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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 (\textit{rbcl} exon, \textit{trnL} intron, \textit{trnL-F} spacer, \textit{matK} exon, \textit{ndhF} exon, \textit{psba-trnH} spacer, and \textit{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 Dendrokingstoniaceae and Monocarpieae, and cryptoaperturate or disulculate 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 rumination 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, Marsypopetalum, Trivalvaria*), which had all been placed in the highly polyphyletic *Polyalthia sensu lato*.


**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
Summary and conclusions

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, meiogynin 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.

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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, percurent 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/disulculate 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. um pangensis*. *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 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*. Fitzalonia has been shown to be nested phylogenetically within Meiogyne (Chapter 2). Meiogyne and Fitzalonia 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 Fitzalonia. Additionally, Meiogyne has lent its name to a dimeric sesquiterpenoid, meiogynin 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.

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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.


**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.

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**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 supratectal elements and the infratectum 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.

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**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 *Marsypopetalum* (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.
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.