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**Phylogenetic and taxonomic studies in *Macaranga*,
Mallotus and other acalyphoid genera (Euphorbiaceae s.s.)**

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MOLECULAR PHYLOGENY OF *MACARANGA*, *MALLOTUS*, AND RELATED GENERA (EUPHORBIACEAE S.S.): INSIGHTS FROM PLASTID AND NUCLEAR DNA SEQUENCE DATA¹

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SUMMARY

Macaranga and *Mallotus* (Euphorbiaceae s.s.) are two closely related, large paleo(sub)tropical genera. To investigate the phylogenetic relationships between and within them, and determine the position of related genera belonging to the subtribe Rottlerinae, one plastid (*trnL-F*) and three nuclear (ITS, *ncpGS*, *phyC*) markers were sequenced for a representative species sample of these genera. The analyses demonstrated the monophyly of *Macaranga* and the paraphyly of *Mallotus* and revealed three highly supported main clades. The genera *Cordemoya* and *Deuteromallotus* and the *Mallotus* sections *Hancea* and *Oliganthae* form a basal *Cordemoya* s.l. clade. The two other clades, the *Macaranga* clade and the *Mallotus* s.s. clade (the latter with *Coccoceras*, *Neotrewia*, *Octospermum* and *Trewia*) are sister groups. In the *Macaranga* clade two basal lineages (comprising mostly sect. *Pseudorottlera*) and a crown group with three geographically homogenous main clades were identified. The phylogeny of the *Mallotus* s.s. clade is less clear because of internal conflict in all four datasets. Many of the sections and informal infrageneric groups of *Macaranga* and *Mallotus* do not appear to be monophyletic. In both the *Macaranga* and *Mallotus* s.s. clades the African and/or Madagascan taxa are nested in Asian clades, suggesting migrations or dispersals from Asia to Africa and Madagascar.

Key words: Afro-Asian distribution, biogeography, *Cordemoya*, Euphorbiaceae, *Macaranga*, *Mallotus*, molecular phylogenetics, Rottlerinae.

INTRODUCTION

Macaranga and *Mallotus* are two paleo(sub)tropical genera of shrubs, trees, and, exceptionally, woody climbers in the angiosperm family Euphorbiaceae s.s. (uniovulate Euphorbiaceae, Wurdack et al. 2005). These large genera (with c. 260 species in *Macaranga*, and c. 150 in *Mallotus*; Whitmore, in press; Radcliffe-Smith, 2001) have a wide habitat range from primary forest understorey to heavily disturbed sites, and from swamp forests to montane forests. They are characteristic components of secondary forests in Southeast Asia, and can be used as indicators of various levels of forest disturbance (Slik et al., 2003a). Moreover, both genera show an array of interesting morphological features, most striking undoubtedly being the ant-housing adaptations

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of myrmecophytic *Macaranga* species (Ridley, 1910). To deepen the knowledge on the evolution of these important ecological and morphological traits, a robust hypothesis about the phylogenetic relationships between and within these two genera is needed. The phylogeny will also help to draw conclusions about the biogeography and taxonomic delimitations in *Mallotus* and *Macaranga*.

In the Euphorbiaceae classifications of Webster (1994b) and Radcliffe-Smith (2001), *Macaranga* and *Mallotus* are placed in the tribe Acalyphaeae of the uniovulate subfamily Acalyphoideae. *Mallotus* is a member of the subtribe Rottlerininae, together with 7 other genera (Table 2.1; additionally, the New World genus *Avellanita* was included by Radcliffe-Smith, 2001), whereas *Macaranga* is placed in the monogeneric subtribe Macaranginae. This classification implies that *Macaranga* and *Mallotus* are clearly distinct, well-separated genera. Morphologically, however, these genera are

Table 2.1. Distribution and some typical morphological characters for *Mallotus*, other genera in the subtribe Rottlerininae, and *Macaranga* (Webster, 1994b). The number of known species per genus and the number of sampled species is given in parentheses following the generic name.

Genus (spp. total / sampled)	Distribution	Typical morphological characters
<i>Coccoceras</i> Miq. (4 / 1)	Myanmar to Borneo	fruits indehiscent and often with horn-like processes (otherwise resembling <i>Mallotus</i>)
<i>Cordemoya</i> Baill. (1 / 1)	Mascarene Is.	conspicuous glandular hairs absent
<i>Deuteromallotus</i> Pax & K.Hoffm. (3 ^a / 3)	Madagascar	conspicuous glandular hairs absent
<i>Macaranga</i> Thouars. (c. 260 / 57)	Africa (26 spp.), Madagascar (10 spp.), Mascarene Is. (1 sp.), Asia to West Pacific	conspicuous glandular hairs present, indument always simple, anthers 3-4-locular
<i>Mallotus</i> Lour. (c. 150 / 31)	Africa & Madagascar (2 spp.), Asia to West Pacific	conspicuous glandular hairs usually present, indument often tufted or stellate ^b , anthers 2-locular
<i>Neotrewia</i> Pax & K.Hoffm. (1 / 1)	Borneo, Sulawesi, Philippines	fruits indehiscent, 1-locular (otherwise resembling <i>Mallotus</i>)
<i>Octospermum</i> Airy Shaw (1 / 1)	New Guinea	fruits indehiscent, 7-9-locular (otherwise resembling <i>Mallotus</i>)
<i>Rockinghamia</i> Airy Shaw (2 / 2 ^c)	Australia (Queensland)	leaves pseudoverticillate, inflorescences regularly bisexual, styles often bifid
<i>Trewia</i> L. (2 / 1)	India to West Malesia	fruits indehiscent, 3-5-locular (rarely dehiscent and 2-locular; otherwise resembling <i>Mallotus</i>)

Notes:

^a: Including *Mallotus spinulosus* McPherson, a species which has never been formally a member of *Deuteromallotus*, but which is clearly part of this group.

