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

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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): apocarpy/syncarpy, polypetaly/sympetaly, 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 ≥ 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 Malmeeae. 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 µg of bovine serum albumin (BSA) in 50 µl 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® 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 ≥ 85%, 70–84 %, and ≤ 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 2×10^7 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 1000th 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 ≥ 0.96, 0.91–0.95, and ≤ 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
rbcL	1F	ATGTCACCACAAACAGAAAC	Olmstead *et al*. (1992)
	724R	TCGCATGTACCTGCAGTAGC	Fay *et al*. (1997)
	636F	GCGTTGGAGAGATCGTTTCT	Fay *et al*. (1997)
	1460R	TCCTTTTAGTAAAAGATTGGGCCGAG	Olmstead *et al*. (1992)
trnLF	C	CGAAATCGGTAGACGCTACG	Taberlet *et al*. (1991)
	D	GGGGATAGAGGGACTTGAAC	Taberlet *et al*. (1991)
	E	GGTTCAAGTCCCTCTATCCC	Taberlet *et al*. (1991)
	F	ATTTGAACTGGTGACACGAG	Taberlet *et al*. (1991)
matK	390F	CGATCTATTCATTCAATATTTC	Cuénoud *et al*. (2002)
	Mint-F	TCCTTTGGAACTGTTCTTGAGC	Pirie *et al*. (2005)
	Mint-R	GATCCTGTGCGGTTGAGACC	Pirie *et al*. (2005)
	1326R	TCTAGCACACGAAAGTCGAAGT	Cuénoud *et al*. (2002)
ndhF	1F	ATGGAACAKACATATSAATATGC	Olmstead & Sweere (1994)
	54F	GCTCGTCGTATGTGGGCTTTTC	Present study
	660R	GTGCAGATTTAGCAACTGCACC	Present study
	481F	CGTTTGTAACGAATCGTGTAGGGG	Present study
	1089R	CCACCCATAAGAACCATGTTCTG	Present study
	972F	GTCTCAATTGGGTTATATGATG	Olmstead & Sweere (1994)
	1087F	GTCAGAACATGGTTCTTATGGG	Present study
	1650R	CGAAGGGAATTCCTATGGACCC	Present study
	1550F	CTCTGACAATAAAGACACTTCC	Present study
	2110R	CCCCCTAYATATTTGATACCTTCTCC	Olmstead & Sweere (1994)
psbA-trnH	F	CGAAGCTCCATCTACAAATGG	Hamilton (1999)
	R	ACTGCCTTGATCCACTTGGC	Hamilton (1999)
ycf1	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)
Polyalthia cerasoides	42	Verrucate(-rugulate)	Finely, densely granular	1?
Polyalthia decora[1]	-	Verrucate-rugulate	-	-
Polyalthia forbesii	29	Fossulate-perforate	Finely, densely granular	2
Polyalthia henrici	31	Rugulate	Finely, densely granular	2
Polyalthia jenkinsii	33	(Weakly) coarsely rugulate to fossulate	Finely, densely granular	2
Polyalthia keraudreniae[1]	-	± Fossulate-perforate	-	-
Polyalthia korinti	33	(Verrucate-)rugulate	Finely, densely granular	?

TABLE 2 (continued).

Taxon	Size (µm; LM)	Ornamentation (SEM)	Infratectum (TEM)	Germ. zone(s) (TEM)
Polyalthia multistamina[1]	-	Fossulate-perforate	-	-
Polyalthia nitidissima	-	Verrucate-rugulate, with two ± psilate areas opposite each other	Finely, densely granular	?
Polyalthia pendula	43	Verrucate	Finely, densely granular	2
Polyalthia perrieri	30	Verrucate(-rugulate)	Finely, densely granular	2
Polyalthia sambiranensis[1]	-	Verrucate-rugulate	-	-
Polyalthia stuhlmannii[2]	-	-	Finely, densely granular	1?
Polyalthia tanganyigensis	32	Verrucate(-rugulate)	Finely, densely granular	1?
Hubera sp. 2	43	Weakly coarsely rugulate to fossulate	Finely, densely granular	2
Hubera sp. 6	46	± Fossulate-perforate	Finely, densely granular	?
Hubera sp. 7	43	± Fossulate-perforate	Finely, densely granular	2
Hubera sp. 8	43	± Fossulate-perforate	Finely, densely granular	1?

