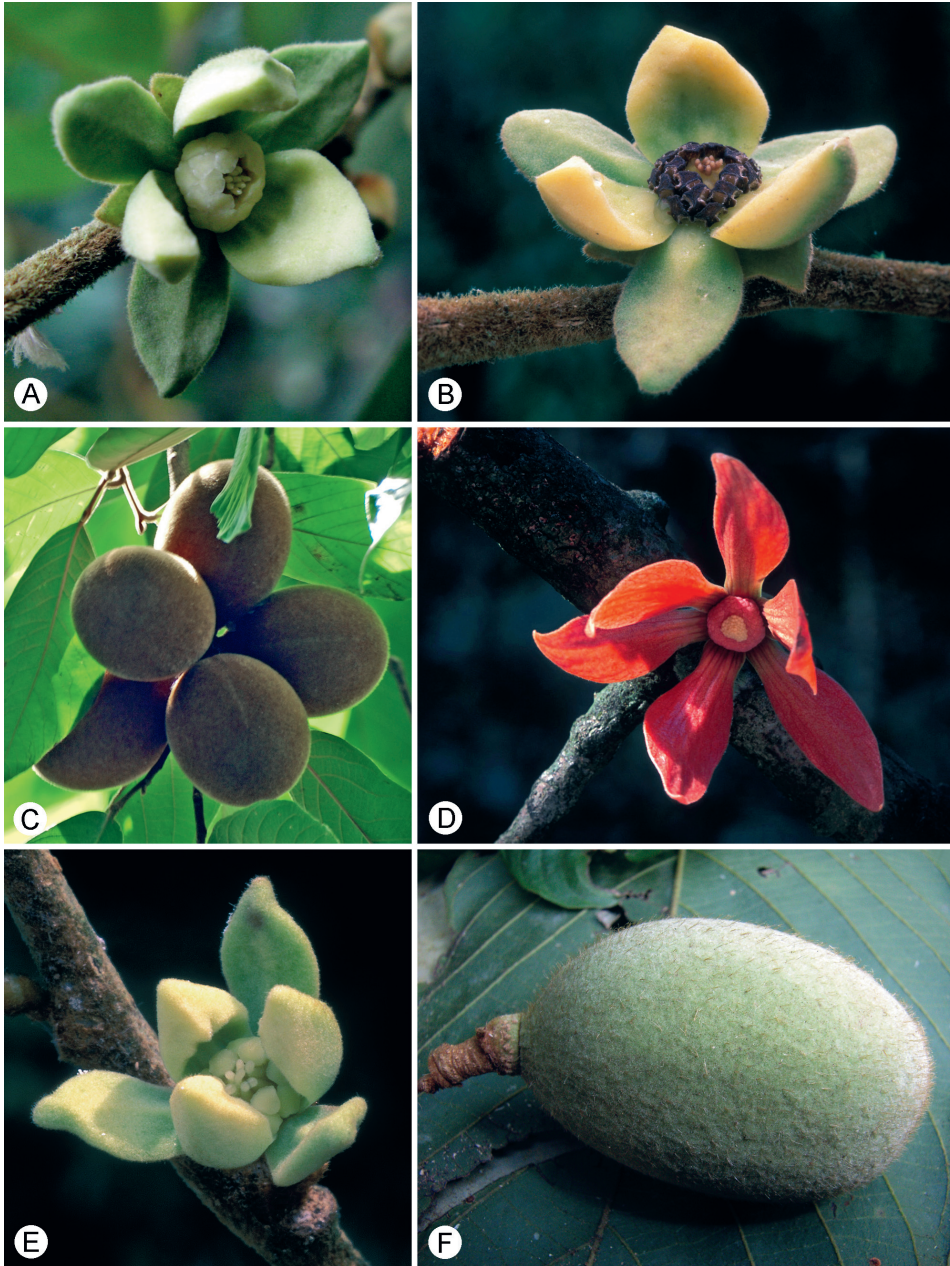
**Habitat and Phenology:**—Occurring in aggrading evergreen/deciduous forests amongst limestone outcrops. Elevation ca. 120 m. Flowering August, December. Fruiting August, December.

**Field notes:**—Bark grey; inner bark brownish-yellow. Petals white maturing yellow. Monocarps pale green with white and pale brown hairs.

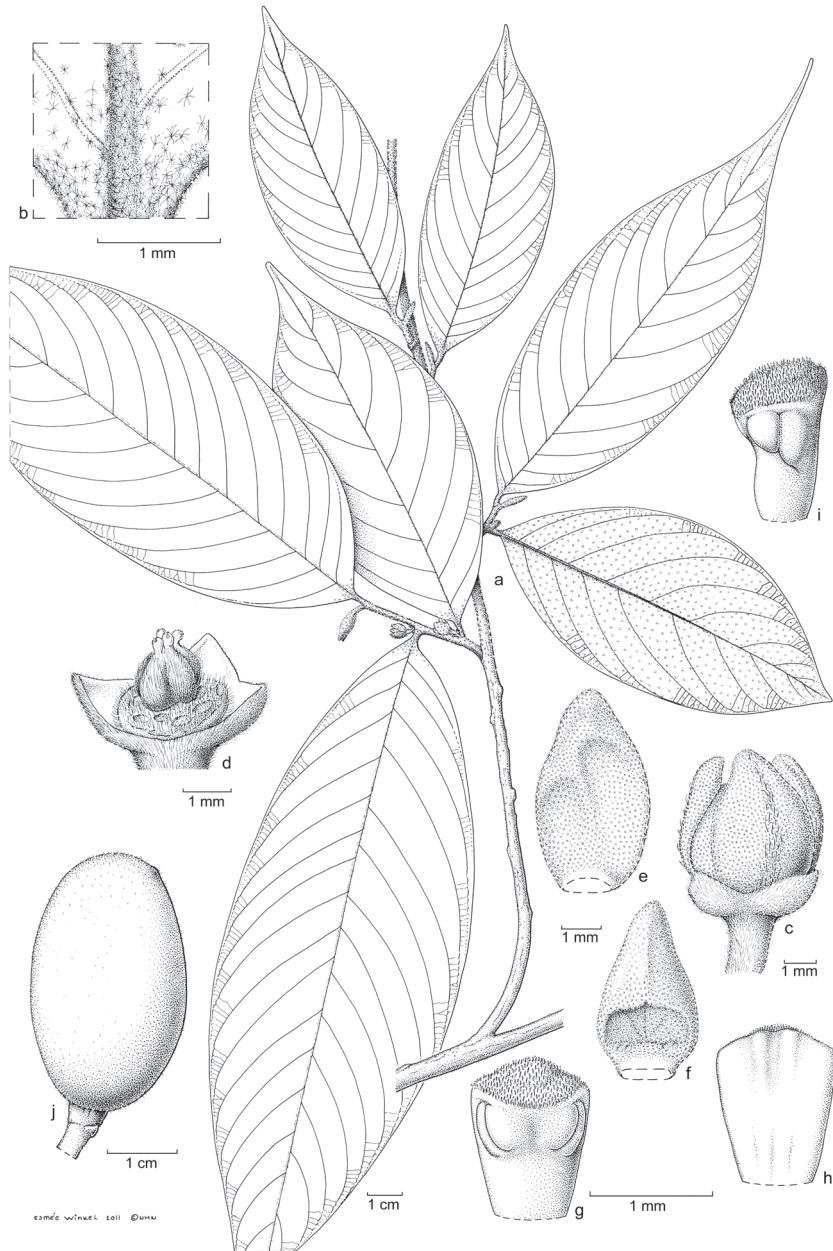
**Etymology:**—The epithet refers to the sparse stellate indumentum on the lower leaf surface.

**Vernacular name:**—Ma-Khiew (Thai).

**Observations:**—This new species seems very close to *Neo-uvaria viridifolia* from the Philippines. They both possess a (very) sparse stellate indumentum on the lower leaf surface whereas it is (much) denser in the other species. In addition, a single stellate hair on the lower leaf surface of both species is relatively (much) smaller than that of the other species. *N. sparsistellata*, however, principally differs from *N. viridifolia* in having smaller petals [outer petals 5.2 × 2.8–3.0 mm, inner petals 4.0–5.0 × 2.5 mm in *N. sparsistellata* vs. outer petals (7.0–)10.2 × (3.6–)4.1 mm, inner petals (6.7–)9.5 × 3.4–3.7 mm in *N. viridifolia*], fewer stamens per flower (12–15 in *N. sparsistellata* vs. ca. 31 in *N. viridifolia*) and carpels per flower (4–6 in *N. sparsistellata* vs. ca. 12 in *N. viridifolia*). Besides, *N. sparsistellata* always has solitary flowers while *N. viridifolia* often have two (or three) flowers per inflorescence.



**FIGURE 1.** Flowers and fruits of *Neo-uvaria* and a flower of *Enicosanthum*. A–C. *Neo-uvaria telopea*: (A) Flower at female anthesis; (B) Flower at male anthesis, showing the blackened stamens; (C) Fruit with five monocarps. D. *Enicosanthum* sp.: (D) Flower at female anthesis. E, F. *N. sparsistellata*: (E) Flower at female anthesis; (F) Fruit with a single monocarp. Photographs: B, D, E, S. Gardner; A, C, S. Punnaadee; F, T. Chaowasku.



**FIGURE 2.** *Neo-uvaria sparsistellata*. a. Habit. b. Lower leaf surface. c. Nearly open flower. d. Flower with petals and stamens removed. e. Inside of an outer petal. f. Inside of an inner petal. g. Stamen, abaxial side. h. Stamen, adaxial side. i. Stamen, lateral view. j. Monocarp. (a–c, Gardner *et al.* ST 1894; d–j, Chaowasku 99).



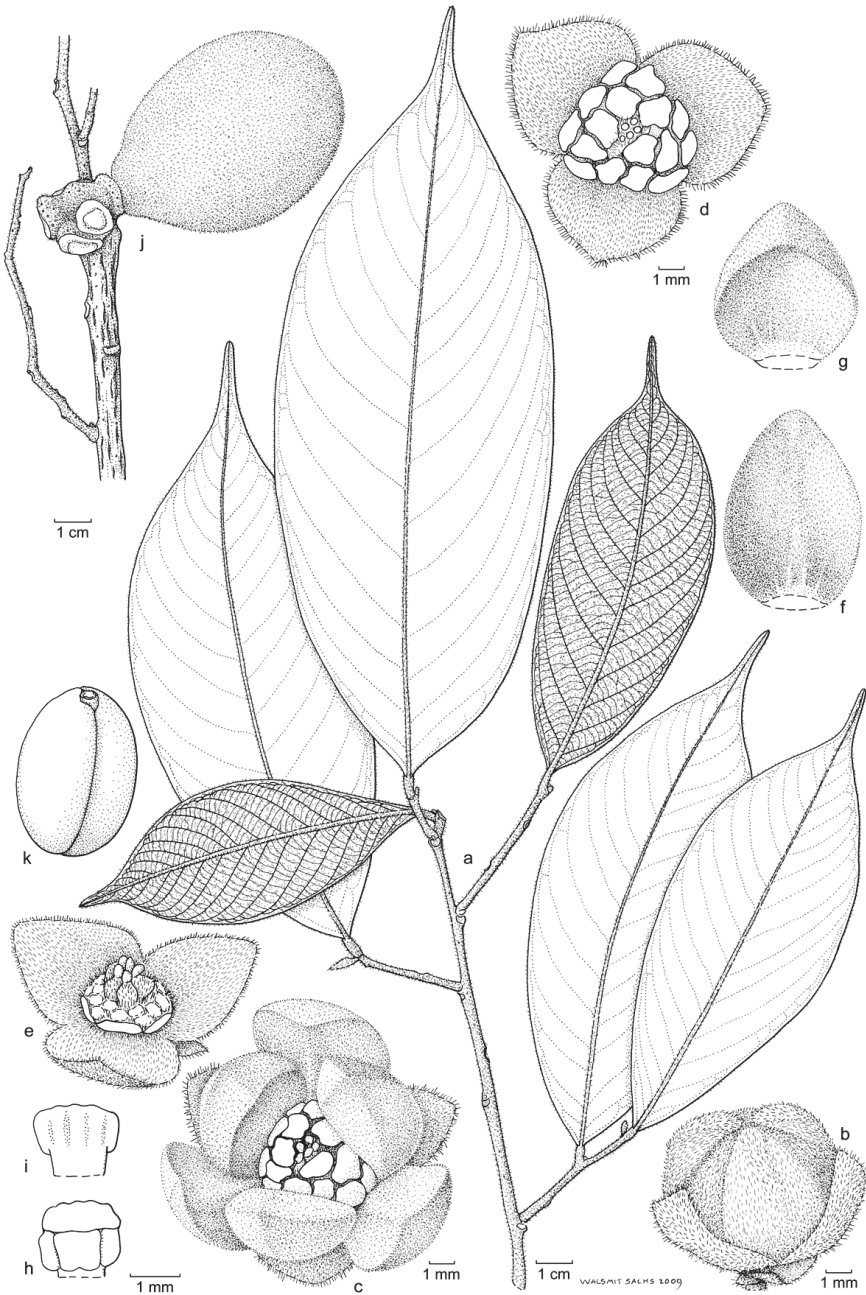
FIGURE 3. Distribution of *Neo-uvaria sparsistellata* (filled inverted triangle) and *N. telopea* (filled upright triangle).