^b: Stellate in broad sense; micromorphological studies (Ž. Fišer, Nationaal Herbarium Nederland, unpublished data) have shown that the seemingly stellate hairs in many *Mallotus* species are, in fact, tufted hairs.

^c: Only sampled for the pilot study (see Materials & Methods).

very similar. Both genera, with few species excepted, possess conspicuous, usually colorful, glandular hairs (also called glandular scales). This character is rare within Euphorbiaceae, and might indicate a common origin for *Macaranga* and *Mallotus*. Furthermore, the only clear-cut difference between them is the number of locules in the anthers (2 in *Mallotus*, 3 or 4 in *Macaranga*).

The seven genera classified with *Mallotus* in the subtribe Rottlerinae (Table 2.1) each only contain 1–5 species. Most of them show close affinities with *Mallotus*, and have been previously treated as congeneric with it. Airy Shaw (1963) already considered *Coccoceras* to belong to *Mallotus*; this view was later morphologically confirmed in the revision of *Mallotus* sect. *Polyadenii* (Bollendorff et al., 2000). Similarly, the Madagascan genus *Deuteromallotus* has been considered congeneric with *Mallotus* (McPherson, 1995). Three Asiatic genera, *Neotrewia*, *Octospermum* and *Trewia*, resemble *Mallotus* closely: they also possess the glandular hairs, and practically the only deviating characters are the fruit type (indehiscent instead of the typically dehiscent capsule of *Mallotus*) and, except in *Trewia*, the number of locules per ovary (Kulju et al., 2007, Chapter 3).

The phylogenetic relationships between *Macaranga*, *Mallotus* and related genera are poorly understood. In a molecular phylogenetic study of the Euphorbiaceae s.s. (Wurdack et al., 2005) *Macaranga*, *Mallotus*, and *Trewia* form a well supported clade, which is sister to *Blumeodendron* (tribe Pycnosomeae). Further, one of the Rottlerinae genera, the Australian *Rockinghamia*, was shown to be unrelated to this clade. However, only limited conclusions can be drawn from this study because of the limited taxon sampling (only 1 or 2 species were sampled from each of the above-mentioned genera, and no other Rottlerinae taxa were included).

Although several studies have focused on the phylogeny of the myrmecophytic *Macaranga* species (Blattner et al., 2001; Davies et al., 2001; Bänfer et al., 2004), only one study tried to clarify the relationship between *Mallotus* and *Macaranga* (Slik & Van Welzen, 2001a). According to this morphological phylogenetic study, *Mallotus* is paraphyletic because of two reasons. First, *Mallotus* sections *Hancea* and *Oliganthae* (Table 2.2) are separated from the rest of the genus by some of the outgroup taxa. Second, *Macaranga* is nested within *Mallotus*, being sister to *Mallotus* sect. *Mallotus*. This study is, however, hampered by a couple of shortcomings. First and foremost, the taxon sampling was limited: only 3 *Macaranga* species, and none of the small genera related to *Mallotus*, were included. Second, the resulting phylogeny was poorly supported, perhaps because of a low characters/taxa ratio (76/50) and a high number of polymorphic characters (50 out of 76). Thus, a comprehensive study of the phylogeny of this interesting plant group is clearly needed before the drastic taxonomic rearrangements suggested by the morphological study (e.g., merging *Macaranga* and *Mallotus*) can be executed (as was already concluded by Slik & Van Welzen, 2001a).

Macaranga, *Mallotus*, and related small genera show an intriguing distribution pattern (Tables 2.1 and 2.2): the group is Asia-centered — most species occurring in an area from the Indian subcontinent through the Malay Archipelago (Malesia) to Australia and the southwest Pacific — but there are several *Macaranga* and a few *Mallotus* species in Africa, Madagascar and the Mascarene Islands as well. Furthermore, the small genera *Cordemoya* and *Deuteromallotus* are endemic to the Mascarene Islands and Madagascar, respectively. This kind of distribution could be explained with various biogeographical

Table 2.2. Distribution, number of known species, and number of species sampled for the infrageneric groups of *Macaranga* and *Mallotus* (Airy Shaw, 1968; Whitmore, in press). The groups occurring on the western side of the Indian Ocean are shown underlined.