[1](Schatz & Le Thomas 1990); [2](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 2x10^7 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 µm.

SEM (Fig. 3A, B, D, E, G, H): Exine ornamentation verrucate to rugulate to fossulate (-perforate). Two opposite, ± 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 ± 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,

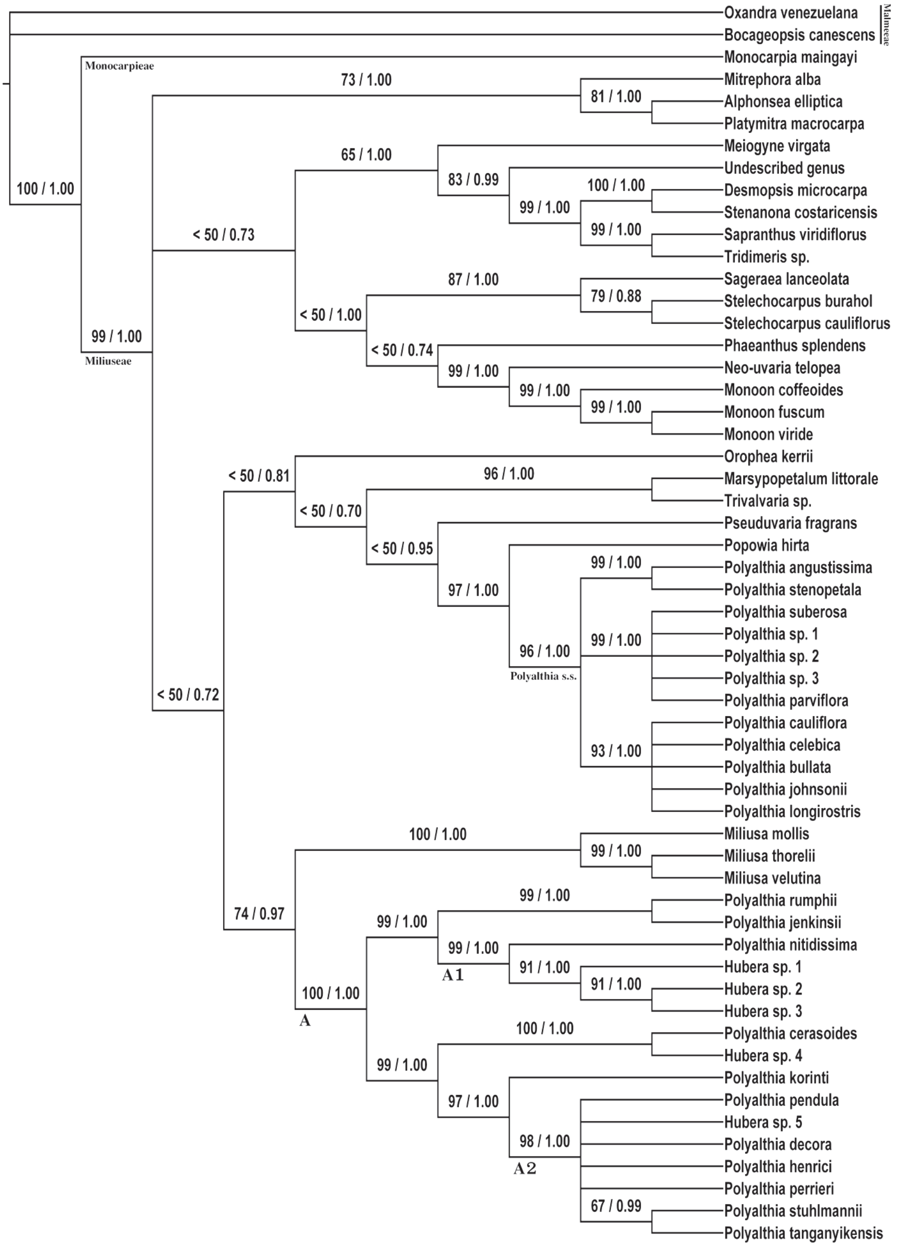

FIGURE 1. Bayesian posterior probability tree, with support indicated above branches: Bayesian posterior probabilities right of slash, maximum parsimony's symmetric resampling values left of slash.