***Neo-uvaria telopea* Chaowasku, sp. nov. (Figs. 1A–C; 3; 4)**

*Neo-uvaria foetida* e *Malaysia peninsulari proxima, precipue foliis plerumque maioribus, venis tertiariis adiacentibus plus distantibus, pilis (plus) densibus, floribus maioribus, staminum numero minore differt.*

**Type:**—THAILAND. Ranong Province: Klong Nakha Wildlife Sanctuary, September 2008, Chaowasku 77 (holotype L!, isotype BKF!), in fruit.

Medium-sized trees, ca. 11 m tall, ca. 12 cm in dbh, all parts generally covered with stellate hairs intermixed with simple hairs. Young twigs densely velvety-villous. Petioles 5–7 mm long, densely velvety-villous. Leaves elliptic, (9.5–)13.0–21.5 × (4.0–)4.7–9.1 cm, base broadly wedge-shaped to obtuse, apex caudate-acuminate, lamina almost glabrous above, (densely) villous below, upper surface of midrib slightly sunken, velvety, lower surface of midrib raised, densely (appressed-)velvety-villous, secondary veins 17–18 pairs



**FIGURE 4.** *Neo-uvaria telopea*. a. Habit. b. Flower bud. c. Flower. d. Flower with petals removed. e. Flower with petals and stamens removed. f. Inside of an outer petal. g. Inside of an inner petal. h. Stamen, abaxial view. i. Stamen, adaxial view. j. Fruiting branch with four monocarps detached. k. Seed. (a, j, k, *Chaowasku 77*; b–i, *Gardner & Sidisunthorn ST 1992*).

per leaf, angle with midrib 45°–50°. Flowers solitary, axillary, (almost) sessile, bracts usually 2 per flower, ovate. Sepals broadly ovate to slightly triangular, ca. 7.5 × 6.5 mm, outside and margin (appressed-)velvety-tomentose, inside (appressed-)tomentose. Outer petals elliptic, 11.5–13.5 × 6.5–8.0 mm, indumentum on outer side and margin similar to sepals, inside shortly cobwebbed with sparser hairs, base (almost) glabrous. Inner petals slightly ovate to broadly elliptic, 8.5–10.0 × 7.5–8.5 mm, apically thickened, outside (appressed-)velvety-tomentose in the middle, sparser towards the margin which is shortly cobwebbed, inside shortly cobwebbed, sparser towards the base which is (almost) glabrous. Stamens 16–20(–22) per flower, 1.6–1.7 mm long, connective tissue flat-topped. Carpels 5–8 per flower, stigmas ellipsoid-cylindrical, ovaries densely villous, ovules 1(–2) per ovary, basal. Torus more or less flat, villous, enlarged in fruit. Monocarps 1–5 per fruit, sessile, ellipsoid-ovoid, 6.5–7.0 × 5.2–5.5 cm, surface (appressed-)velvety-tomentose. Seeds 1 per monocarp, ellipsoid(-ovoid), 5.0 × 3.4–3.5 cm.

**Distribution:**—Peninsular Thailand [Ranong Province (Fig. 3)] [only known from two collections (*Chaowasku* 77 and *Gardner & Sidisunthorn* ST 1992) collected from the same individual].

**Habitat and Phenology:**—Occurring in understory of semi-disturbed lowland evergreen forests. Elevation ca. 120 m. Flowering December (collection *Gardner & Sidisunthorn* ST 1992). Fruiting September (collection *Chaowasku* 77).

**Field notes:**—Crown monopodial with horizontal branching. Bark dark brown, smooth with very shallow horizontal cracks with raised edges; middle bark indistinct; inner bark cream, fibrous. Petals pale green aging greenish-yellow, thick and fleshy; staminal mass pale yellow, blackened at male anthesis (Fig. 1B). Monocarps brown with velvety hairs.

**Etymology:**—The epithet refers to the huge size of the monocarps (Fig. 1C), which therefore can be easily seen from afar.

**Vernacular name:**—Ma-Neng (Thai).

**Observations:**—Odor of rotten fish was emitted from (nearly) dried monocarps. This smell was also detected in those of *N. foetida* (pers. obs. TC). This new species seems to be closely related to *N. foetida* occurring in Peninsular Malaysia. It chiefly differs in having generally larger leaves [(9.5–)13.0–21.5 × (4.0–)4.7–9.1 cm in *N. telopea* vs. 7.7–15.7(–18.0) × 2.5–5.0(–6.0) cm in *N. foetida*] with wider distance of the adjacent tertiary veins, denser indumentum on nearly all parts, larger flowers [sepals ca. 7.5 × 6.5 mm, outer petals 11.5–13.5 × 6.5–8.0 mm, inner petals 8.5–10.0 × 7.5–8.5 mm in *N. telopea* vs. sepals ca. 4.0 × 3.6 mm, outer petals ca. 9.2 × 5.4 mm, inner petals, ca. 6.7 × 5.4 mm in *N. foetida*], fewer stamens per flower [16–20(–22) in *N. telopea* vs. (26–)27 in *N. foetida*], and generally fewer carpels per flower [5–8 in *N. telopea* vs. 7–11 in *N. foetida*]. Vegetatively, *N. telopea* resembles *N. acuminatissima* in the density of the indumentum, but the latter species has much smaller monocarps and flowers. The fruits of *N. parallelivenia* are unknown but its flowers are noticeably different from those of *N. telopea*, especially in the much smaller sepals and the non-thickened apex of the inner petals of the former.

## New combinations

***Neo-uvaria merrillii* (C.B.Rob.) Chaowasku, comb. nov.**

Basionym: *Mitrephora merrillii* C.B.Rob. (1908: 67). *Griffithianthus merrillii* (C.B.Rob.)

W.H. Brown ex Merrill (1915: 231). *Mitrephora ferruginea* Merrill (1904: 16), *nom. illeg.*

**Type:**—THE PHILIPPINES. Luzon, Bataan Province: Mt. Mariveles, January 1904, Merrill 3728 [PNH (destroyed), lectotype (here designated) NY!, isotypes BM!, P!], in fruit.

**Observations:**—Originally, the syntypes of this species consist of disparate elements as stated by Merrill (1915) and Weerasooriya & Saunders (2010). Nevertheless, no one has designated a lectotype, therefore it is done here. *Neo-uvaria merrillii* primarily differs from *N. acuminatissima* in having usually (broadly) wedge-shaped leaf base (whilst usually obtuse to rounded in *N. acuminatissima*), larger sepals (4.5–5.0 × 4.0–4.5 mm in *N. merrillii* vs. ca. 2.6 × 2.9 mm in *N. acuminatissima*) and wider monocarps (ca. 2.2 cm wide in *N. merrillii* vs. ca. 1.7 cm wide in *N. acuminatissima*).

***Neo-uvaria viridifolia* (Elmer) Chaowasku, *comb. nov.***

Basionym: *Mitrephora viridifolia* Elmer (1913: 1716).

**Type:**—THE PHILIPPINES. Mindanao, Agusan Province: Cabadbaran (Mt. Urdaneta), October 1912, Elmer 14184 [PNH (destroyed), lectotype (here designated) NY!, isotypes BM!, BP, DS, E!, G!, GH, LI, NA, P!, U!], in flower.

**Observations:**—*N. viridifolia* is considerably different from *N. acuminatissima*, especially in the much sparser stellate indumentum on the lower leaf surface. Moreover, its pedicels are longer than those of *N. acuminatissima* [(4–)5–6 mm long in *N. viridifolia* vs. nearly absent in *N. acuminatissima*]. The leaf base of *N. viridifolia* is usually (broadly) wedge-shaped in contrast to *N. acuminatissima* which usually has obtuse to rounded leaf base. *N. acuminatissima* was very rarely collected concerning the flowers. Of all specimens investigated, only the collection Sinclair *et al.* 9250 [this collection seems to have generally smaller leaves with more obtuse base and wider distance of the adjacent tertiary veins compared to those of Elmer 21112 and the type of *N. acuminatissima*, however, all other morphology of the three collections is similar] has flowers which are smaller than those of *N. viridifolia* [outer petals ca. 3.6 × 2.5 mm, inner petals ca. 3.8 × 3.0 mm in *N. acuminatissima* vs. outer petals (7.0–)10.2 × (3.6–)4.1 mm, inner petals (6.7–)9.5 × 3.4–3.7 mm in *N. viridifolia*].

**Provisional key to seven recognized species of *Neo-uvaria***

(monocarps and petals are unknown in *N. parallelevenia* and *N. merrillii*, respectively)

1. Lower leaf surface (very) sparsely covered with stellate indumentum..... 2
  - Lower leaf surface (moderately to) densely covered with stellate indumentum..... 3 or 6
2. Flowers solitary. Outer petals 5.2 × 2.8–3.0 mm, inner petals 4.0–5.0 × 2.5 mm, stamens 12–15 per flower, carpels 4–6 per flower ..... ***N. sparsistellata***
  - Flowers solitary or in an inflorescence with 2 (or 3) flowers. Outer petals (7.0–)10.2 × (3.6–)4.1 mm, inner petals (6.7–)9.5 × 3.4–3.7 mm, stamens ca. 31 per flower, carpels ca. 12 per flower ..... ***N. viridifolia***
3. Monocarps > 4 cm long..... 4
  - Monocarps < 4 cm long..... 5
4. Leaves (9.5–)13.0–21.5 × (4.0–)4.7–9.1 cm. Sepals ca. 7.5 × 6.5 mm, outer petals 11.5–13.5 × 6.5–8.0 mm, inner petals 8.5–10.0 × 7.5–8.5 mm, stamens 16–20(–22)

- per flower, carpels 5–8 per flower ..... ***N. telopea***
- Leaves 7.7–15.7(–18.0) × 2.5–5.0(–6.0) cm. Sepals ca. 4.0 × 3.6 mm, outer petals ca. 9.2 × 5.4 mm, inner petals ca. 6.7 × 5.4 mm, stamens (26–)27 per flower, carpels 7–11 per flower.....*N. foetida*
5. Leaf base usually (broadly) wedge-shaped. Sepals 4.5–5.0 × 4.0–4.5 mm. Monocarps ca. 2.2 cm wide ..... ***N. merrillii***
- Leaf base usually obtuse to rounded. Sepals ca. 2.6 × 2.9 mm. Monocarps ca. 1.7 cm wide .....*N. acuminatissima*
6. Outer petals ca. 3.6 × 2.5 mm, inner petals ca. 3.8 × 3.0 mm.....*N. acuminatissima*
- Outer and inner petals ≥ 5.4 mm long and wide..... 7
7. Apex of the inner petals not thickened..... *N. parallelivenia*
- Apex of the inner petals conspicuously thickened..... 4

### Pollen morphology

Walker (1971b) described the pollen of one *Neo-uvaria* species (identified as *N. acuminatissima*), using LM, as: solitary, apolar, radiosymmetric, inaperturate, globose, medium-sized (longest axis 32 µm), tectate (columellae indistinct) and microbaculate. We studied several more species (Table 1), using LM/SEM/TEM. In general, their pollen is similar to that of *Neo-uvaria* sp. described by Walker (1971b). The following description of *Neo-uvaria* pollen summarizes our observations (Tables 2, 3).

