The Miliuseae revisited : phylogenetic, taxonomic, and palynological studies in a major clade of Annonaceae

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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 ± 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 (Avise & 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 ruminate endosperm, Annonaceae are easily recognizable both in the field and as herbarium specimens.

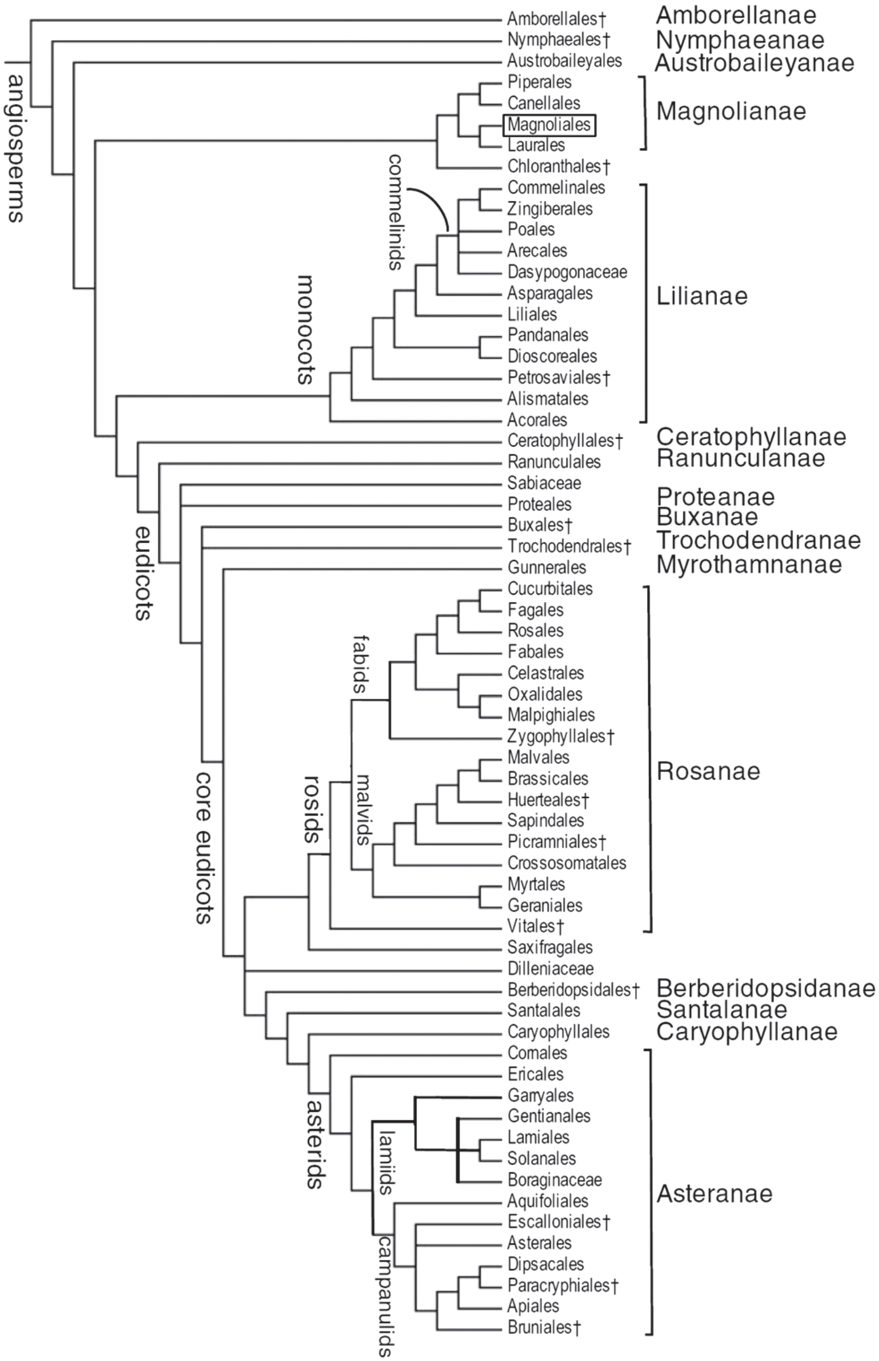

FIGURE 1. Phylogenetic position of Magnoliales (rectangle) and Magnolianae in angiosperm phylogeny. Adapted from Chase & Reveal (2009).

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 subfamilies 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), *Miliusa* Lesch. ex A.DC., *Orophea* Blume, *Phoenicanthus* Alston, and *Platymitra* Boerl. (Keßler 1993), which

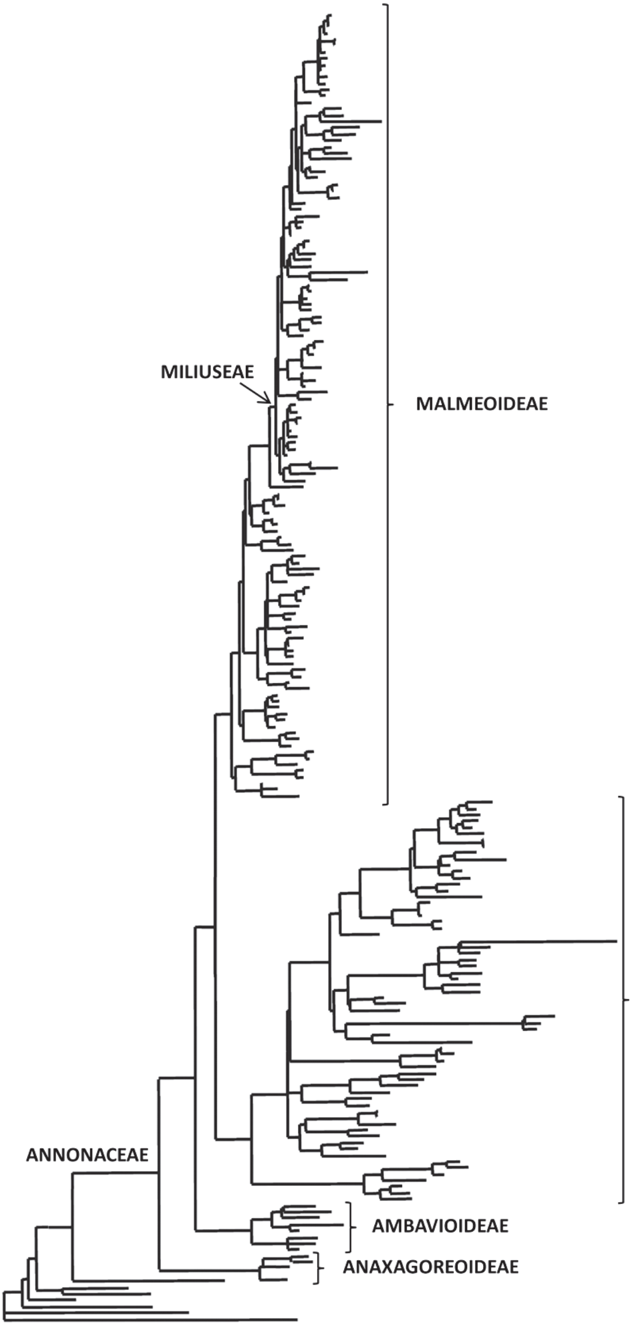

FIGURE 3. Phylogeny of Annonaceae, showing four subfamilies and position of Miliuseae. Adapted from Richardson *et al*. (2004).

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 *Miliusa*) 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 *Enicosanthum* Becc. (now synonymized with *Monoon*), are presented.