Genus	Distribution	Spp. total	Spp. sampled
<i>Macaranga</i>			
“African” ^a	<u>Africa</u>	26	9
Angustifolia	From Sulawesi to New Guinea and Australia	13	1
Bicolor	From Thailand to Philippines	6	2
Brunneofloccosa	Sulawesi (1 sp.) and New Guinea	20	2
Conifera	India to Sulawesi	5	2
Coriacea	New Caledonia	6	1
Denticulata	India to Sumatra and Java	6	3
Dioica	Sulawesi to New Guinea, Australia, Vanuatu and Micronesia	24	5
Gracilis	New Guinea	7	1
Javanica	From southern China and Thailand to Sulawesi and Philippines	13	1
Longistipulata	From Sulawesi and Philippines to New Guinea	19	3
Mappa	From Sulawesi and Philippines to New Guinea and Oceania	21	1
<u>Mauritiana</u>	<u>Mauritius</u>	1	1
<u>Oblongifolia</u>	<u>Madagascar, Comoros</u>	10	5
sect. <i>Pachystemon</i>	From Nicobars and Indochina to Borneo and Philippines	25	4
sect. <i>Pruinosae</i>	From Burma, Andamans and Nicobars to Borneo and Sulawesi	9	3
sect. <i>Pseudorottlera</i>	From India to New Guinea and Australia	15	6
Tanarius	From Sulawesi to Fiji, Samoa and Tonga	14	4
Winkleri	Borneo	2	1
<i>Mallotus</i>			
sect. <i>Axenfeldia</i>	Asia	≥17 ^b	3
sect. <i>Hancea</i>	From Southern China to New Guinea	12(+5) ^c	3(+2) ^c
sect. <i>Mallotus</i>	From India to Australia and Solomon Islands	c.10	4
sect. <i>Oliganthae</i>	From Burma to Borneo and Java	1	1
sect. <i>Philippinenses</i>	From Pakistan to Australia and New Caledonia	5(+3) ^d	4(+1) ^d
sect. <i>Polyadenii</i>	From India to Australia and Solomon Islands	8	1
<u>sect. <i>Rottleropsis</i></u>	Asia, <u>Africa (2 spp.)</u>	≥40 ^b	9
sect. <i>Stylanthus</i>	From India to Australia and Solomon Islands	6	3

Notes:

^a: African *Macaranga* species were not designated to groups (Whitmore, in press).

^b: sections poorly known in parts of continental Asia

^c: in parentheses the species excluded from sect. *Hancea* (Slik & Van Welzen, 2001b).

^d: in parentheses species related to *M. chromocarpus*, a species excluded from sect. *Philippinenses* (Sierra et al., 2005).

scenarios (e.g., vicariance following the break-up of the super-continent Gondwana or dispersal/migration from the ancestral distribution area). As the first step to resolve these biogeographical questions, the phylogenetic relationships between species occurring at western and eastern sides of the Indian Ocean need to be investigated.

For both *Macaranga* and *Mallotus*, infrageneric classifications exist (Table 2.2), but they are far from satisfactory, and not based on a phylogenetic framework. In their revision of *Macaranga*, Pax & Hoffmann (1914; 1919; 1931) divided the genus rather artificially into 36 sections. Their circumscription was criticized by Airy Shaw (1969; 1971), and recently Davies suggested, based on a phylogenetic analysis, new delimitations for the sections *Pachystemon* and *Pruinosae* (Davies, 2001; Davies et al., 2001). The genus *Macaranga* was Whitmore's long-time research subject (e.g., 1965; 1969; 1980), but the monograph of it could not be finished during his life time. However, in the manuscript, being published as a *prodromus* (Whitmore, in press), a new subdivision of *Macaranga* is presented. Apart from three previously clearly established sections *Pachystemon*, *Pruinosae*, and *Pseudorottlera*, Whitmore could not classify all species in proper sections, but instead recognized 15 "natural species groups" (*Gestalt* groups). These preliminary groupings (for which diagnostic characters were not clearly given) have only a limited correspondence to the sections of Pax & Hoffmann.

The first sectional delimitations of *Mallotus* were made by Müller (1865; 1866; 5 sections) and by Pax & Hoffmann (1914; 10 sections). The classification was later refined by Airy Shaw (1968) to contain eight sections. This subdivision has been used, with slight modifications, as the basis for revisions of a part of the genus (Bollendorff et al., 2000; Slik & Van Welzen, 2001b; Sierra & Van Welzen, 2005; Sierra et al., 2005; Van Welzen & Sierra, 2006; Van Welzen et al., 2006; Sierra et al., 2007). Unfortunately, the infrageneric division is based on only a few, and sometimes dubious, characters. For example, a diverse group of opposite-leaved *Mallotus* species is divided into two sections only by the character of penninerved or tripli/palminerved leaves. In the morphological phylogenetic analysis (Slik & Van Welzen, 2001a) many of the sections were indicated to be non-monophyletic, but low levels of support prevented definitive conclusions to be made.

The aim of this study was to reconstruct the phylogeny of *Mallotus*, *Macaranga*, and related small genera in order to address the following questions:

(1) are *Macaranga* and *Mallotus* monophyletic, or is *Macaranga* nested within *Mallotus* as suggested by Slik & Van Welzen (2001a)?

(2) is the merging of *Coccoceras* and *Deuteromallotus* with *Mallotus* (see above) justified, and what is the phylogenetic position of the other small genera?

(3) what are the main infrageneric clades, and how do they relate to the infrageneric groupings of Airy Shaw (1968) and Whitmore (in press)?