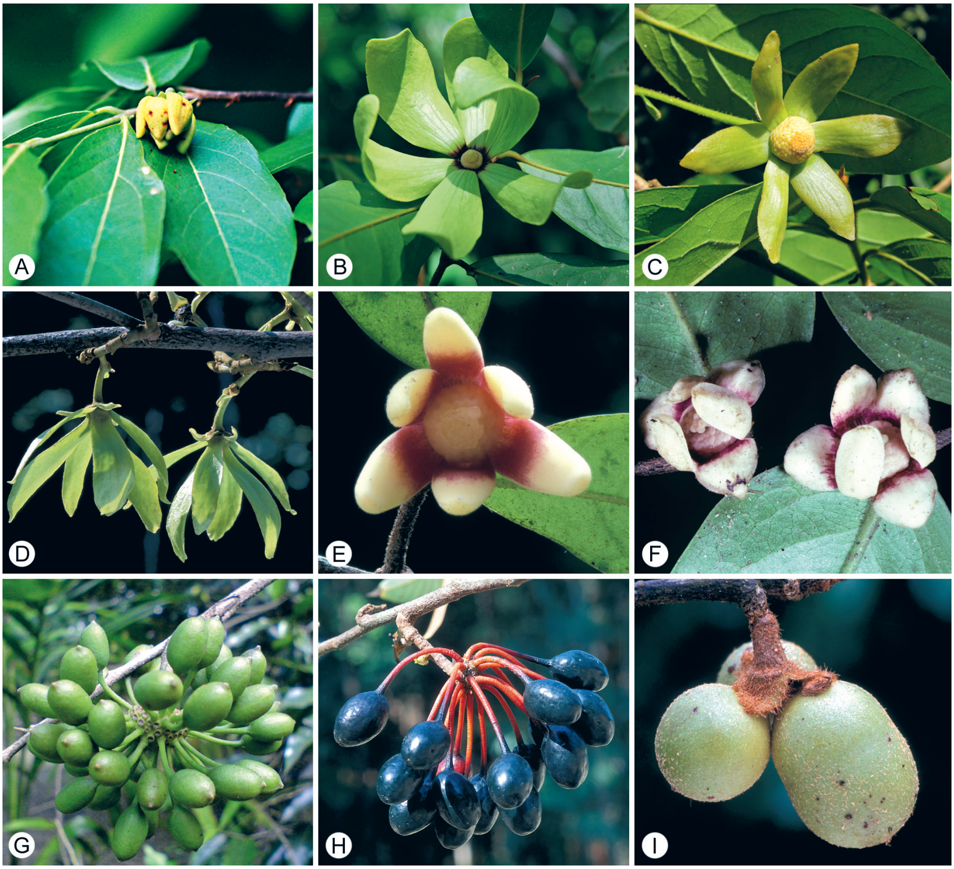

FIGURE 2. Flowers/fruits of representative species of clade A, *Monoon*, and *Polyalthia sensu stricto*. A–C, G. Clade A. A. *Polyalthia cerasoides*. B, G. *Polyalthia jenkinsii*. C. *Hubera* sp. 4. D, H. *Monoon*. *Monoon* sp. E, F, I. *Polyalthia sensu stricto*. E, I. *Polyalthia parviflora*. F. *Polyalthia 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 ± 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 ± columellate) infratectum and tubular exintine. Scale bars: 10 µm (D, G, M), 5 µm (A, J), 1 µm (B, C, E, F, H, I, K, L, N, O). Abbreviations: b, basal layer; en, endintine; ex, exintine; i, infratectum; t, tectum.