**TABLE 1.** Origin of *Encisanthum* and *Neo-uvaria* pollen samples, and applied techniques.

	Voucher	LM	SEM	TEM
<i>Encisanthum fuscum</i>	<i>Kostermans 774</i> , Thailand (L)	X	X	X
<i>E. membranifolium</i>	<i>KEP/FRI 98720</i> , Peninsular Malaysia (L)	X	-	-
<i>E. paradoxum</i>	<i>Ambriansyah &amp; Arifin B 1520</i> , Borneo (L)	X	X	X
<i>Neo-uvaria acuminatissima</i>	<i>Sinclair et al. 9250</i> , Borneo (L)	-	-	X
<i>N. foetida</i>	<i>Rogstad 950</i> , Peninsular Malaysia (KEP)	-	X	X
<i>N. merrillii</i>	not available	-	-	-
<i>N. parallelivenia</i>	<i>Keßler sub IV-H-73</i> , Bogor, Java (L)	X	X	X
<i>N. sparsistellata</i>	<i>Chaowasku 99</i> , Thailand (L)	-	X	X
<i>N. telopea</i>	<i>Gardner &amp; Sidisunthorn ST 1992</i> , Thailand (L)	X	X	X
<i>N. viridifolia</i>	<i>Elmer 14184</i> , the Philippines (L)	X	X	-
<i>Neo-uvaria</i> sp. 1	<i>PNH 91908</i> , the Philippines (L)	X	X	X
<i>Neo-uvaria</i> sp. 2	<i>Binhasun 1</i> , Thailand (L)	-	X	X

**TABLE 2.** LM and SEM observations of pollen grain size and ornamentation in *Encisanthum* and *Neo-uvaria*. L (long axis) and B (short axis) in µm.

	L	B	L/B	Ornamentation
<i>Encisanthum fuscum</i>	39	35	1.11	Scabrate
<i>E. membranifolium</i>	26	22	1.18	(Micro)echinate
<i>E. paradoxum</i>	39	37	1.05	Scabrate

TABLE 2 (continued).

	L	B	L/B	Ornamentation
<i>Neo-uvaria foetida</i>	16	16	1.00	(Micro)echinate
<i>N. parallelivenia</i>	26	24	1.08	Microechinate
<i>N. sparsistellata</i>	17	15	1.13	(Micro)echinate
<i>N. telopea</i>	24	22	1.06	Microechinate
<i>N. viridifolia</i>	29	25	1.16	(Micro)echinate
<i>Neo-uvaria</i> sp. 1	25	22	1.14	(Micro)echinate
<i>Neo-uvaria</i> sp. 2	21	19	1.10	Microechinate

TABLE 3. TEM observations of thickness of pollen wall sublayers in *Enicosanthum* and *Neo-uvaria*. Exine, supratectal elements (height), exintine and endintine in  $\mu\text{m}$ ; tectum, infratectum and basal layer as proportions of exine thickness.

	Exine	Supratectal elem.	Tectum	Infratectum	Basal layer	Exintine	Endintine
<i>Enicosanthum fuscum</i>	1.1	-	1/3	1/2	Thin	c. 1.1	0.5
<i>E. paradoxum</i>	1.0	-	1/3	1/2	Thin	c. 1.0	0.5
<i>Neo-uvaria acuminatissima</i>	0.35	$\leq 1.4$	1/2	1/4	1/4	c. 1.1	0.5
<i>N. foetida</i>	0.25	$\leq 1.05$	1/5	3/5	1/5	c. 0.3	c. 0.3
<i>N. parallelivenia</i>	0.2	$\leq 0.3$	1/3	1/3	1/3	c. 0.7	0.3
<i>N. sparsistellata</i>	0.2	$\leq 0.4$	1/2	1/2	Thin	c. 0.8	Thin
<i>N. telopea</i>	0.2	$\leq 0.6$	1/3	1/3	1/3	c. 0.6	0.2
<i>Neo-uvaria</i> sp. 1	0.3	$\leq 0.5$	1/3	1/3	1/3	c. 0.7	0.5
<i>Neo-uvaria</i> sp. 2	0.4	$\leq 0.65$	1/4	1/2	1/4	c. 0.8	?

elem. = elements

**LM:** Pollen grains apolar, (spheroidal to) subspheroidal, inaperturate monads, L (long axis) 16–29  $\mu\text{m}$ , B (short axis) 15–25  $\mu\text{m}$ , L/B 1.00–1.16.

**SEM (Fig. 5A–F):** Exine ornamentation microechinate to echinate.

**TEM (Fig. 6A–F):** Exine inaperturate, tectate, 0.2–0.4  $\mu\text{m}$  thick. Supratectal elements: (micro)echinae up to 1.4  $\mu\text{m}$ . Tectum usually distinct, 1/5–1/2 of exine thickness. Infratectum usually distinct, 1/4–3/5 of exine thickness, consisting of  $\pm$  distinct granules. Basal layer usually distinct, very thin to 1/3 of exine thickness, sometimes distinctly lamellate. Intine continuous, without recognizable germination zone(s), consisting of a 0.3–1.1  $\mu\text{m}$  thick tubular exintine and a 0.2–0.5  $\mu\text{m}$  thick homogeneous endintine.

## Discussion

### Growth architecture consideration

Johnson (2003) demonstrated the importance of growth architecture in Annonaceae. He distinguished two types: 1. spiral and 2. distichous branching patterns. These types are

(micro)echinate exine; (B) Detail of exine. C, D. *N. sparsistellata*: (C) Pollen grain, showing (micro)echinate exine; (D) Detail of exine. E, F. *N. telopea*: (E) Pollen grain, showing microechinate exine; (F) Detail of E, showing ornamentation. G, H. *Enicosanthum paradoxum*: (G) Pollen grain, showing scabrate exine; (H) Detail of G, showing ornamentation. Scale bars — 5  $\mu\text{m}$  (A, C, E, G), 1  $\mu\text{m}$  (B, D, F, H).

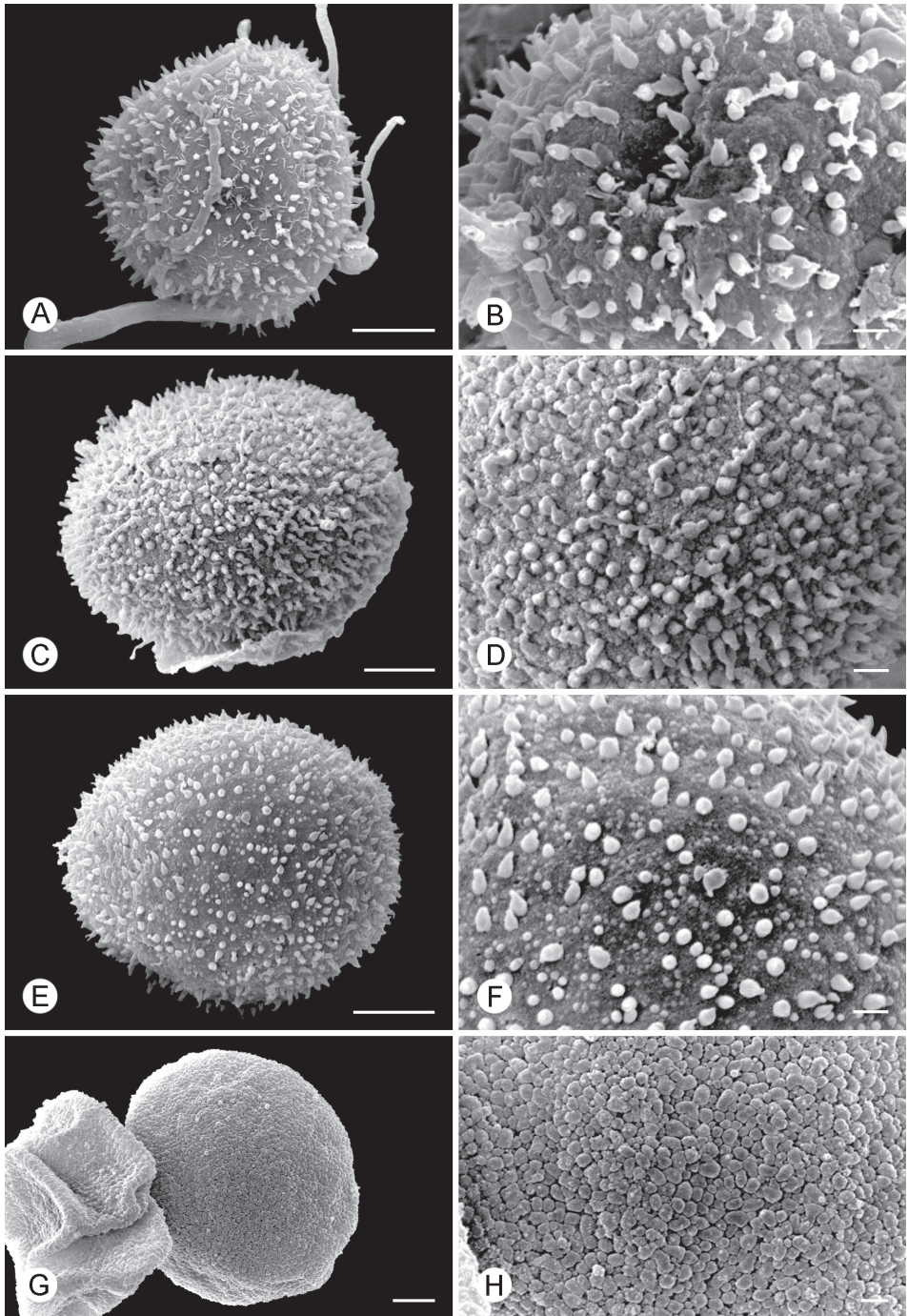


FIGURE 5. Pollen of *Neo-uvaria* and *Encicosanthum*, SEM. A, B. *Neo-uvaria foetida*: (A) Pollen grain, showing

...continued on page 156

considered as a diagnostic/synapomorphic character of particular clades. For example, an Afro-Asian subclade (uvarioid clade + African long branch clade) of the LBC (Couvreur *et al.* 2008), consisting of woody climbers, shrubs and trees, is easily recognizable by its distichous architecture. Similarly, as observed so far, members of the SBC exhibit spiral branching. It is likely that this trait had evolved in the common ancestor of the SBC and hence is expected to occur also in the other SBC genera where the growth architecture has not been observed. This correlation can greatly help to identify major Annonaceae clades in the field. *Neo-uvaria* shows spiral branching, and therefore is unlikely to be allied with *Uvaria* (a member of the uvarioid clade). The branching architecture, in combination with pollen morphology (discussed below), corroborates the systematic position of *Neo-uvaria* as belonging to the miliusoid clade, which is a subclade of the SBC.