(4) how are the species occurring in Africa, Madagascar and the Mascarene Islands related to those occurring in the Asia-Pacific, and what kind of biogeographical scenario could explain this Afro-Asian distribution pattern?

To answer these questions we have sequenced a representative taxon sample of these genera for four DNA markers, both from plastid and nuclear genomes. Two of the markers, plastid *trnL-F* (consisting of *trnL* intron and *trnL-F* spacer) and nuclear rDNA ITS have been commonly used to infer plant phylogenies at low taxonomic

levels (e.g., Kathriarachchi et al., 2006; Samuel et al., 2006), whereas two other markers are relatively novel fragments of low-copy number nuclear genes. The chloroplast-expressed glutamine synthetase gene (*nepGS*) plays a role in the nitrogen metabolism in chloroplasts and it has been shown to exhibit more sequence divergence than ITS between closely-related *Oxalis* species (Emshwiller and Doyle, 2002). The second low-copy number marker used in this study, a photoreceptor gene phytochromeC (*phyC*) has been used for the family-level phylogeny of the Phyllanthaceae, a family closely related to Euphorbiaceae s.s. (Samuel et al., 2005).

These four markers showed various levels of sequence divergence, and their analysis provided, in most parts, a robust phylogeny illuminating the evolution of this plant group, and providing a framework for taxonomic rearrangements and for further studies.

MATERIALS AND METHODS

Taxon sampling and outgroup choice—A pilot study was conducted to investigate whether all genera in the subtribe Rottlerinae (sensu Webster, 1994b) are, in fact, closely related to *Macaranga* and *Mallotus*. Representatives of these genera were sequenced for *rbcL* and/or *trnL-F* genes (data not shown). A maximum parsimony analysis with the large uniovulate Euphorbiaceae dataset (Wurdack et al., 2005) showed that all these taxa, except the genus *Rockinghamia*, form a well-supported clade, which is sister to the genus *Blumeodendron* (see Appendix 2.1). Therefore, *Rockinghamia* was excluded from subsequent analyses, and *Blumeodendron* selected as outgroup. Additional analyses of the individual gene datasets with more distant outgroup taxa (e.g., *Cleidion*; data not shown) were either cumbersome due to divergent, barely alignable outgroup sequences, or showed results highly similar to those presented here.

Taxon names, voucher information, and GenBank accession numbers of the samples used in this study are listed in the Appendix 2.2 (see also the number of species sampled per genus or infrageneric group in Tables 2.1 and 2.2). The taxon sampling includes nearly all satellite genera (except doubtfully included *Avellanita*), 57 species of *Macaranga* and 31 of *Mallotus*, covering all the species groups of *Macaranga* (Whitmore, in press) and the sections of *Mallotus* (Airy Shaw, 1968). All *Mallotus* species from Africa and Madagascar and a considerable sample of *Macaranga* species from these areas were sampled. For several species more than one specimen was sequenced to determine possible infra-specific variation.

Laboratory methods—Total DNA was extracted from leaf tissue using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). For silica-dried material manufacturer instructions were followed; for samples from herbarium specimens a modified protocol was used (a prolonged lysis step with proteinase K and β -mercaptoethanol added; Wurdack et al., 2004). Additionally, a few samples were extracted in collaborative laboratories with various other methods. Some of the herbarium specimen extracts were further diluted (10–100 \times) or cleaned with PCR cleaning columns (see below) to facilitate PCR.

trnL-F was amplified with primer pairs *c+d* (*trnL* intron) and *e+f* (*trnL-F* intergenic spacer; Taberlet et al., 1991). For the ribosomal ITS region the primer pair *ITS5+ITS4* was mostly used; additionally, ITS1 and ITS2 regions of certain

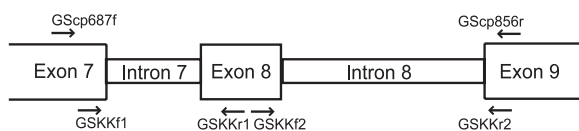


Fig. 2.1. Primers used in PCR and sequencing of *ncpGS*. Published primers (Emshwiller and Doyle, 1999) above, and the newly designed primers below.

degraded templates were amplified separately with primer pairs *ITS5+ITS2* and *ITS3+ITS4* (White et al., 1990). A fragment of *ncpGS* containing introns 7 and 8 was initially amplified with primers *GScp687f* and *GScp856r* (Emshwiller & Doyle, 1999). However, because these primers proved to work poorly with some taxa, a set of new primers was designed for the study group (Fig. 2.1):

GSKKf1 [5'-GGC ACC AAT GGA GAG GTT AT-3'],

GSKKf2 [5'-GAT CAC ATC TGG TGT GCW AG-3'],

GSKKr1 [5'-AGC TTC AAT TCC CAC RCT GG-3'], and

GSKKr2 [5'-YAA CAC CAG CYT GTT CWG TGA-3'].

Most taxa were amplified with primer pair *GSKKf1+GSKKr2*, but in some cases other combinations were used. The *phyC* fragment (part of exon 1) was primarily amplified with primer pair *PHYC-F+PHYC-R* (Samuel et al., 2005), although for a few degraded samples a newly designed forward primer *PHYCiF2* [5'-GGGTTTRGTGGTYTGCYAYCA-3'] was used in combination with *PHYC-R* to amplify a shorter fragment.

