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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 (≥ 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.

Key words: 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; *Miliusa* 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 ≥ 85%, 70–84%, and ≤ 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 1000th 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 ≥ 0.96, 0.91–0.95, and ≤ 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 *Stelechocapus 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 (± 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. ± colu-

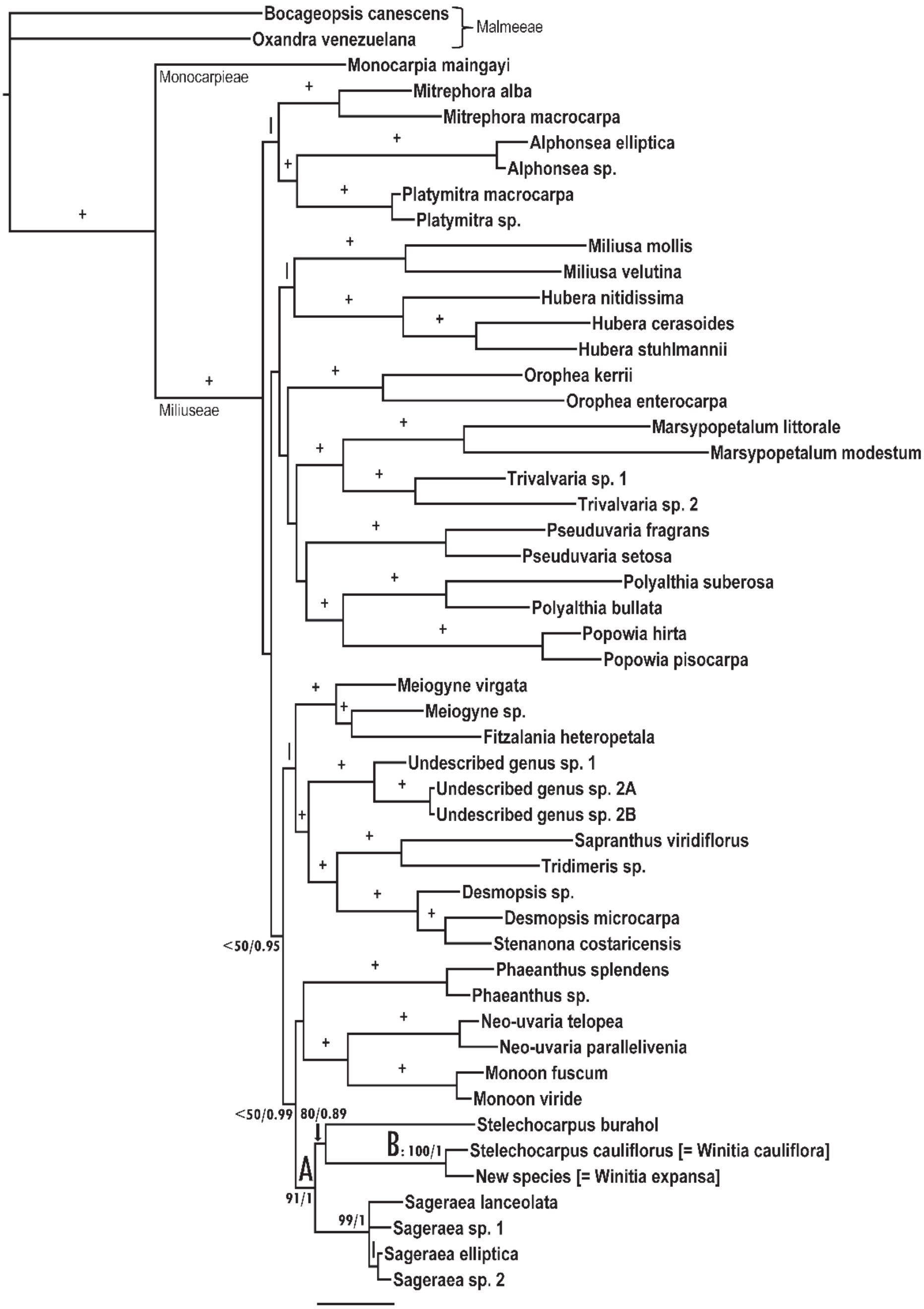


FIGURE. 1. 50% majority-rule consensus phylogram derived from Bayesian analysis of combined seven plastid markers. Clade support: left of slash – parsimony symmetric resampling values (SR) corresponding to clades recovered in Bayesian tree, right of slash – Bayesian posterior probabilities (PP); + = 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	*Stelechocarpus burahol*	Clade B (= *Winitia gen. nov.*)	*Sageraea*
- Secondary veins on lower leaf surface	Prominent	Prominent	± Indistinct[a]
- Upper surface of leaf midrib	(Slightly) raised	(Slightly) raised	Slightly sunken[a]
- Petal color	Usually green to yellow/cream[b]	Usually ± rose-colored	White to yellow/cream to reddish(-pink) to purple to brown[a]
- 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 *Sageraea elliptica*)
- 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)[a]
- Stigma shape	Bilobed (± heart-shaped)	Multicolumellar [5–9(–10) columns per stigma]	One (small) orbicular lobe, bilobed, cylindrical or capitate[a]
- Male torus	(Conical-)cylindrical	± Hemispheroid	Nearly flat (observed only for *Sageraea elliptica*)
- Seed raphe	Slightly grooved on a (slight) ridge	(Slightly) ridged	(Flat to) ± grooved[c]
- 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

[a](Van Heusden 1997a); [b](Van Heusden 1995); [c](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].