### *Intergeneric relationships*

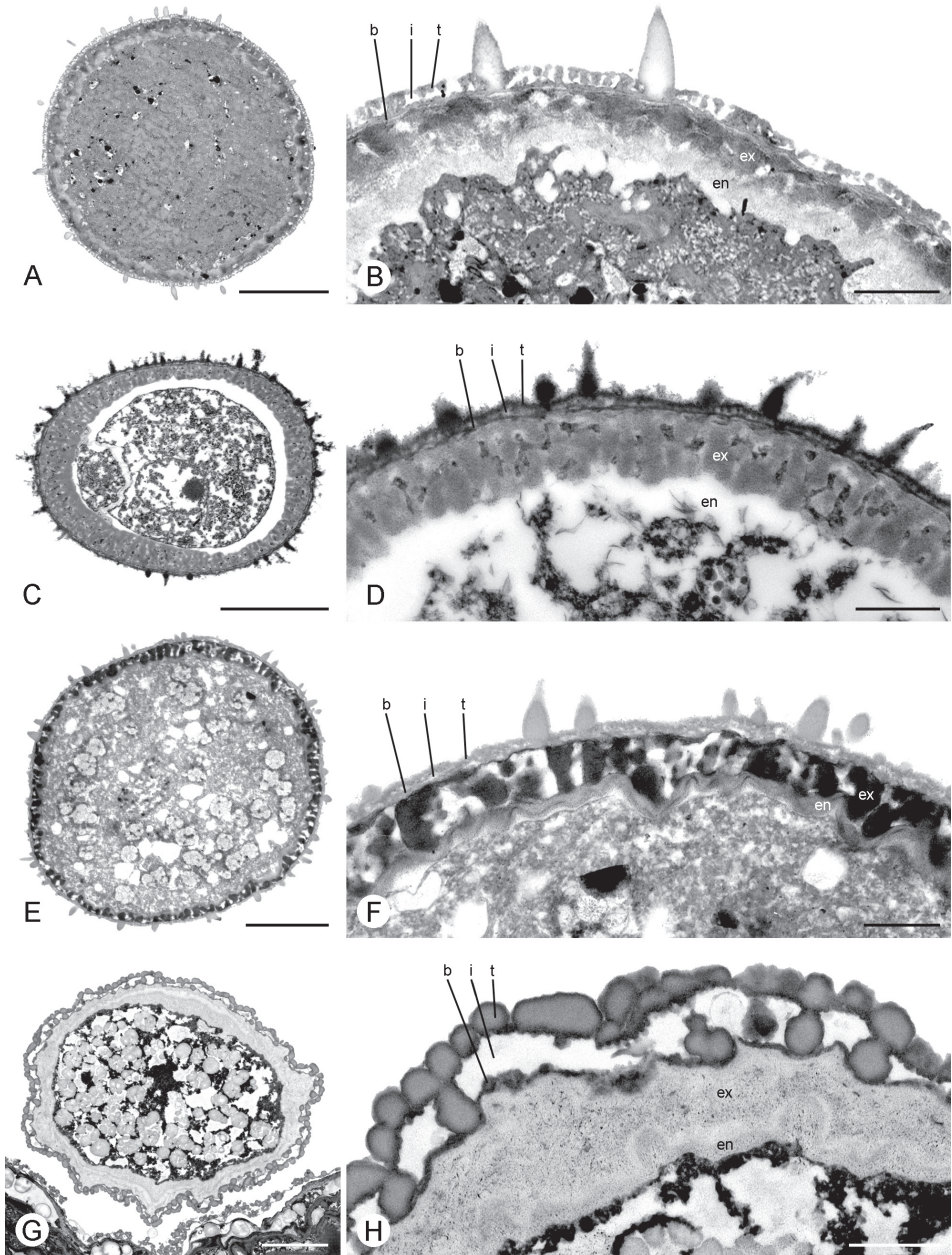
The genus *Enicosanthum sensu lato* [Fig. 1D; including certain species assigned to *Polyalthia* Blume (1830: 68), these species will be eventually transferred to *Enicosanthum* elsewhere] appeared to be sister to *Neo-uvaria* (Mols *et al.* 2004a, 2004b, Richardson *et al.* 2004, Chaowasku *et al.* unpublished study). *Enicosanthum* and *Neo-uvaria* share the following (synapomorphic) characters:

- Percurrent tertiary venation of the leaves (though some species of *Enicosanthum* have reversed to possess less percurrent/more reticulate tertiary venation)
- Axillary flowers/inflorescences
- An ovary bearing a single basal ovule (though sometimes two ovules were observed in *N. telopea*; Hooker & Thomson (1872), Airy Shaw (1939) and Sinclair (1955) stated that *N. foetida* usually has two ovules per ovary, but only one ovule per ovary was observed in the present study)
- Relatively large (much larger in *N. telopea* and *N. foetida*) single seed per monocarp with obvious raphe seen as a longitudinal groove
- Four-part-lamellate ruminations of the endosperm (in cross section)

*Neo-uvaria* differs from *Enicosanthum* in having much thicker petals, relatively larger stamens [which look more or less like those of *Sageraea* Dalzell (1851: 207) and *Stelechocarpus* Hooker & Thomson (1855: 94), according to Van Heusden 1995, 1997a], a reduced number of stamens and carpels, and importantly the stellate indumentum. The last character is peculiar and has so far not been (consistently) observed in any other genera in the miliusoid clade, or even the SBC. Additionally, *Neo-uvaria* usually possesses sessile monocarps whilst they are usually markedly stipitate in *Enicosanthum*.

The sister genera *Sageraea* and *Stelechocarpus* are possibly allied to *Neo-uvaria* and *Enicosanthum* because they all share the axillary flowers/inflorescences and seeds with

exine and intine sublayers. E, F. *N. telopea*: (E) Cross-section of pollen grain, showing continuous exine and intine without recognizable germination zone(s); (F) Detail of cross-section, showing exine and intine sublayers. G, H. *Enicosanthum paradoxum*: (G) Cross-section of pollen grain, showing continuous exine and intine without recognizable germination zone(s); (H) Detail of cross-section, showing exine and intine sublayers. Scale bars — 5 µm (A, C, E, G), 1 µm (B, D, F, H); b = basal layer, en = endintine, ex = exintine, i = infratectum, t = tectum.



**FIGURE 6.** Pollen of *Neo-uvaria* and *Ecosanthum*, TEM. A, B. *Neo-uvaria foetida*: (A) Cross-section of pollen grain, showing continuous exine and intine without recognizable germination zone(s); (B) Detail of cross-section, showing exine and intine sublayers. C, D. *N. sparsistellata*: (C) Cross-section of pollen grain, showing continuous exine and intine without recognizable germination zone(s); (D) Detail of cross-section, showing

...continued on page 158

a grooved<sup>5</sup> raphe and four-part-lamellate ruminations of the endosperm (pers. obs. TC; Mols *et al.* 2004a; see also Van Setten & Koek-Noorman 1992). Nevertheless, *Sageraea* and *Stelechocarpus* possess an ovary with many ovules arranged in two rows and lack the percurrent tertiary venation of the leaves (pers. obs. TC; Mols *et al.* 2004a). The most recent and comprehensive phylogenetic analyses of the SBC (= the Malmeoideae, Chaowasku *et al.* unpublished study) also reveal this relation, but only with moderate support in Bayesian analysis while no support in the analysis under maximum parsimony criterion. The genus *Phaeanthus* Hooker & Thomson (1855: 146) appears to become part of a clade comprising the mentioned four genera. It shares all characters<sup>6</sup> with *Neo-uvaria* and *Enicosanthum* except the axillary flowers/inflorescences (terminal in *Phaeanthus*) and the similarly-sized outer petals and inner petals (similarly-sized sepals and outer petals in *Phaeanthus*). Further study is required to unravel the relationships of *Neo-uvaria*/*Enicosanthum*, *Sageraea*/*Stelechocarpus* and *Phaeanthus*.

### Pollen morphology

Pollen morphologically the genus *Neo-uvaria* is rather homogeneous. Pollen of the transferred species *N. viridifolia* and the newly described *N. sparsistellata* and *N. telopea* agrees well with those of *N. acuminatissima*, *N. foetida* and *N. parallelivenia*. Also pollen of *Neo-uvaria* sp. 1 and *N. sp. 2* fits well in the generic description. *Enicosanthum*, the closest relative of *Neo-uvaria*, has more or less similar pollen, showing inaperturate monads with scabrate [*E. fuscum* (King 1893: 10) Airy Shaw (1939: 277), *E. paradoxum* Beccari (1871: 184); Fig. 5G, H, Fig. 6G, H] or (micro)echinate [*E. membranifolium* Sinclair (1955: 191)] ornamentation and a continuous intine without recognizable germination zone(s) (see also Mols *et al.* 2004a). However, in comparison to the *Neo-uvaria* species, the scabrate *Enicosanthum* species have larger pollen grains (39 µm vs. 16–29 µm) with a much thicker exine (1.0–1.1 µm vs. 0.2–0.4 µm). Unfortunately, no TEM data of the echinate *E. membranifolium* are available.

### Conclusion

*Neo-uvaria* is a small genus in the miliusoid clade of the SBC. It consists of 9–15 species, seven of which are now formally recognized, including the two newly described species, *N. sparsistellata* and *N. telopea*, and the transferred *N. merrillii* and *N. viridifolia*. The genus *Enicosanthum* is the closest relative of *Neo-uvaria* based on macromorphology, pollen morphology, and molecular phylogenetics. *Neo-uvaria* is presently circumscribed by having the following characters: 1) leaves with percurrent tertiary veins, 2) stellate hairs generally covering all parts, 3) thick and fleshy petals, 4) reduced number of stamens and carpels, 5) usually single ovule per ovary and thus one seed per monocarp, 7) usually sessile monocarps, 8) relatively large seeds with distinct longitudinal groove, 8)

<sup>5</sup> It appeared that the seed raphe of *Winitia* Chaowasku (a recently described genus separated from *Stelechocarpus*; see Chapter 4) is not grooved but (slightly) ridged, following subsequent detailed observations.

<sup>6</sup> Like *N. telopea*, sometimes two ovules per ovary are also observed in *Phaeanthus* (see Chapter 2 and reference therein).

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lamelliform ruminations of the endosperm, divided into four equal parts (in cross section), and 9) inaperturate (micro)echinate pollen grains dispersed as monads. Taxonomic study covering the entire distribution area of *Neo-uvaria* is still needed. Up to now, *Neo-uvaria* is known from southern Thailand, Peninsular Malaysia, Sumatra, Borneo and the Philippines.



# Chapter 10

## Summary and conclusions

This thesis presented eight studies in the field of systematic botany, with a focus on the molecular phylogenetics, taxonomy, and pollen morphology of the tribe Miliuseae (*sensu* Chatrou *et al.* 2012; formerly known as the miliusoid clade *sensu* Mols *et al.* 2004b) of the pantropical flowering plant family Annonaceae. The general aims were:

(1) to investigate intertribal relationships of the tribe Miliuseae, (2) to clarify generic delimitations by investigating the intergeneric relationships within the tribe Miliuseae, and (3) to understand patterns of character evolution within the tribe Miliuseae.

To achieve these aims, a molecular phylogeny of a subclade of the subfamily Malmeoideae consisting of the tribes Maasieae, Malmeeae, Fenerivieae, Dendrokingstonieae (sampled for the first time), Monocarpieae, and Miliuseae was reconstructed using a much expanded taxon and chloroplast DNA marker sampling in comparison to previous studies. In addition, detailed taxonomic and/or palynological studies of the monogeneric tribes Dendrokingstonieae and Monocarpieae, and several genera in Miliuseae have been presented. The evolution of selected macromorphological and palynological characters was investigated using ancestral character-state reconstructions. Generic delimitations within Miliuseae, in light of the results of the molecular phylogenetic studies, were discussed, and two new genera, *Hubera* and *Winitia*, have been characterized based on macromorphology and pollen morphology.