PCR amplifications were carried out in 50 μ L reactions using 0.2–2 μ L of total DNA extract as template. The reaction mixture contained also 1 \times PCR Buffer (Qiagen, Hilden, Germany), 20 pmol of each primer, 5 nmol dNTPs, 0.5 μ g bovine serum albumin (BSA; Promega, Madison, Wisconsin, USA) and 1 U Taq DNA polymerase (Qiagen, Hilden, Germany). The concentration of $MgCl_2$ was 2.5 mM for *trnL-F*, 2 mM for *ITS*, and 1.5 mM for *ncpGS* and *phyC*. The PCR program consisted of 4 min initial denaturation at 94°C, and 30–36 cycles of 30 s denaturation at 94°C, 30 s annealing at 52.5°C (48°C for *phyC*), and 1 min extension at 72°C, followed by a final extension of 5 min at 72°C.

PCR products were checked for length and yield by electrophoresis on 1% agarose gels and cleaned with either QIAquick PCR Cleanup (Qiagen, Hilden, Germany) or Nucleospin Extract II (Macherey-Nagel, Düren, Germany) columns. The latter was also used to recover fragments of correct size from agarose gels when multiple bands were present. In cases of degraded templates yielding very weak PCR products, one of two approaches was taken. Either the products from several parallel PCR reactions were pooled in the cleaning step, or gel-excised products were used as template in a re-amplification PCR. The cleaned PCR products were sequenced either on an ABI 377 automated sequencer using the ABI BigDye Terminator chemistry for cycle sequencing (Applied Biosystems, Forster City, California, USA) and Sephadex G50 AutoSeq columns (GE Healthcare, Diegem, Belgium) for reaction cleaning, or by external service (using ABI 3730xl; Applied Biosystems, Forster City, California, USA).

Generally, samples were sequenced with both forward and reverse PCR primers, though additional internal *ITS* and *ncpGS* primers were used as needed. The chromatograms were inspected, and sequence contigs assembled, with Sequencher v4.1.4 (Gene Codes Corp., Ann Arbor, Michigan, USA). In this process, special

attention was paid to sites with overlapping nucleotide peaks, possibly indicating infra-individual variation (polymorphisms). If an obviously overlapping signal was detected at both forward and reverse chromatograms, then the site was deemed to be putatively polymorphic between alleles or copies, and coded with IUPAC ambiguity codes.

Cloning of PCR products was carried out to facilitate the sequencing of a few difficult samples, and to further determine infra-individual polymorphism in ITS and *ncpGS*. The pGEM-T Easy Vector System (Promega, Madison, Wisconsin, USA) was used following the instructions of the manufacturer. Bacterial cells picked up from insert-containing colonies were directly used as template for standard PCR with M13 forward and reverse primers, and the resulting products were size-selected using agarose gel electrophoresis. Three clones per individual were sequenced as above.

Sequence alignment and indel characters—The sequences were aligned either completely by eye using MacClade v4.08 (Maddison & Maddison, 2001) and Bioedit v7.0.5.2 (Hall, 1999), or with the multiple sequence alignment algorithm of ClustalW v1.81 (Thompson et al., 1994) followed by extensive manual adjustments. In the alignment process both sequence similarity and mechanisms of molecular evolution were taken into account (Kelchner, 2000). Specifically, the following guidelines were used:

(1) Indels were assumed to occur less likely than substitutions, i.e., a gap was inserted only if otherwise at least two substitutions had to be assumed.

(2) The length variation in long mononucleotide repeats (here defined to be at least 6 bp long), and possible substitutions within, were considered to have uncertain homologies, and were excluded from the alignment.

(3) If the gap could be clearly postulated to have resulted from an insertion or deletion of a multinucleotide tandem repeat, this information was used to place the gap.

(4) As undetected inverted repeats can bias phylogenetic analysis (Quandt et al., 2003), attention was paid to visually detect them from the alignment.

(5) In the cases of overlapping gaps, the gaps were placed in a way which minimized the total number of indel events.

(6) Sometimes a gap could be placed equally probably in two or several positions. If the choice could possibly affect the phylogenetic analyses, question marks or ambiguity codes were introduced in the data matrix to take this uncertainty into account, still preserving as much phylogenetic information as possible.

(7) Ambiguously alignable regions with uncertain homologies were excluded.

The indel information of alignments was incorporated into parsimony analyses with the program SeqState (Müller, 2005a). The indel coding algorithm of SeqState (Müller, 2006) automates the coding of indel characters, outputting a NEXUS file containing the original data matrix followed by an extra character block comprising the indel characters. The simple indel coding (SIC; Simmons & Ochoterena, 2000) was used. Additionally, inverted repeats were coded as binary characters.

Phylogenetic analyses—The phylogenetic analyses were generally conducted using both maximum parsimony (MP) and Bayesian inference (BI) methods. Additionally, a maximum likelihood (ML) analysis was used in a specific case as alternative model-based phylogeny method.