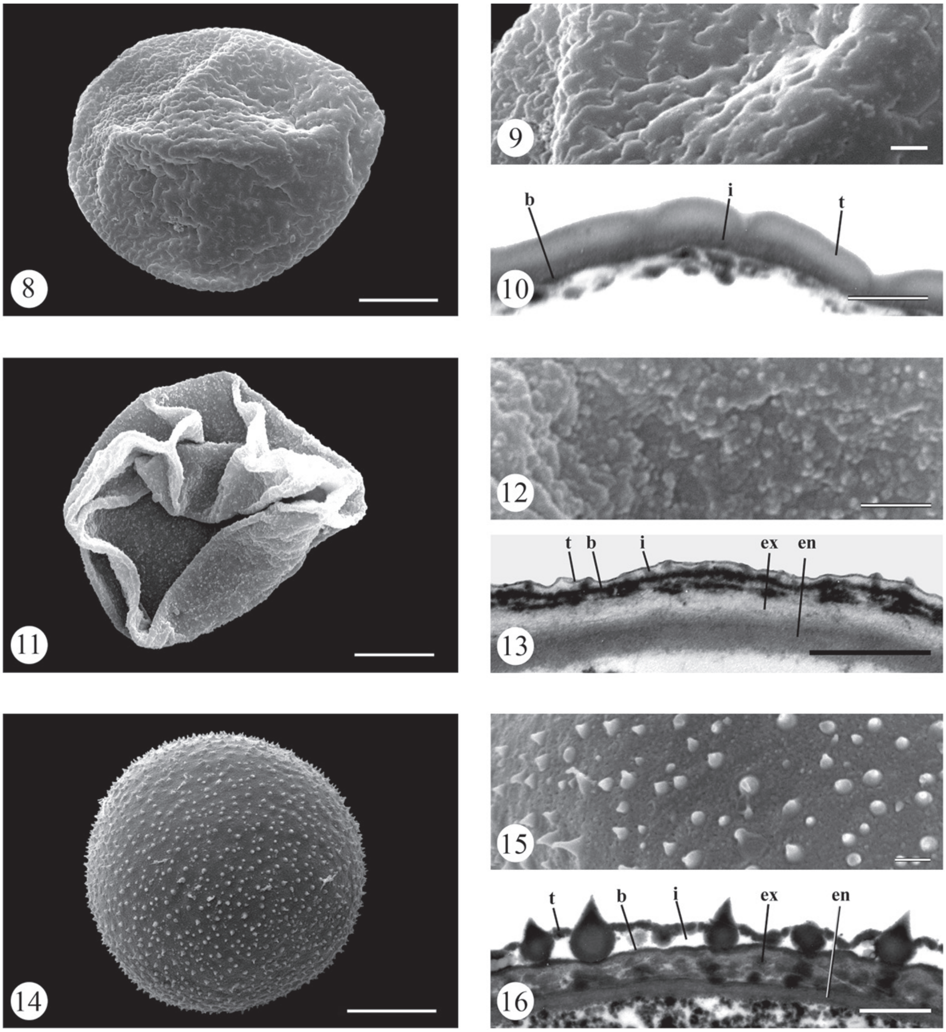
Discussion

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

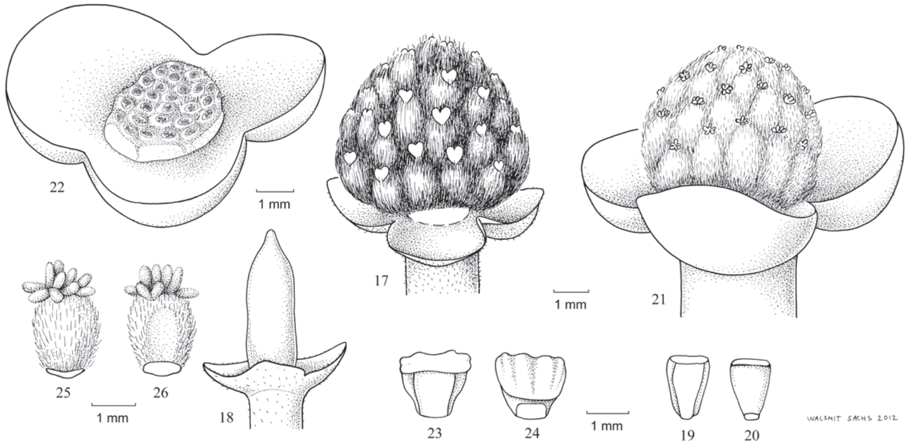
FIGURES 2–7. Flowers of *Stelechocarpus burahol* (2, 3), clade B (= *Winitia gen. nov.*), and *Sageraea*. 4, *Winitia cauliflora comb. nov*. (= *Stelechocarpus cauliflorus*). 5, 6, *Winitia expansa sp. nov*. 7, *Sageraea bracteolata*. (Photographs: 2, 3 = ©C. Pradubpet; 4 = ©N. Panitvong; 5, 6 = ©T. 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 ± 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 ± indistinct secondary veins on the lower leaf surface of *Sageraea*. In Miliuseae, multicolumellar stigmas (≥ 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 supratectal 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 supratectal 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 (± 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 ± 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 & Phengklai 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 infratectum (Fig. 10). In Miliuseae, such an infratectum 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 ± columellate/coarsely granular infratectum (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 ± thick leaves and the biseriately 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; ± 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 ± columellate/coarsely granular (Chaowasku *et al*. unpublished data[1]).

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 ± (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

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[1] = 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 ± the midpoint) of pedicels; perianths of male and female flowers same shape and ± same size. Sepals (broadly) triangular-ovate, ± semicircular, or shortly linguiform; ± connate. Petals usually ± 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 ± broadened, seldom reduced. Carpels 26–32 per female flower, ± 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 ± 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, ± subglobose (collapsed in Fig. 11), apolar, longest axis ca. 18–19 µm, exine ornamentation ± scabrate-microgemmate; TEM (Fig. 13) – Exine inaperturate, tectate, ca. 0.24 µm thick, tectum very thin, supratectal elements present, short, infratectum ± 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 acetotysis.