**Chapter 2** presented molecular phylogenetic reconstructions of a subclade of the subfamily Malmeoideae comprising the tribes Maasieae, Malmeeae, Fenerivieae, Monocarpieae, and Miliuseae (with ca. 89% of generic diversity sampled), using maximum parsimony and Bayesian analyses of seven chloroplast markers (*rbcl* exon, *trnL* intron, *trnL-F* spacer, *matK* exon, *ndhF* exon, *psbA-trnH* spacer, and *ycf1* exon). Notably, data of accessions of the monogeneric tribe Dendrokingstonieae, never previously sampled for molecular phylogenetic analyses, were included. The analyses showed Dendrokingstonieae, Monocarpieae, and Miliuseae to form a strongly supported clade, and Dendrokingstonieae to be sister to a weakly to moderately supported clade comprising Monocarpieae and Miliuseae. The tribes Maasieae, Malmeeae, and Fenerivieae formed a polytomy with the Dendrokingstonieae-Monocarpieae-Miliuseae clade. Most of the genera in Miliuseae having two or more species sampled were recovered as monophyletic, except for the paraphyletic genus *Desmopsis* within which *Stenanona* is nested. The phylogenetic position in Miliuseae of an undescribed genus occurring in Thailand was confirmed. Reconstructions of the ancestral character states of selected characters, which are diagnostically important at the generic or tribal level, were performed for Miliuseae using Bayesian and maximum parsimony approaches. Pollen morphological variation corresponded well

to the molecular phylogeny, i.e. monosulcate pollen grains occurred in all tribes recovered as being outside the Miliuseae, including Dendrokingstonieae and Monocarpieae, and cryptoaperturate or disulcate pollen grains were inferred to be a synapomorphy for the Miliuseae. Multi-ovuled ovaries, showy outer petals, non-clawed inner petals, bisexual flowers, spiniform to flattened peg-like ruminations of the endosperm, pollen dispersed as monads at maturity, and pollen with a columellate to coarsely granular infratectum were inferred as the ancestral character states of Miliuseae. The analyses indicated considerable degrees of homoplasy of most analyzed characters (maximum ovule number per carpel, outer petal appearance, inner petal base, flower sexuality, endosperm ruminations type, pollen dispersal unit, and pollen infratectal type) within Miliuseae.

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**Chapter 3** characterized the genus *Hubera* (formerly known as the *Polyalthia cerasoides* group), a new genus of the tribe Miliuseae, using a combination of macromorphology, pollen morphology, and molecular phylogenetics (based on the same DNA regions and analyses as those described in Chapter 2). *Hubera* was strongly supported as a monophyletic group and its sister relationship to *Miliusa* was moderately to strongly supported, but synapomorphies uniting the two genera have not yet been identified. Main characters used for circumscribing this new genus are the reticulate tertiary venation of the leaves, axillary inflorescences, a single ovule per ovary, seeds with a flat to slightly raised raphe, spiniform(-flattened peg) ruminations of the endosperm, and pollen with a finely and densely granular infratectum. A number of *Hubera* species (12 of the 27 transferred species) possess domatia in the form of a tuft of aggregated hairs on the lower leaf surface in the between the secondary veins and primary vein. The domatia distinguish these *Hubera* species from species of *Polyalthia sensu stricto* and other genera (*Monoon*, *Marsyopetalum*, *Trivalvaria*), which had all been placed in the highly polyphyletic *Polyalthia sensu lato*.

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**Chapter 4** provided an assessment of the generic circumscriptions of *Stelechocarpus* and *Sageraea* using a combination of molecular phylogenetics (based on the same DNA regions and analyses as were used in Chapter 2), macromorphology, and pollen morphology. The results supported recognition of a new genus, *Winitia*, chiefly characterized by (1) multicolumellar stigmas, a synapomorphy of the genus, and (2) pollen grains with a very thin tectum, a more or less columellate/coarsely granular infratectum, and a very distinct basal layer. The new genus comprises two species: *W. cauliflora* comb. nov. and *W. expansa* sp. nov.; the former is distributed in Vietnam, southern Thailand, Peninsular Malaysia, and Borneo, while the latter is endemic to southern Thailand. *Winitia* was weakly to moderately supported as sister to *Stelechocarpus*, which consists of only a single species, *Stelechocarpus burahol*, with three autapomorphic features: (1) a separated distribution of male and female flowers in the same individual (mixed in *Winitia*), (2) different sizes of male and female flowers (for the same individual; flowers of both sexes same size in *Winitia*), and (3) a finely and

densely granular infratectum of the pollen. *Stelechocarpus* and *Winitia* share two vegetative traits: a prominent secondary venation on the lower leaf surface and a (slightly) raised upper surface of the leaf midrib. A clade composed of *Stelechocarpus* and *Winitia* was strongly supported as sister to *Sageraea*. *Sageraea* possesses one diagnostically important vegetative feature: more or less indistinct secondary veins on the lower leaf surface.

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**Chapter 5** presented a proposal to conserve the name *Meiogyne* against the earlier name *Fitzalania*. *Fitzalania* has been shown to be nested phylogenetically within *Meiogyne* (Chapter 2). *Meiogyne* and *Fitzalania* share two diagnostic characters: a corrugated or grooved area at the base of the adaxial side of the inner petals and elongated connective tissue of the inner stamens. *Meiogyne* has a wider distribution area and contains more species than *Fitzalania*. Additionally, *Meiogyne* has lent its name to a dimeric sesquiterpenoid, meioyginin A, isolated from the bark of *Meiogyne cylindrocarpa*, which has significant potential as an anti-cancer agent. Therefore, to maximize the stability of names, *Meiogyne* was proposed for conservation.

Published in *Taxon* 60: 1522–1523. 2011.

**Chapter 6** presented a taxonomic revision of the genus *Dendrokingstonia* (classified in the monogeneric tribe Dendrokingstonieae) including detailed investigations of its pollen morphology and that of the supposedly allied genus *Monocarpia* (classified in the monogeneric tribe Monocarpieae). Both genera share a combination of character states rarely found in the family, i.e. highly reduced number of carpels per flower, considerably enlarged stigmas that are more or less peltate, percurrent tertiary venation of the leaves, and huge and hard-walled monocarps. The two genera also share monosulcate pollen with a more or less bulging intine and a columellate infratectum. *Dendrokingstonia* resembles *Monocarpia*, but differs, however, in having axillary inflorescences (terminal in *Monocarpia*) and four-parted lamelliform ruminations of the endosperm (spiniform in *Monocarpia*). On the basis of pollen morphology, the phylogenetic position of *Dendrokingstonia* was likely to fall outside the tribe Miliuseae, which exhibits cryptoaperturate/disulcate pollen. *Monocarpia* has been hypothesized to be closely allied to *Dendrokingstonia* based on macromorphology and pollen morphology. *Dendrokingstonia* as here circumscribed consists of three species: (1) *D. acuminata* comb. nov., occurring in Sumatra, (2) *D. gardneri* sp. nov., occurring in southern Thailand and northern Peninsular Malaysia, and (3) *D. nervosa*, occurring in Peninsular Malaysia.

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**Chapter 7** provided an alpha-taxonomic contribution to our knowledge of the type genus of the tribe Miliuseae, *Miliusa*. Seven new species were described from Thailand: *M. fragrans*, *M. hirsuta*, *M. intermedia*, *M. nakhonsiana*, *M. sessilis*, *M. thailandica*, and *M. umpangensis*. *Miliusa* has been circumscribed by a combination of characters: (1) sepals similar in size to the outer petals, both of which are much smaller than the inner

petals, (2) a densely hairy torus, (3) ‘miliusoid’ stamens, i.e. loosely arranged stamens without conspicuously dilated connective tissue covering the anther thecae, and (4) four-parted lamellate ruminations of the endosperm. The morphological circumscription of four informal infrageneric groups which have previously been proposed for *Miliusa*, was expanded. The newly described *M. fragrans*, *M. intermedia*, *M. nakhonsiana*, and *M. sessilis* exhibit semicircular to crescent-shaped glandular structures on the inner base of the inner petals, which is an important character used for circumscribing the *M. mollis* group. The remaining three new species, *M. hirsuta*, *M. thailandica*, and *M. umpangensis*, possess inner petals that are tightly appressed from the base to more or less the midpoint at anthesis, a character unique to the *M. campanulata* group. A key to all 19 species of *Miliusa* in Thailand was provided, and complete taxonomic nomenclature, including basionyms, synonyms, and lectotypifications of the 12 previously known species, was given.

Published in *Nordic Journal of Botany* 31: 680–699. 2013.

**Chapter 8** presented detailed palynological studies (using light microscopy, LM; scanning electron microscopy, SEM; and transmission electron microscopy, TEM) of *Miliusa* and several other genera that had been placed in the polyphyletic tribe Miliuseae *sensu* Keßler (1993): *Alphonsea*, *Mezzettia*, *Orophea*, and *Platymitra* as well as the *Polyalthia cerasoides* group (now recognized as the genus *Hubera*, see Chapter 3), which had been identified as the sister group of *Miliusa* on the basis of molecular phylogenetic analyses. As previously mentioned in Chapter 7, four informal macro-morphological groups of *Miliusa* had been defined. Pollen morphology, however, does not distinguish these groups, as pollen features of all four groups are largely indistinguishable and the pollen morphology of *Miliusa* is also quite similar to that of *Alphonsea*, *Orophea*, and *Platymitra*. All mentioned genera, including *Miliusa*, possess pollen grains that are released as monads at maturity. The exine is tectate, with verrucate, rugulate, fossulate or scabrate ornamentation, and a columellate to coarsely granular infratectum. The pollen ornamentation of the *Polyalthia cerasoides* group and of *Mezzettia* agrees well with that of the other four genera; however, the *Polyalthia cerasoides* group deviates in having the exine with a finely and densely granular infratectum, whereas *Mezzettia* deviates in having the exine without an infratectum, i.e. the exine is differentiated into only scabrae/verrucae and a basal layer. The pollen of all genera investigated generally shows the presence of two germination zones characterized by the enlargements and reductions of the intine sublayers, while the overlying exine shows no indication of apertures (= cryptoaperturate) or is sometimes depressed (= disulculate). It was concluded that the tribe Miliuseae *sensu* Keßler (1993) cannot be defined by pollen morphology alone because other allied genera based on molecular phylogenetic analyses also possess similar pollen features.

Published in *Grana* 47: 175–184. 2008.

**Chapter 9** provided a contribution to the systematics of *Neo-uvaria*, a genus unique

in subfamily Malmeoideae in always possessing stellate hairs. Two new species were described from southern Thailand (*N. sparsistellata* and *N. telopea*) and combinations were made for two Philippine species (*N. merrillii* and *N. viridifolia*). A provisional key to the seven recognized species of *Neo-uvaria* was provided. In addition, the pollen morphology of several species of *Neo-uvaria* as well as a few species of *Enicosanthum* (recently reduced under *Monoon*, see Xue *et al.* 2012), which is a sister of *Neo-uvaria* based on molecular phylogenetic analyses, was investigated. Apart from the unique stellate hairs, other morphological features of *Neo-uvaria* (e.g. axillary inflorescences, relatively large single seed per monocarp, a distinctly grooved seed raphe, and four-parted lamelliform ruminations of the endosperm) are similar to those of *Enicosanthum*, except for the relatively thicker/fleshier petals and the reduced number of stamens and carpels per flower in *Neo-uvaria*. *Neo-uvaria* pollen was found to be quite homogeneous; all species investigated possess pollen with (micro)echinate ornamentation. Using TEM, the (micro)echinae appear to be supracteal elements and the infractectum consists of more or less distinct granules (equivalent to coarsely granular in Chapter 2). The pollen of *Enicosanthum* is similar to that of *Neo-uvaria*; however, scabrate ornamentation was observed in the pollen of *E. paradoxum* and *E. fuscum*. Macromorphology, pollen morphology, and molecular phylogenetics supported the sister relationship of *Neo-uvaria* and *Enicosanthum* that had previously been shown in other studies.