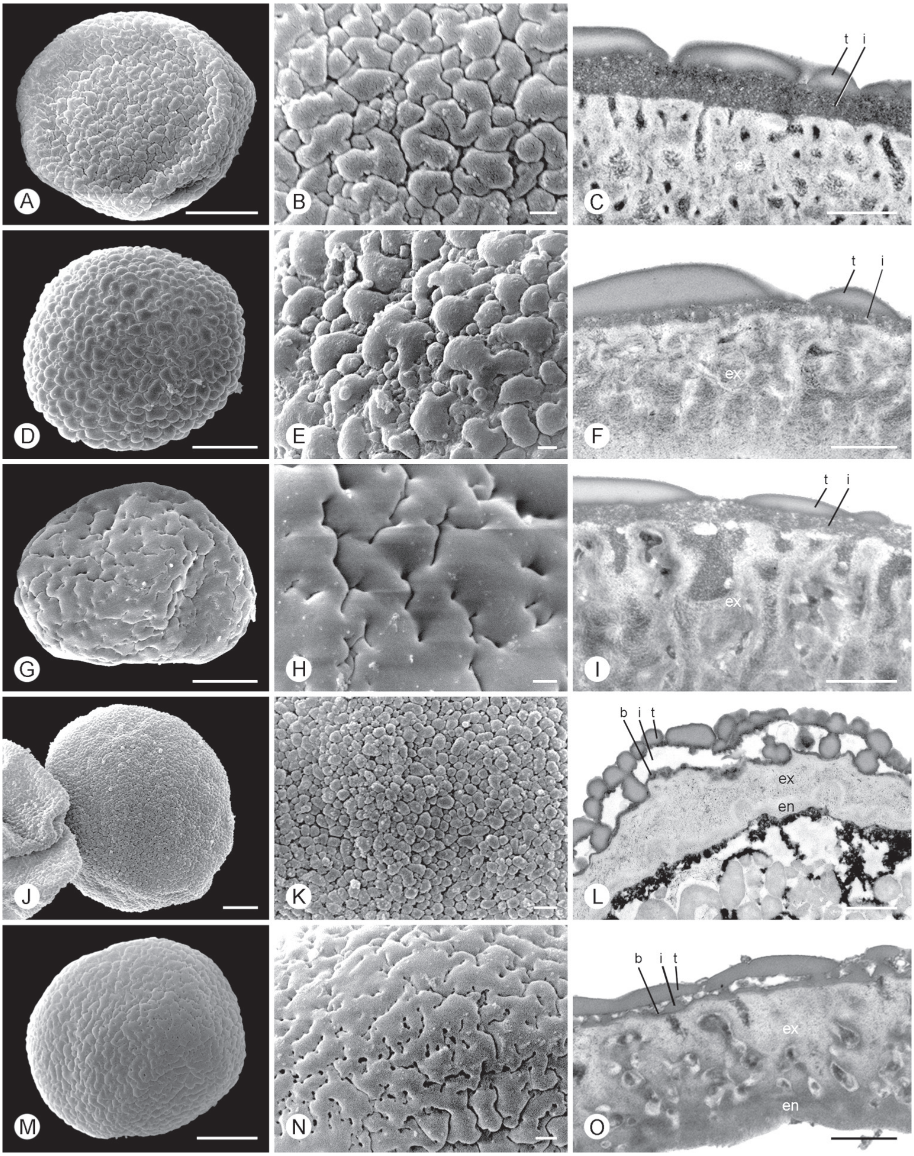

FIGURE 3. Pollen of representative species of clade A, *Monoon*, and *Polyalthia sensu stricto*.; SEM (columns 1 and 2) and TEM (column 3). A–I. Clade A. A–C. *Polyalthia cerasoides* (A, B, *Vajravelu 36762*; C, *Larsen et al*. *33731*). A. Pollen grain. B. Detail showing verrucate(-rugulate) ornamentation. 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) ± 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 ± 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	*Monoon*	*Polyalthia sensu stricto*
- 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 ± (sub) cordate; generally ± 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	± Columellate or coarsely granular	± 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

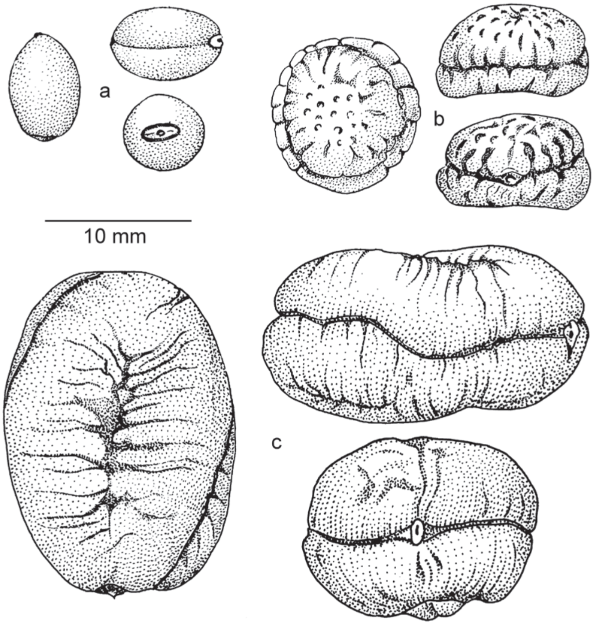

FIGURE 4. Seeds of representative species of clade A, *Monoon*, and *Polyalthia sensu stricto*. Clade A. a. *Polyalthia cerasoides*. *Monoon*. c. *Monoon fuscum*. *Polyalthia sensu stricto*. b. *Polyalthia subcordata*. All reproduced from Van Setten & Koek-Noorman (1992).