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 ≥ 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.

Holo- Iso- Lecto- Syn- **TYPE**

Winitia expansa Chaowasku

Winitia expansa Chaowasku

det.
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BKF ROYAL BOTANIC GARDENS KEW **Field Guide to Trees of Southern Thailand** DARWIN INITIATIVE TOYOTA

Family ANNONACEAE **SpNo ST1183**

Species Stelechocarpus sp. cauliflorus

Province Phatthalung **District** Si Ban Phot **Lat.** 7°40' **Long.** 99°52'

Location Khao Pu/Khao Ya National Park, headquarters, Pha Phung Cliff. **Alt.** 120 m

Ecology Understorey of evergeen/deciduous forest at base of limestone cliff.

Habit Tree **Hgt** 11 m **DBH** 24 cm

Notes CROWN: monopodial; branching horizontal; trunk knobbly, swollen at base. BARK: dark grey-brown, roughened and fissured; inner bark cream to pale orange. FLOWER: from swollen base of trunk; tepals dark pink; centre of flower paler pink.

NOTE: same species as ST0817, from Klong Pranom, Surathani.

Collector S.Gardner & P.Tippayasri **Date** 02-Dec-2004

Duplicate 3 **Other material** spirit, drawing, photo **Det by**

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 *Stelechocarpae* 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), ± 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, ± flask-shaped or slightly ovoid, a bit bending inward; stigmas bilobed, ± 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 ± shallowly verruculose, sometimes verruculae scale-like. Seeds 2–6 per monocarp, ellipsoid or ± 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 µm, exine ornamentation verrucate-scabrate to rugulate to fossulate(-perforate); TEM (Fig. 10) – Exine inaperturate, tectate, ca. 0.40 µm thick, tectum considerably thick, supratectal elements absent, infratectum 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 infratectum 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 infratectum.

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).