Published in *Phytotaxa* 32: 27–42. 2011.

## Future perspectives

The results of the research presented in this thesis provide various new insights into the intertribal and infratribal relationships, and generic circumscriptions of tribe Miliuseae. Parts of the backbone of the framework phylogeny remain poorly supported, however, clearly necessitating further molecular phylogenetic work. Next generation sequencing data based on a genome skimming approach using total genomic DNA of multiple species of Miliuseae have recently been generated in the Saunders Lab at the University of Hong Kong (pers. comm. D.C. Thomas). These data will provide an overview of plastome marker variation and facilitate the development of additional plastome and suitably variable nuclear markers, which apparently seem required to completely resolve this most recalcitrant clade.

A well resolved phylogeny of Miliuseae and closely related tribes will be crucial to further investigate the historical biogeography of the Malmeoideae. The monogeneric tribes Monocarpieae and Dendrokingstonieae show distributions restricted to western Malesia (Peninsular Malaysia, Sumatra, plus Borneo for Monocarpieae) and southern Thailand. Dendrokingstonieae is currently recovered as the earliest divergent group within the Dendrokingstonieae-Monocarpieae-Miliuseae clade, which might have some implications on the reconstruction of the historical biogeography of this clade. However, the precise sister relationship of the Dendrokingstonieae-Monocarpieae-Miliuseae clade to the tribes Malmeae, Maasieae, and Fenerivieae is not yet resolved. Resolving the sister relationship of the Dendrokingstonieae-Monocarpieae-Miliuseae clade and a denser sampling of various Asian genera in the tribe Miliuseae will allow to address

several biogeographical questions:

- What is the geographic origin of the Dendrokingstonieae-Monocarpieae-Miliuseae clade and the tribe Miliuseae?
- Is an origin in continental Southeast Asia or western Malesia and subsequent dispersal to eastern Malesia and Australia as hypothesized for *Pseuduvaria* (Su & Saunders 2009) and *Meiogyne* (Thomas *et al.* 2012) a predominant biogeographical pattern for the genera in Miliuseae?
- What is the origin of the Indian representatives of Miliuseae genera; do they form early divergent clades or are they nested within clades of predominantly Southeast Asian taxa?

There are certain lineages that need in-depth phylogenetic analyses. The genera *Sapranthus* and *Desmopsis sensu lato* (including *Stenanona*) require phylogenetic studies based on a dense species sample because generic circumscriptions and differentiation of the two genera based solely on macromorphology are obscure. An integrated generic reappraisal, as performed in Chapters 3 and 4, will shed light on generic delimitation in the *Sapranthus-Tridimeris-Desmopsis s.l.* clade.

Apart from large scale phylogenetic and biogeographic analyses of the tribe Miliuseae, additional taxonomic baseline work is still needed. In the molecular phylogenetic analyses (Chapter 2), there is one particular clade representing an undescribed genus known to occur in Thailand. More material, especially flowers, of this genus is needed to shed light on its identity and to provide a complete formal description. The obscure circumscriptions of species-rich Miliuseae genera formerly placed in the polyphyletic *Polyalthia s.l.* complex (*Hubera*, *Monoon*, and *Polyalthia s.s.*) have hindered their revisionary studies. Studies by Xue *et al.* (2012) and Chaowasku *et al.* (2012a; Chapter 3) have clarified the generic boundaries of these genera, but the species of these genera are badly in need of taxonomic revision. Smaller genera with obvious diagnostic characters such as *Marsyopetalum* (Xue *et al.* 2011), *Trivalvaria* (Van Heusden 1997b; Xue *et al.* 2011), and *Neo-uvaria* (Chaowasku *et al.* 2011a; Chapter 9) should also be thoroughly taxonomically studied, as there are a number of species still unknown to science (pers. obs.). Another genus that has been neglected is *Popowia*. An unpublished regional treatment was completed by Moeljono (2009), but a complete revision of the genus is currently not available.

Reconstructions of the ancestral character states have identified cryptoaperturate/disulculate pollen as a synapomorphy of the tribe Miliuseae (Chapter 2). This character syndrome, however, still needs further investigation using critical point drying to retain the original shape and configuration of the pollen as much as possible. Disulculate pollen may just represent cryptoaperturate pollen that is partially collapsed at the exine where the intinous germination zones lie beneath. Moreover, the pollen grains of Miliuseae members that apparently lack the germination zones altogether need to be further investigated to clarify whether (1) sections previously made and investigated did not cross the germination zones, or (2) the pollen taken was still in an ontogenetic stage that does not exhibit the germination zones, or (3) such pollen is really inaperturate (omniaperturate). Functional studies of pollen germination may also be helpful to elucidate some of these issues.

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Recent advances in sequencing technology such as hybridization-based targeted enrichment strategies (Stull *et al.* 2013) and reduced-representation approaches such as restriction site associated DNA sequencing (Davey *et al.* 2011) facilitate the generation of huge quantities of DNA sequencing data at reasonable costs, and have great potential to resolve both recent and ancient rapid radiations (e.g. Nadeau *et al.* 2012). By capitalising on these methodological advances and continuing thorough morphological studies and alpha-taxonomic efforts, resolution of the phylogenetic relationships and a modern taxonomic treatment of the recalcitrant Miliuseae are within reach.



## Samenvatting en conclusies

Dit proefschrift op het gebied van de plantensystematiek omvat acht deelonderzoeken waarin de moleculaire fylogenie, de taxonomie en de pollenmorfologie van de tribus Miliuseae (*sensu* Chatrou *et al.* 2012; voorheen de 'miliusoid clade' *sensu* Mols *et al.* 2004b) worden behandeld. Deze tribus behoort tot de pantropische bloemplantenfamilie Annonaceae (Zuurzakfamilie). De algemene doelstellingen van het onderzoek waren:

(1) het vaststellen van de verwantschapsrelaties van de tribus Miliuseae met andere tribussen, (2) het ophelderen van de geslachtsomgrenzingen binnen de tribus Miliuseae door de verwantschapsrelaties tussen de geslachten te onderzoeken, en (3) het begrijpen van de kenmerkevolutie binnen de tribus Miliuseae.

Om deze doelstellingen te bereiken, werd voor de onderfamilie Malmeoideae een moleculaire fylogenie gereconstrueerd waarbij, vergeleken met eerdere studies, meer chloroplast DNA-markers werden gebruikt en een veel uitgebreidere selectie van taxa werd onderzocht. Deze selectie omvatte vertegenwoordigers van de tribussen Maasieae, Malmeeae, Fenerivieae, Dendrokingstonieae (voor het eerst bemonsterd), Monocarpieae en Miliuseae. Tevens werden de twee tribussen Dendrokingstonieae en Monocarpieae, die beide slechts één geslacht omvatten, en een aantal genera van de Miliuseae grondig taxonomisch gerevideerd of gedetailleerd palynologisch onderzocht. Voor het onderzoek naar de evolutie van macromorfologische en palynologische kenmerken werd gebruik gemaakt van reconstructies van voorouderkenmerken. Geslachtsomgrenzingen binnen de Miliuseae werden bediscussieerd aan de hand van de resultaten van het moleculaire fylogenetische onderzoek. Op grond van macromorfologische kenmerken en pollenmorfologie werden binnen deze tribus twee nieuwe geslachten onderscheiden: *Hubera* en *Winitia*.

**Hoofdstuk 2** behandelt fylogenetische reconstructies van een tak binnen de onderfamilie Malmeoideae die zijn gebaseerd op moleculair onderzoek met zeven chloroplast markers (*rbcL* exon, *trnL* intron, *trnL-F* spacer, *matK* exon, *ndhF* exon, *psbA-trnH* spacer, *ycf1* exon). Deze reconstructies omvatten vertegenwoordigers van de tribussen Maasieae, Malmeeae, Fenerivieae, Monocarpieae en Miliuseae (waarvan ca. 89% van de geslachten zijn bemonsterd) en zijn tot stand gekomen door gebruik te maken van het principe van maximale spaarzaamheid (maximum parsimony) en Bayesiaanse analyses. Het onderzoek was inclusief de tribus Dendrokingstonieae, dat slechts één geslacht omvat en waarvan nooit eerder materiaal voor moleculair onderzoek was gebruikt. De fylogenetische analyses lieten zien dat de Dendrokingstonieae, Monocarpieae en Miliuseae een goed ondersteunde tak van de fylogenetische boom vormen, en dat de Dendrokingstonieae een zustergroep is van een zwak tot matig ondersteunde tak die bestaat uit de Monocarpieae en Miliuseae. De tribussen Maasieae, Malmeeae en Fenerivieae vormen een polytomie met de Dendrokingstonieae-Monocarpieae-Miliuseae tak. De meeste geslachten van de Miliuseae die met twee of meer soorten in de analyses vertegenwoordigd waren, bleken monofyletisch te zijn, met uitzondering van het parafyletische geslacht *Desmopsis*, waarin *Stenanona* genesteld is. De gevonden fylogenie bevestigt bovendien dat er binnen de Miliuseae een nog onbeschreven

geslacht bestaat dat in Thailand voorkomt. Voor de Miliuseae werden bovendien van de kenmerken die op tribus- of geslachtsniveau onderscheidend zijn de voorouderlijke kenmerktoestanden gereconstrueerd. Hiervoor werd eveneens gebruik gemaakt van een Bayesiaanse benadering en het principe van maximale spaarzaamheid. De morfologische variabiliteit bij pollenkorrels bleek vervolgens goed overeen te komen met de moleculaire fylogenie. Cryptoaperturaat of disulculaat pollen kan beschouwd worden als een synapomorfie voor de Miliuseae, terwijl in alle andere tribussen, inclusief de Dendrokingstonieae en de Monocarpieae, monsulcaat pollen voorkomt. De volgende kenmerktoestanden kunnen als voorouderlijke kenmerktoestanden van de Miliuseae worden beschouwd: vruchtbeginsels met veel zaadknoppen, buitenste kroonbladen met een opvallend uiterlijk, ongeklauwde binnenste kroonbladen, tweeslachtige bloemen, stekelvormige tot afgeplat wigvormige intrusies in het endosperm, afgifte van rijp pollen in de vorm van losse pollenkorrels, en pollenkorrels met een columellair tot grofkorrelig infratectum. De fylogenetische analyses laten zien dat de meeste onderzochte kenmerken (maximum aantal zaadknoppen per vruchtblad, mate van opvallen van de buitenste kroonbladeren, binnenste kroonbladbasis, geslacht van de bloem, ruminatie van het endosperm, de verspreidingseenheid van rijp pollen, infratectum-type van pollenkorrels) binnen de Miliuseae veel homoplasie vertonen.

In druk in *American Journal of Botany*.

**Hoofdstuk 3** behandelt het geslacht *Hubera*, een nieuw geslacht binnen de tribus Miliuseae. Dit geslacht stond voorheen bekend als de *Polyalthia cerasoides*-groep en krijgt de status van geslacht op grond van een combinatie van macromorfologie, pollenmorfologie en moleculaire fylogenie (gebaseerd op dezelfde DNA-regio's en dezelfde analyses als beschreven in Hoofdstuk 2). *Hubera* is een sterk ondersteunde monofyletische groep. De zustergroep-relatie met *Milium* wordt matig tot sterk ondersteund, maar synapomorfieën (gemeenschappelijke afgeleide kenmerken) die de twee geslachten gemeen hebben zijn tot dusver nog niet gevonden. De belangrijkste onderscheidende kenmerken van *Hubera* zijn netvormige adering van de bladeren (door de fijnste nerfjes), okselstandige bloeiwijzen, één zaadknop per vruchtbeginsel, een vlakke tot iets verhoogde zaadnerf, stekelvormige (tot afgeplat wigvormige) intrusies in het endosperm, en pollenkorrels met een fijn en dicht korrelig infratectum. Een aantal *Hubera*-soorten (12 van de 27 in dit geslacht ondergebrachte soorten) heeft aan de onderzijde van het blad in de nerfoksels tussen de hoofdnerf en de zijnerven (van de eerste orde) een dicht toefje haren, waarmee deze *Hubera*-soorten zich onderscheiden van soorten van *Polyalthia sensu stricto* en andere geslachten (*Monoon*, *Marsypopetalum*, *Trivalvaria*), die voorheen waren ondergebracht in de sterk polyfyletische *Polyalthia sensu lato*.