For MP analyses, PAUP* v4.0b10 (Swofford, 2003) was used, treating nucleotide characters unordered and unweighted, and the polymorphic character states as uncertainties. Gaps in the alignment were treated as missing data, and the indel character block from SeqState was either included or excluded to assess the effect of indel characters. The parsimony ratchet (Nixon, 1999) was used to search for the most parsimonious trees. The ratchet batch files for PAUP* were generated with PRAP v.1.21 (Müller, 2004). In a ratchet run, each of 20 starting trees built with Random Addition Sequence (RAS) and TBR branch swapping underwent 50 iterations (25% of characters given double weight). This fast search strategy proved to be thorough enough for our data sets; experiments with more extensive ratchet searches and further swapping of trees found by ratchet did not result in shorter trees or changes in the strict consensus. Support for clades was assessed by bootstrap analysis (BS; Felsenstein, 1985) running 2000 pseudoreplicates. Since only a moderate exploration of tree space is necessary for estimating bootstrap and jackknife support values (Farris et al., 1996; Freudenstein et al., 2004; Müller, 2005b), only a single tree, resulting from one replication of RAS+TBR, was saved per pseudoreplicate.

Bayesian inference (BI) of phylogeny with posterior probabilities (PP) as support measure was done with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The model of molecular evolution for each gene dataset was selected using the Akaike Information Criterion (AIC) as implemented in MrModeltest v.2.2 (Nylander, 2004; for advantages of AIC over hierarchical likelihood ratio test (hLRT) see Posada & Buckley, 2004). The selected models were: GTR+G for *trnL-F*, GTR+G+I for ITS (except GTR+G in the *Cordemoya s.l.* clade analysis; see below), HKY+G for *ncpGS* (except HKY in the *Macaranga* clade and *Cordemoya s.l.* clade analyses), and HKY+G for *phyC*. The default priors of MrBayes were used. For each analysis two simultaneous runs were done (starting from random trees), having 3 heated and 1 cold chain with default temperature (0.2). Markov chains were sampled every 100th generation. Analyses were run until average standard deviation of split frequencies approached 0.02, indicating that two runs converged onto a stationary distribution. Additionally, the plot of generation vs. log probability was inspected after the run to ensure that stationarity was reached, and to determine the burn-in. Depending on the dataset, 1 000 000–4 000 000 generations were run, and typically c. 10% of the samples were discarded as burn-in.

An additional maximum likelihood (ML) bootstrap analysis for combined dataset of the *Mallotus s.s.* clade (see Results) was conducted with PHYML v.2.4.4 (Guindon & Gascuel, 2003) with the GTR+G+I model using four rate categories and 500 pseudoreplicates. Model parameters were estimated from data for the whole concatenated dataset (as PHYML does not allow splitting a dataset into partitions with different models).

Both MP and BI analyses were done with two different taxon-sampling strategies. First, all the taxa were analyzed together, with *Blumeodendron* as outgroup. Second, each of the three main clades found in the analyses of all taxa (see Results) were analyzed individually, selecting 1 or 2 species from two other clades to serve as an outgroup (indicated in the Appendix 2.2). In both of these cases, four markers were first analyzed separately, and the results screened for hard incongruences (i.e., incongruences with bootstrap support > 70%; Hillis & Bull, 1993) before the combined analysis of all four

datasets. Statistical tests for incongruence (e.g., incongruence length difference test; Farris et al., 1994) were not conducted, because these tests have been shown to be unreliable in certain conditions (e.g., Dolphin et al., 2000; Yoder et al., 2001; Darlu & Lecointre, 2002; see also Hipp et al. 2004). Moreover, because combining incongruent datasets can sometimes lead to a more robust phylogeny (Sullivan, 1996; Flynn & Nedbal, 1998; Wiens, 1998), we think that automatic rejection to combine them is a too strict approach. Instead, we take the view advocated by Wiens (1998): datasets with hard incongruences can be combined, but parts of the resulting tree being in strongly supported conflict between datasets should be regarded as questionable.

The internal conflict within each dataset was inspected with the consensus network approach (Holland & Moulton, 2003; Holland et al., 2005). This tree-based method visualises the conflict between input trees in a network. By selecting the trees from bootstrap pseudoreplicates as input trees, a consensus network provides a view to the character conflict in the dataset. For this analysis, SplitsTree v4.3 was used (Huson & Bryant, 2006), with threshold proportion $x=0.1$.

RESULTS

Sequence characteristics—Properties of the sequence datasets of each marker are given in Table 2.3. For a few taxa, some of the nuclear markers could not be sequenced (see Appendix 2.2), mainly due to difficulties in amplifying low copy number nuclear genes from degraded samples. These taxa with missing data were nevertheless included in the combined phylogenetic analyses. Generally, the forward and reverse sequencing reactions covered the sequence contigs fully. In this respect *ncpGS* was more problematic: in several occasions the chromatogram quality dropped drastically after mononucleotide repeats, and consequently, parts of the contigs were based on single direction only. In these cases the chromatograms were inspected with special attention, and the sequencing reaction repeated, if necessary for an unambiguous result.

As expected, the markers with both coding and non-coding parts (ITS+5.8S, *ncpGS*) showed more variation in non-coding regions. Also, the exons of protein coding genes *ncpGS* and *phyC* were most variable on the third codon positions. Furthermore, the inspection of amino acid translations revealed several stop codons in *phyC* sequences of *Mallotus discolor* and *Octospermum pleiogynum*; thus these sequences were putatively regarded as pseudogenes and subsequently excluded from the phylogenetic analyses of the *Mallotus* s.s. clade (see below).