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	AY518807	JQ690401	JQ690402	JQ690403
***Alphonsea* sp.** - *Keßler PK 3186*, Thailand (TISTR, Bangkok)	---	AY319082	AY518808	JQ690404	JQ690405	JQ690406
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
***Desmopsis* sp.** - *Rainer 1593*, Mexico (WU)	---	AY841701	KC857552*	KC857553*	KC857554*	KC857555*
Fitzalania heteropetala - *Kemp TH 7267*, Australia (L)	---	KC857556*	KC857557*	KC857558*	KC857559*	KC857560*
Hubera cerasoides - *Chalermglin 440214-4*, Thailand (L)	AY319017	AY319131	AY518854	JQ889985	JQ889980	JQ889975
Hubera nitidissima - *Ford AF 4967*, Australia (L)	---	JQ889988	JQ889989	JQ889986	JQ889981	JQ889976
Hubera stuhlmannii - *Luke & Robertson 1424*, Kenya (K)	---	AY319149	AY518853	JX544882	JX544862	JX544852
Marsypopetalum littorale - *Rastini 153*, Bogor Bot. Gard. (L)	AY319026	AY319140	AY518835	JX544827	JX544804	JX544813
Marsypopetalum modestum - *Keßler PK 3192*, Thailand (L)	AY318980	AY319092	AY518834	KC857561*	KC857562*	KC857563*
Meiogyne virgata - *Keßler PK 2751*, Borneo (L)	AY318982	AY319094	AY518798	JX544769	JX544784	JX544756

APPENDIX 1 (continued).

Taxon - Voucher, location (herb.)	Molecular phylogenetic study (GenBank accession numbers)					
	rbcL	*trnLF*	*matK*	*ndhF*	*psbA-trnH*	*ycf1*
***Meiogyne* sp.** - *Gardner et al. ST 2014*, Thailand (L)	KC857564*	KC857565*	KC857566*	KC857567*	KC857568*	KC857569*
Miliusa mollis - *Keßler PK 3207*, Thailand (L)	---	AY319102	AY518851	JQ690503	JQ690504	JQ690505
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
Mitrephora macrocarpa - *Mols 8*, Bogor Bot. Gard. (L)	---	AY319107	AY518859	JQ889984	JQ889979	JQ889974
Monocarpia maingayi - *Kaewruang 1*, Thailand (L)	JQ690395	JQ690396	JQ690397	JQ690398	JQ690399	JQ690400
Monoon fuscum - *Keßler PK 3222*, Thailand (L)	AY318973	AY319085	AY518787	JX544779	JX544792	JX544767
Monoon viride - *Chalermglin 440214-3*, Thailand (L)	AY319040	AY319154	AY518784	JX544780	JX544793	JX544768
Neo-uvaria telopea - *Chaowasku 77*, Thailand (L)	JX544755	JX544783	JX544751	JX544778	JX544791	JX544766
Neo-uvaria parallelivenia - *Keßler sub IV-H-73*, Bogor Bot. Gard. (L)	AY319000	AY319113	AY518794	KC857570*	KC857571*	KC857572*
Orophea enterocarpa - *Chalermglin 440403*, Thailand (TISTR, Bangkok)	AY319006	AY319119	AY518815	JQ690416	JQ690417	JQ690418
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

APPENDIX 1 (continued).

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

APPENDIX 1 (continued).

Taxon - Voucher, location (herb.)	Molecular phylogenetic study (GenBank accession numbers)					
	rbcL	*trnLF*	*matK*	*ndhF*	*psbA-trnH*	*ycf1*
Sageraea lanceolata						
- *Ridsdale DV-M2-1692*, Borneo (L)	AY319050	AY319164	AY518799	JX544774	JX544787	JX544762
- *Ambriansyah & Arbainsyah AA 1673*, Borneo (L) {SEM, TEM}						
***Sageraea* sp. 1**						
- *Slik 3868*, Borneo (L)	---	KC857591*	KC857592*	KC857593*	KC857594*	KC857595*
***Sageraea* sp. 2**						
- *Gardner & Sidisunthorn ST 1006*, Thailand (L)$^{\#}$	---	KC857596*	KC857597*	KC857598*	KC857599*	KC857600*
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
- *Achmad 1036*, Sumatra (L)$^{\#}$						
- *De Wilde & De Wilde-Duyfjes 19866*, Sumatra (L)$^{\#}$						
- *Dransfield 2542*, Java (L)$^{\#}$						
- *Forbes 1604a*, Sumatra (L)$^{\#}$						
- *Kostermans s.n.*, Java (L)$^{\#}$						
- *Lörzing 5585*, Sumatra (L)$^{\#}$						
- *Lörzing 11332*, Sumatra (L)$^{\#}$ {SEM, TEM}						
- *Pradubpet 1*, Thailand (L)$^{\#}$						
- *S 40553*, Borneo (L)$^{\#}$						
- *Unknown 7692*, Bogor Bot. Gard. (L)$^{\#}$						
- *Van Balgooy 5249*, Bali (L)$^{\#}$						
- *Van Steenis 5706*, Sumatra (L)$^{\#}$						
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

APPENDIX 1 (continued).

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

herb. = herbarium