it is ± 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 ± 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 ≥ 40 per flower, apex of anther connective flat-topped, covering the thecae. Carpels generally ≥ 10 per flower, cylindrical or occasionally flask-shaped, peripheral ones sometimes slightly incurved; stigmas ± globose, (ellipsoid-)obovoid, ellipsoid(-ovoid) or ellipsoid-cylindrical, generally ± hairy; ovaries hairy, 1 ovule per ovary, sub-basal. Torus (nearly) flat or (slightly) raised with flat-topped (or with rarely rounded) apex, generally ± hairy, often ± 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 ± obovoid, apex sometimes (slightly) apiculate. Seed per monocarp 1, (sub)globose, ellipsoid, cylindrical or rarely ± 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[1]).

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[1] 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/disulculate 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.

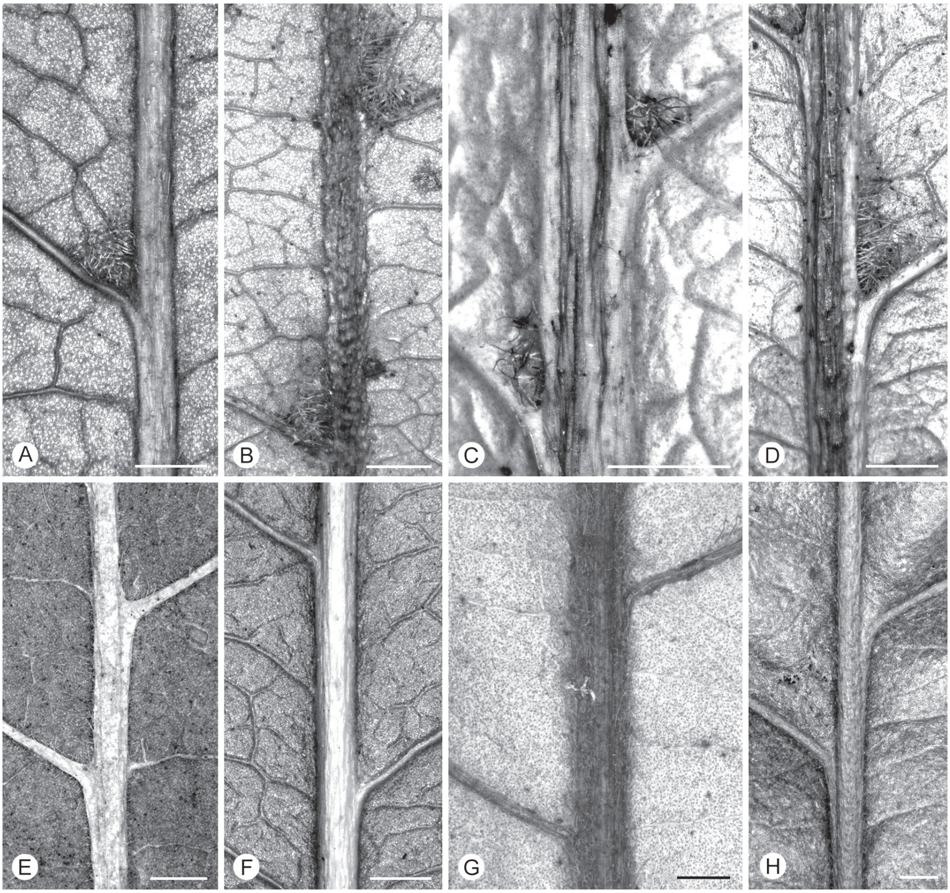

FIGURE 5. Lower leaf surface of representative species of *Hubera* with domatia (A–D) and *Hubera* (E, F), *Monoon* (G), and *Polyalthia sensu stricto* (H) without domatia. A. *Hubera nitidissima* (*Forster PIF 28246*). B. *Hubera vitiensis* (*Smith 8203*). C. *Hubera korinti* (*Kostermans 24916*). D. *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 (± 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 - Voucher, location (herb.)	Molecular phylogenetic study (GenBank accession numbers)					
	rbcL	*trnLF*	*matK*	*ndhF*	*psbA-trnH*	*ycf1*
Alphonsea elliptica						
- *Van Balgooy 5141*, Bogor Bot. Gard. (L)	AY318966	AY319078	AY519907	JQ690401	JQ690402	JQ690403
Bocageopsis canescens						
- *Maas et al. 9243*, Brazil (U)	JQ690407	JQ690408	JQ690409	JQ690410	JQ690411	JQ690412
Desmopsis microcarpa						
- *Chatrou et al. 85*, Costa Rica (U)	AY319059	AY319173	AY518804	JX544771	AY841461	JX544758
Hubera amoena						
- *Smith 6423*, Fiji (BISH)$^{\#*}$						
Hubera capillata						
- *Smith 4581*, Fiji (BISH)$^{\#*}$						
Hubera cerasoides						
- *Chalermglin 440214-4*, Thailand (L)$^{\#}$	AY319017	AY319131	AY518854	JQ889985	JQ889980	JQ889975
- *Larsen et al. 33731*, Thailand (L) {LM, SEM, TEM}						