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**Hoofdstuk 4** onderzoekt de geslachtsomgrenzingen van *Stelechocarpus* en *Sageraea* met behulp van een combinatie van moleculaire fylogenie (gebaseerd op dezelfde DNA-regio's en dezelfde analyses als beschreven in Hoofdstuk 2), macromorfologie en

pollenmorfologie. Het onderzoek resulteerde in de herkenning van een nieuw geslacht, *Winitia*, dat voornamelijk wordt gekenmerkt door: (1) veelzuilige stempels, een synapomorfie voor dit geslacht, en (2) pollenkorrels met een erg dun tectum en een min of meer columellair/grofkorrelig en een erg duidelijk te onderscheiden basale laag. Het nieuwe geslacht omvat twee soorten: *W. cauliflora* comb. nov. en *W. expansa* sp. nov. De eerste soort komt voor in Vietnam, zuidelijk Thailand, West-Maleisië (Maleisisch deel van het Malakkaschiereiland) en Borneo, de tweede is een endem van zuidelijk Thailand. *Winitia* wordt matig tot sterk ondersteund als zustergroep van *Stelechocarpus* (dat slecht één soort omvat: *Stelechocarpus burahol*) met drie autapomorfieën: (1) mannelijke en vrouwelijke bloemen op dezelfde plant staan gescheiden van elkaar (bij *Winitia* staan ze bij en door elkaar), (2) mannelijke en vrouwelijke bloemen op dezelfde plant verschillen in grootte (bij *Winitia* hebben mannelijke en vrouwelijke bloemen dezelfde afmetingen), en (3) pollenkorrels met een fijn en dicht korrelig infratectum. *Stelechocarpus* en *Winitia* hebben twee vegetatieve eigenschappen met elkaar gemeen: de opvallende zijnerf aan de onderzijde van het blad en het (licht) verhoogde oppervlak van de hoofdnerf. Een tak van de fylogenetische boom met *Stelechocarpus* en *Winitia* wordt sterk ondersteund als zustergroep van *Sageraea*. *Sageraea* heeft slechts één onderscheidend vegetatief kenmerk: de min of meer onduidelijke zijnerf aan de onderzijde van het blad.

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**Hoofdstuk 5** betreft een voorstel om de naam *Meiogyne* te behouden (conserveren) vanwege de stabiliteit van namen. De naam *Meiogyne* wordt veel gebruikt en vervangend door de oudere naam *Fitzalania*, wat volgens de nomenclatuurregels eigenlijk zou moeten, zal tot verwarring leiden. *Meiogyne* en *Fitzalania* hebben twee onderscheidende kenmerken met elkaar gemeen: een in de lengterichting gerimpeld of gegroefd gebied aan de basis van de bovenzijde van de binnenste kroonbladeren en een verlengd helmbindsel van de binnenste meeldraden. Het geslacht *Fitzalania* is, fylogenetisch gezien, genesteld in *Meiogyne* (Hoofdstuk 2). *Meiogyne* omvat echter meer soorten en heeft een groter areaal dan *Fitzalania*. Bovendien is de naam meiogynine A, een dimere sesquiterpenoïde met veelbelovende antikanker-eigenschappen die wordt geïsoleerd uit de bast van *Meiogyne cylindrocarpa*, afgeleid van de naam *Meiogyne*.

Gepubliceerd in *Taxon* 60: 1522–1523. 2011.

**Hoofdstuk 6** omvat een taxonomische revisie van het geslacht *Dendrokingstonia* (het enige geslacht van de tribus Dendrokingstonieae) en tevens een gedetailleerde pollenmorfologische studie van zowel dit geslacht als van het vermoedelijk verwante geslacht *Monocarpia* (het enige geslacht van de tribus Monocarpieae). Beide geslachten hebben een binnen de familie Annonaceae zelden voorkomende combinatie van kenmerktostanden met elkaar gemeen: een sterk gereduceerd aantal vruchtbladen per bloem, sterk vergrote en min of meer schildvormige stempels, bladeren die over de gehele lengte van de bladschijf door fijne nerfjes geaderd zijn, en hardwandige en grote enkelvoudige vruchten. De twee geslachten hebben ook monosulcate pollenkorrels met een min of meer uitstulpende intine en een columellair infratectum met elkaar gemeen.

*Dendrokingstonia* lijkt op *Monocarpia*, maar heeft okselstandige bloeiwijzen (eindstandig in *Monocarpia*) en vierdelige plaatvormige intrusies in het endosperm (stekelvormig in *Monocarpia*). Op grond van de pollenmorfologie valt te verwachten dat *Dendrokingstonia* in de fylogenetische boom buiten de tribus Miliuseae wordt geplaatst, die immers gekenmerkt wordt door cryptoaperturaat/disulculaat pollen. Vanwege macromorfologische en pollenmorfologische kenmerken wordt echter wel verondersteld dat *Monocarpia* nauw verwant is met *Dendrokingstonia*. Zoals hier omschreven omvat *Dendrokingstonia* de volgende drie soorten: (1) *D. acuminata* comb. nov., die voorkomt op Sumatra, (2) *D. gardneri* sp. nov., die voorkomt op zuidelijk Thailand en het noordelijk deel van West-Maleisië, en (3) *D. nervosa*, die voorkomt in West-Maleisië.

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**Hoofdstuk 7** omvat een alfa-taxonomische bewerking van Thaise vertegenwoordigers van het typegeslacht van de tribus Miliuseae, het geslacht *Miliusa*. Er werden zeven nieuwe soorten beschreven voor Thailand: *M. fragrans*, *M. hirsuta*, *M. intermedia*, *M. nakhonsiana*, *M. sessilis*, *M. thailandica* en *M. umpangensis*. *Miliusa* wordt omschreven door een combinatie van kenmerktostanden: (1) kelkbladeren en buitenste kroonbladeren zijn even groot en veel kleiner dan de binnenste kroonbladeren, (2) een dichtbehaarde bloembodem, (3) ‘miliusoïde’ meeldraden, dat wil zeggen los verspreid staande meeldraden zonder opvallend opgezwollen helmbindsel dat de helmknoppen bedekt, en (4) vierdelige plaatvormige intrusies in het endosperm. De morfologische omgrenzing van vier bestaande informele groepen binnen het geslacht *Miliusa* werd uitgebreid. De vier nieuw beschreven soorten *M. fragrans*, *M. intermedia*, *M. nakhonsiana* en *M. sessilis* hebben halfronde tot halvemaanvormige klierstructuren aan de basis van de bovenzijde van de binnenste kroonbladeren, wat een onderscheidend kenmerk is voor de *M. mollis*-groep. De andere drie nieuwe soorten, *M. hirsuta*, *M. thailandica* en *M. umpangensis*, hebben binnenste kroonbladeren die, bij het receptief worden van de stigma’s en het rijpen van de meeldraden (de bloem gaat daarbij maar beperkt open), van de bladbasis tot ongeveer het midden van het kroonblad dicht samengedrukt opeen staan, een kenmerktostand die uniek is voor de *M. campanulata*-groep. In de bewerking is een sleutel opgenomen voor alle 19 *Miliusa*-soorten van Thailand. Tevens wordt een compleet overzicht gegeven van de taxonomische nomenclatuur, inclusief basioniemen, synoniemen en lectotypificaties, van de 12 soorten die al van Thailand bekend waren.

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**Hoofdstuk 8** behandelt palynologisch onderzoek van *Miliusa* en een aantal andere geslachten van de polyfyletische tribus Miliuseae *sensu* Keßler (1993): *Alphonsea*, *Mezzettia*, *Oropheia* en *Platymitra*, en tevens de *Polyalthia cerasoides*-groep (nu beschouwd als een apart geslacht, *Hubera*, zie Hoofdstuk 3), die op grond van moleculaire fylogenetische analyses als een zustergroep van *Miliusa* kan worden beschouwd. Voor dit onderzoek werd gebruik gemaakt van een lichtmicroscop (LM), een raster (scanning)elektronenmicroscop (SEM) en een transmissie-elektronenmicroscop (TEM). Zoals al in Hoofdstuk 7 werd opgemerkt, worden binnen *Miliusa* vier informele

groepen onderscheiden. Dit onderscheid wordt niet terug gevonden in pollenmorfologie, omdat de pollenkorrels van de vier groepen morfologisch nauwelijks van elkaar verschillen en de pollenmorfologie van *Miliusa* veel overeenkomsten vertoont met die van *Alphonsea*, *Orophea* en *Platymitra*. Bij alle eerder genoemde geslachten, inclusief *Miliusa*, wordt het rijpe pollen afgegeven in de vorm van losse pollenkorrels. De exine heeft een tectum met verrucate, rugulate, fossulate of scabrate ornamentatie en een columellair tot grofkorrelig infratectum. De ornamentatie van de pollenkorrels van de *Polyalthia cerasoides*-groep en *Mezzettia* komt goed overeen met die van de andere vier genera, maar bij de *Polyalthia cerasoides*-groep heeft de exine een fijn en dicht korrelig infratectum, terwijl de exine bij *Mezzettia* geen infratectum heeft, wat wil zeggen dat de exine alleen een basale laag met scabrae/verrucae heeft. Pollenkorrels van alle onderzochte geslachten hebben in het algemeen twee kiemzones, die worden gekenmerkt door vergrotingen en reducties van de intine, terwijl de daarboven liggende exine geen aperturen vertoont (cryptoaperturaat) of soms ingedrukt is (disulculaat). Als conclusie kan worden gesteld dat de tribus Miliuseae *sensu* Keßler (1993) niet uitsluitend op grond van pollenmorfologie kan worden onderscheiden, omdat volgens moleculaire analyses verwante geslachten een overeenkomstige pollenmorfologie hebben.

Gepubliceerd in *Grana* 47: 175–184. 2008.

**Hoofdstuk 9** draagt bij aan de kennis van de systematiek van *Neo-uvaria*, een geslacht dat vanwege diens sterharen uniek is binnen de onderfamilie Malmeoideae. Er worden twee nieuwe soorten voor zuidelijk Thailand beschreven (*N. sparsistellata* en *N. telopea*) en nieuwe combinaties gemaakt voor twee Filipijnse soorten (*N. merrillii* en *N. viridifolia*). In deze bijdrage is ook een voorlopige sleutel voor de *Neo-uvaria*-soorten opgenomen. Tevens werd de pollenmorfologie onderzocht van een aantal *Neo-uvaria*-soorten en van een paar soorten van *Enicosanthum* (recentelijk ondergebracht in het geslacht *Monoon*, zie Xue *et al.* 2012), op grond van moleculaire analyses een zustergroep van *Neo-uvaria*. Met uitzondering van de voor *Neo-uvaria* unieke sterharen, de relatief dikkere/vlezigere kroonbladeren en het gereduceerde aantal meeldraden en vruchtbladen per bloem, zijn de andere morfologische kenmerken van *Neo-uvaria* (bijv. okselstandige bloeiwijzen, één relatief groot zaad per enkelvoudige vrucht (monocarp), een opvallend gegroefde zaadnerf, vierdelige plaatvormige intrusies in het endosperm) hetzelfde als bij *Enicosanthum*. Pollen van *Neo-uvaria* blijkt vrij homogeen te zijn; alle onderzochte soorten hebben pollenkorrels met een (micro)echinate ornamentatie. Onderzoek met de transmissie-elektronenmicroscop (TEM) laat zien dat de (micro)echinae (stekeltjes) structuren zijn die op het tectum staan en dat het infratectum bestaat uit min of meer duidelijk te onderscheiden korreltjes (wat overeenkomt met de term ‘grofkorrelig’ in Hoofdstuk 2). Het pollen van *Enicosanthum* ziet er hetzelfde uit als dat van *Neo-uvaria*, maar pollenkorrels van *E. paradoxum* en *E. fuscum* hebben een scabrate ornamentatie. De macromorfologie, de pollenmorfologie en de moleculaire fylogenie ondersteunen de in eerder onderzoek aangetoonde zusterrelatie tussen *Neo-uvaria* en *Enicosanthum*.