Sequence alignment and the indel characters—The alignments are available from the authors. Insertion of gaps was required to align all non-coding regions; moreover, two gaps (3 and 12 bp long) were needed to align the *phyC* exon. The other coding regions (the exons of *ncpGS*, and 5.8S of ITS), were gap-free. The most extensive length variation was observed in *ncpGS*: a number of long and overlapping gaps were needed in its intron 8. Because of these indel events, the *ncpGS* sequences of the *Cordemoya* s.l. and *Macaranga* clades (see phylogenetic results below) were much shorter than the sequences of the *Mallotus* s.s. clade (c. 300 bp instead of c. 600 bp).

The inclusion of indel characters into the MP analyses had only limited impact on the phylogenetic results. Considering the results within the three main clades (see

below), indel characters had no or very little effect in the *Cordemoya* s.l. and *Mallotus* s.s. clades, but in the *Macaranga* clade they provided additional resolution and support. Here we report only the cladograms based on analyses including the indel characters, and, where necessary, we mention the differences with analyses where the indels were omitted.

Infra-specific and -individual polymorphism—Two or three separate specimens (collected from different parts of the distribution of the species, if possible) were sequenced for eight species to assess the infra-specific variation. The acquired sequences were either identical or highly similar, and the specimens were always placed together in the phylogenetic analyses (result not shown). For the subsequent analyses only one of the specimens was chosen to represent the species.

Polymorphic sites with overlapping nucleotide peaks were detected with direct sequencing in all nuclear datasets, but their number was generally low (Table 2.3), and visual inspection of the alignments revealed no clear additive patterns possibly indicating hybridisation (e.g., Sang et al., 1995). Two ITS sequences showing a relatively high number of these putatively polymorphic sites (*Mallotus griffithianus* and *M. lackeyi*) were cloned. The clone sequences confirmed the presence of either all (*M. griffithianus*, 10 out of 10) or some (*M. lackeyi*, 2 out of 5) of the putative polymorphisms (data not shown). Additionally, one 2 bp long indel polymorphism was found in *M. griffithianus*. Several additional differences between clones were also observed; some of them do not appear to be Taq errors and could be traced back to weak, previously unnoticed, overlapping peaks in the chromatograms. In a phylogenetic analysis of ITS data (result not shown) the clone sequences were placed in the vicinity of the corresponding direct sequence. Moreover, using the direct sequence or any of the clones resulted in the same phylogenetic position for the specimen in question.

Table 2.3. Summary of alignment properties.

DNA region	Number of taxa sampled	Sequence length	Number of polymorphic sites / sequence (average)
<i>trnL-F</i>	99	897–1160	0
ITS (+5.8S)	96	712–747	0–10 (1.3)
<i>ncpGS</i>	94	320–910	0–6 (0.4)
<i>phyC</i>	91	632–644	0–14 (0.9)

DNA region	Nucleotide characters			Indel characters	
	Alignment length	Number excluded	Number informative	Number total	Number informative
<i>trnL-F</i>	1343	179	108 (9.3%)	53	28
ITS (+5.8S)	812	85	241 (33.2%)	54	22
<i>ncpGS</i>	1000	38	161 (16.7%)	56	32
<i>phyC</i>	644	0	119 (18.5 %)	2	0

Two *ncpGS* samples were also cloned to confirm infra-individual polymorphisms, and, especially, to investigate whether sequencing problems related to mononucleotide repeats (see above) were caused by alleles with a difference in the number of repeated nucleotides. However, the chromatograms of all the clone sequences suffered from the same deteriorated signal after the repeats as the direct sequences. Thus, this phenomenon is likely due to technical problems in the sequencing reactions, and not because of infra-individual polymorphisms.

Analysis with all taxa and the major relationships—Most of the single-marker analyses, as well as the combined analysis of all four markers, revealed the same three highly supported main clades: 1) a *Cordemoya* s.l. clade, consisting of the genera *Cordemoya* and *Deuteromallotus*, and the *Mallotus* sections *Hancea* and *Oliganthae*; 2) a *Mallotus* s.s. clade with the remaining *Mallotus* species and the genera *Coccoceras*, *Neotrewia*, *Octospermum*, and *Trewia*; 3) a *Macaranga* clade with all sampled *Macaranga* species.

The relationships between, and support for, these clades are summarized in Fig. 2.2 (for detailed trees see Appendices 2.3–2.6). *TrnL-F*, *phyC*, and BI analysis of ITS support the sister group relationship of the *Macaranga* and *Mallotus* s.s. clades, placing the *Cordemoya* clade in a basal position. In contrast, in the MP analysis of ITS the *Macaranga* clade is highly nested inside the *Mallotus* s.s. clade, and, in particular, sister to a clade consisting of *Mallotus* sect. *Mallotus*, *Mallotus discolor*, and *Octospermum pleiogynum*. Analysis of *ncpGS* also gave deviating results: the three main clades are present in the MP analysis, but the *Macaranga* and *Cordemoya* s.l. clades are now sister groups. Moreover, BI analysis of *ncpGS* fails to separate the members of the *Macaranga* and *Cordemoya* clades.