- *Maxwell 90-744*, Thailand (L)$^{\#}$						
- *Vajravelu 36762*, India (L)$^{\#}$ {LM, SEM}						
Hubera decora						
- *Phillipson 2971*, Madagascar (WAG)$^{\#}$	---	JX544869	---	JX544879	JX544859	JX544849
Hubera forbesii						
- *Forbes 836* [B (photograph)]$^{\#*}$						
- *Kostermans 2787*, New Guinea (L)$^{\#}$ {LM, SEM, TEM}						
Hubera gracilis						
- *Versteeg 1408* [B (photograph)]$^{\#*}$						
Hubera henrici						
- *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)					
	rbcL	*trnLF*	*matK*	*ndhF*	*psbA-trnH*	*ycf1*
Hubera hirta						
- *Zippelius s.n.* [B (photograph)]$^{\#*}$						
Hubera jenkinsii						
- *Chaowasku 60*, Thailand (L)$^{\#}$	---	JX544803	---	JX544842	JX544812	JX544821
- *Gardner & Sidisunthorn ST 0974* (L)$^{\#}$						
- *Geesink et al. 7761*, Thailand (L)$^{\#}$ {LM, SEM, TEM}						
Hubera korinti						
- *Ratnayake 2/03*, Sri Lanka (HKU)	---	EU522179	---	JX544877	EU522124	JX544847
- *Kostermans 24580*, Sri Lanka (L)$^{\#}$ {LM, SEM, TEM}						
- *Kostermans 24916*, Sri Lanka (L)$^{\#}$						
- *Kostermans 25503*, Sri Lanka (L)$^{\#}$						
- *Kostermans 27618*, Sri Lanka (L)$^{\#}$						
Hubera leptopoda						
- *Ledermann 6585* [B (photograph)]$^{\#*}$						
Hubera loriformis						
- *Gillespie 3639*, Fiji (BISH)$^{\#*}$						
- *Greenwood 1125*, Fiji (BISH)$^{\#}$						
- *Parham 7182*, Fiji (BISH)$^{\#}$						
- *Smith 601*, Fiji (BISH)$^{\#}$						
Hubera mossambicensis						
- *Torre & Correia 17062*, Mozambique (P)$^{\#}$						
Hubera nitidissima						
- *Ford AF 4967*, Australia (L)	---	JQ889988	JQ889989	JQ889986	JQ889981	JQ889976
- *Forster PIF 28246*, Australia (L)$^{\#}$ {SEM, TEM}						
- *Gray 1959*, Australia (L)$^{\#}$						
Hubera pendula						
- *Rabevohitra 2386*, Madagascar (WAG)$^{\#}$ {LM, SEM, TEM}	---	AY319144	AY518852	JQ889987	JQ889982	JQ889977

APPENDIX 1 (continued).

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

APPENDIX 1 (continued).

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

APPENDIX 1 (continued).

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

APPENDIX 1 (continued).

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

APPENDIX 1 (continued).

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

herb. = herbarium