Gepubliceerd in *Phytotaxa* 32: 27–42. 2011.

## Toekomstig onderzoek

Het resultaat van het onderzoek dat ten grondslag ligt aan dit proefschrift geeft nieuwe inzichten in de geslachtsomgrenzingen van de tribus Miliuseae en de verwantschapsrelaties boven en onder het tribusniveau. Basale delen van de fylogenetische boom blijven echter zwak ondersteund en verder moleculair-fylogenetisch onderzoek is dan ook noodzakelijk. Het Saunders Lab van de Universiteit van Hong Kong heeft door middel van een nieuwe sequentietechniek ('next generation sequencing'), waarbij zeer grote hoeveelheden DNA relatief snel kunnen worden gesequenced en die hier werd toegepast op het hele genoom van een groot aantal Miliuseae-soorten, sequentiegegevens verkregen die nieuw inzicht zullen geven in de variabiliteit van de markers van het plastoom (plastiden-DNA). Analyse van deze gegevens zal ook bijdragen tot de ontwikkeling van nieuwe markers, voor zowel het plastoom als het nucleair genoom (celkern-DNA), met de juiste mate van variabiliteit voor evolutionair onderzoek aan de Miliuseae. Nieuwe markers zijn nodig om de fylogenie van deze lastigste tak van de fylogenetische boom volledig op te lossen.

Een goed opgeloste fylogenie van de Miliuseae met nauw verwante tribussen is essentieel om de historische biogeografie van de onderfamilie Malmeoideae te kunnen onderzoeken. De tribussen Monocarpieae en Dendrokingstonieae, die beide slechts één geslacht omvatten, zijn in hun verspreiding beperkt tot westelijk Malesië (West-Maleisië, Sumatra, plus Borneo voor de Monocarpieae) en zuidelijk Thailand. De Dendrokingstonieae worden momenteel gezien als de vroegst afgesplitste groep van de Dendrokingstonieae-Monocarpieae-Miliuseae tak van de fylogenetische boom, wat mogelijk implicaties kan hebben voor de reconstructie van de historische biogeografie van deze tak. De precieze zustergroep-relatie van de Dendrokingstonieae-Monocarpieae-Miliuseae tak met de tribussen Malmeeae, Maasieae en Fenerivieae is nog niet opgelost. Het oplossen van de zustergroep-relaties van de Dendrokingstonieae-Monocarpieae-Miliuseae tak en fylogenetische analyses met een bredere selectie van vertegenwoordigers van de Aziatische geslachten van de tribus Miliuseae zal het mogelijk maken de volgende biogeografische vraagstukken te onderzoeken:

- Waar zijn de Dendrokingstonieae-Monocarpieae-Miliuseae tak en de tribus Miliuseae ontstaan?
- Is verspreiding vanuit een continentaal Zuidoost-Aziatisch of westelijk Malesisch oorsprongsgebied naar oostelijk Malesië en Australië, zoals dat voor *Pseuduvaria* (Su & Saunders 2009) en *Meiogyne* (Thomas *et al.* 2012) wordt verondersteld, een overheersend biogeografisch patroon voor de geslachten van de Miliuseae?
- Wat is de herkomst van de Indiase vertegenwoordigers van de Miliuseae-geslachten; vormen deze vroeg afsplitsende taken van de fylogenetische boom of zijn ze genesteld in takken die voornamelijk uit Zuidoost-Aziatische geslachten bestaan?

Er zijn bepaalde afstammingslijnen waarvoor diepgaand fylogenetisch onderzoek nodig is. Voor de geslachten *Sapranthus* en *Desmopsis sensu lato* (inclusief *Stenanona*) is fylogenetische onderzoek gebaseerd op een veel bredere selectie van soorten

noodzakelijk, omdat het onderscheid tussen de twee geslachten dat nu enkel is gebaseerd op macromorfologische kenmerken onduidelijk is. Nieuw fylogenetisch onderzoek dat de geïntegreerde benadering van Hoofdstuk 3 en 4 volgt zal inzicht geven in de geslachtsomgrenzingen binnen de *Sapranthus-Tridimeris-Desmopsis sensu lato* tak. Naast een grootschalige aanpak van toekomstige fylogenetische en biogeografische analyses van de tribus Miliuseae is aanvullend basaal taxonomisch onderzoek nodig. In de moleculaire fylogenetische analyses (Hoofdstuk 2), komt een tak naar voren die een voor Thailand onbekend en een voor de wetenschap onbeschreven geslacht vertegenwoordigt. Meer materiaal van dit geslacht, vooral van bloemen, is nodig om meer duidelijkheid te verkrijgen over de identiteit van dit geslacht en om het formeel te kunnen beschrijven. De onduidelijke omschrijvingen van soortenrijke geslachten van de Miliuseae, voorheen ondergebracht in het polyfyletische *Polyalthia sensu lato*-complex (*Hubera*, *Monoon*, *Polyalthia sensu stricto*) hebben taxonomische revisies belemmerd. Recent onderzoek van Xue *et al.* (2012) en Chaowasku *et al.* (2012a; Hoofdstuk 3) heeft weliswaar de omgrenzingen van deze geslachten verduidelijkt, maar de soorten van deze geslachten moeten nog steeds taxonomisch worden gerevideerd, en dat is voor goed fylogenetisch en biogeografisch vervolgonderzoek ook beslist noodzakelijk. Ook voor kleinere geslachten met duidelijke onderscheidende kenmerken, zoals *Marsypopetalum* (Xue *et al.* 2011), *Trivalvaria* (Van Heusden 1997b; Xue *et al.* 2011) en *Neo-uvaria* (Chaowasku *et al.* 2011a; Hoofdstuk 9), is een grondige taxonomische revisie noodzakelijk vanwege een aantal soorten dat nog onbekend is voor de wetenschap (eigen waarnemingen). Een ander geslacht dat nog moet worden bewerkt is *Popowia*. Er is een ongepubliceerde regionale bewerking van de hand van Moeljono (2009), maar een complete revisie van het geslacht is nog niet beschikbaar.

Reconstructies van de voorouderlijke kenmerktoestanden geven aan dat cryptoaperturaat/disulculaat pollen een synapomorfie is voor de tribus Miliuseae (Hoofdstuk 2). Dit kenmerksyndroom moet echter verder worden onderzocht met de rasterelektronenmicroscopie (SEM) in combinatie met kritisch puntdrogen om de oorspronkelijke vorm en toestand van de pollenkorrels zoveel mogelijk intact te houden. Het zou immers kunnen dat disulcaat pollen niet veel meer is dan cryptoaperturaat pollen waarbij de exine deels is ingeklapt ter hoogte van de kiemzones.

Bovendien moet pollen van vertegenwoordigers van de Miliuseae waarbij de pollenkorrels kennelijk geen kiemzones hebben verder worden onderzocht om te achterhalen of (1) eerder gemaakte en onderzochte doorsneden de kiemzones hebben gemist, (2) het onderzochte pollen in een stadium van ontwikkeling was waarin de kiemzones nog niet waren aangelegd, (3) zulk pollen inderdaad echt inaperturaat (omniaperturaat) is. Onderzoek naar het kiemen van pollenkorrels kan helpen om een aantal van deze vragen op te helderen.

Recente ontwikkelingen in sequentietechnologie, zoals de methoden en technieken die recent door Stull *et al.* (2013) en Davey *et al.* (2011) zijn voorgesteld, vergemakkelijken het genereren van enorme hoeveelheden DNA-sequentiegegevens tegen aanvaardbare kosten, en bieden daardoor grote mogelijkheden om zowel snelle soortvorming en radiatie uit het recente verleden als zulke gebeurtenissen uit het verre verleden te ontrafelen (bijv. Nadeau *et al.* 2012). Door van deze methodologische

ontwikkelingen gebruik te maken en deze te combineren met grondig morfologisch onderzoek en alfa-taxonomische revisies, zal een moderne taxonomische bewerking van de weerbarstige tribus Miliuseae en het volledig ontrafelen van diens fylogenetische verwantschapsrelaties tot de mogelijkheden gaan behoren.

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## CURRICULUM VITAE

Tanawat Chaowasku was born on 24 September 1980 in Nakhon Pathom, Thailand. During his childhood, his parents moved from province to province and the plant diversity in the different provinces he traveled (i.e. from dipterocarp to deciduous to evergreen forests) has greatly inspired him. Flowering plants fascinated him from his earliest years, but when he was only 14 years old, species of the Annonaceae or custard apple family got his special attention. He obtained the Bachelor of Science degree from Chulalongkorn University in 2003. He chose pharmacognosy and pharmaceutical botany as specialties. His bachelor's project was entitled "Chemical constituents of the stem bark of *Mitrephora tomentosa* (Annonaceae)" and resulted in his first academic publication. After graduation, he worked as a hospital pharmacist for four months. Apart from phytochemistry of Annonaceae, he has a strong interest in Annonaceae systematics, which has become his current research field. In 2004 he passed the Royal Thai Government examination and was awarded a full scholarship for his Master's and PhD studies. In 2006 he obtained the degree of Master of Science at Leiden University for his work on the taxonomy and palynology of the genus *Miliusa*. Afterwards, he expanded his studies into the complex tribe Miliuseae for his PhD project. He hopes that he will be able to continue his research on Annonaceae systematics after his PhD graduation. One of his aims is to start up a pollination biological study on Annonaceae in the near future. He will work as a lecturer and researcher of the Department of Biology, Faculty of Science, Chiang Mai University.

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- Supudompol B, **Chaowasku T**, Kingfang K, Burud K, Wongseripipatana S & Likhitwitayawuid K. 2004. A new pimarane from *Mitrephora tomentosa*. *Natural Product Research* 18: 387–390.
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- Sawasdee K, **Chaowasku T**, Lipipun V, Dufat TH, Michel S, Jongbunprasert V & Likhitwitayawuid K. 2014. Geranylated homogentisic acid derivatives and flavonols from *Milium umpangensis*. *Biochemical Systematics and Ecology* 54: 179–181.
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### Conference participations

- Annonaceae Workshop, Vienna, Austria, 2005, poster presentation “Character analysis and floristic revision of Thai and Indochinese *Milium* (Annonaceae)”
- 7<sup>th</sup> Flora Malesiana Symposium, Leiden, The Netherlands, 2007, oral presentation

“Taxonomic study on *Miliusa* (Annonaceae)”

- Systematics [BioSystEU], Leiden, The Netherlands, 2009, oral presentation

“Congruence between molecular and palynological data in a major clade of Annonaceae”

- 19<sup>th</sup> AETFAT Congress, Antananarivo, Madagascar, 2010, poster presentation

“Systematics of Afro-Malagasy *Polyalthia* (Annonaceae)”

- 15<sup>th</sup> Flora of Thailand Meeting, Chiang Mai, Thailand, 2011, oral presentation

“Diversity of *Miliusa*, *Marsypopetalum*, *Trivalvaria*, *Neo-uvaria*, and two new genera (Annonaceae) in Thailand”

- 9<sup>th</sup> Flora Malesiana Symposium, Bogor, Indonesia, 2013, oral presentation “Molecular phylogenetics of the tribe Miliuseae: Insights into generic delimitations and character evolution”.



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