Individual analyses of three main clades—Aligning the datasets for individual analyses of main clades (without the more distant outgroup *Blumeodendron*) was easier than datasets with all taxa and resulted in fewer excluded characters, especially in the ITS region. The phylogenies produced are generally similar to the analyses with all taxa and show no hard incongruences with them. The effect of analysing *Cordemoya* s.l. and *Mallotus* s.s. clades individually was very small; however, the individual analysis of the *Macaranga* clade resulted in a more resolved strict consensus and additional support for several clades. Only the results of the individual clade analyses are discussed below (the results of the single-marker analyses discussed below are not depicted, but the reader can refer to the Appendices 2.3–2.6 for the similar results from the analyses of all taxa).

Cordemoya s.l. clade—All single-marker analyses (not shown) resulted in similar trees without hard incongruences. The combined analysis (Fig. 2.3) also shows the same pattern of two highly supported subclades: one with *Cordemoya integrifolia* and all three *Deuteromallotus* species, and the other with the *Mallotus* sections *Hancea* and *Oliganthae*.

Macaranga clade—The single-marker analyses (not shown) show varying degrees of resolution and support, *ngpGS* being least, and ITS most resolved. There are no hard

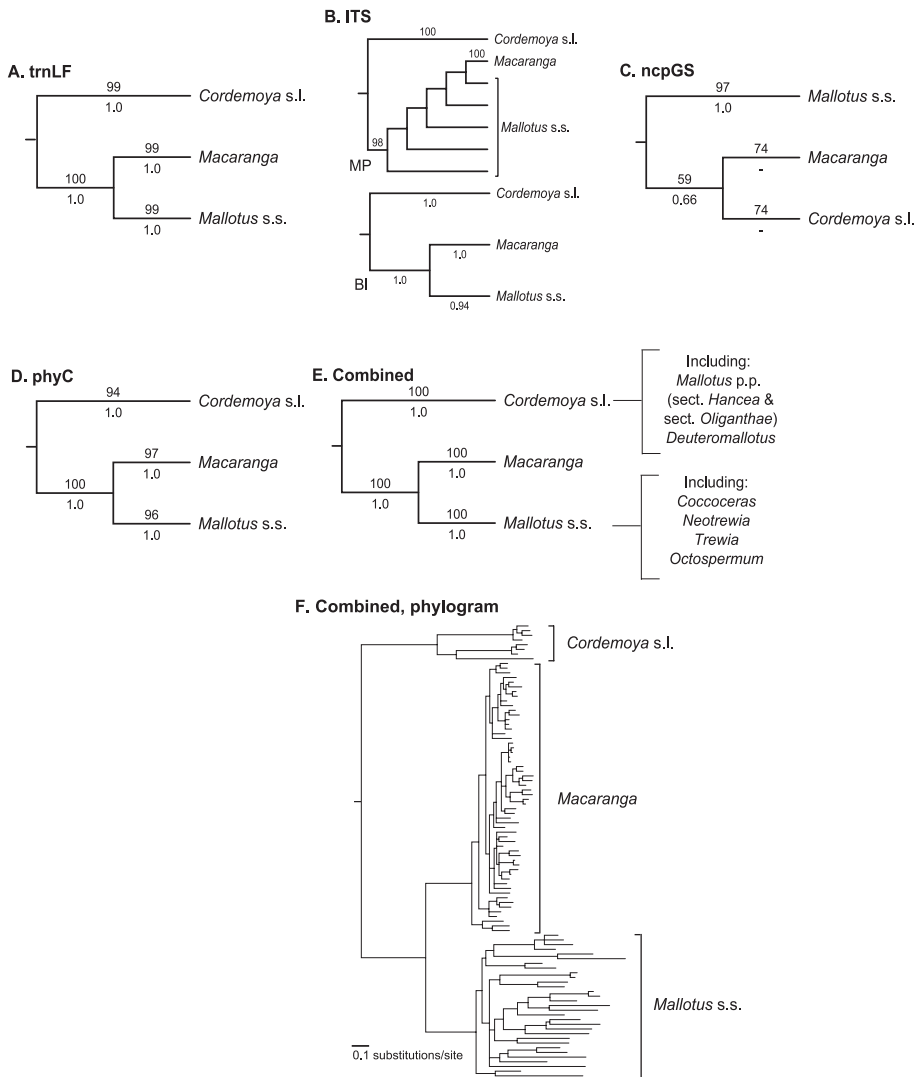


Fig. 2.2. Summary of the phylogenetic analyses with all sampled taxa, showing the relationships of the major clades. (A-D) Single-marker analyses. (E) Combined analysis of all four markers. (F) Bayesian phylogram from the combined analysis. MP, maximum parsimony; BI, Bayesian inference. MP bootstrap values shown above the branches, BI posterior probabilities below. Outgroup (*Blumeodendron*) not shown.

incongruences between these datasets, and combining them (Fig. 2.4) notably increased resolution and support. Both MP and BI analyses gave highly similar results revealing five main clades: two small basal clades (B1 and B2 in Fig. 2.4) and a large crown group of three clades (C1, C2 and C3) and *M. trichocarpa* on a branch of its own.

There are few topological differences between the results of the BI and MP analyses. First, the BI analysis groups clades C1 and C2 together (PP 1.00), whereas MP unites

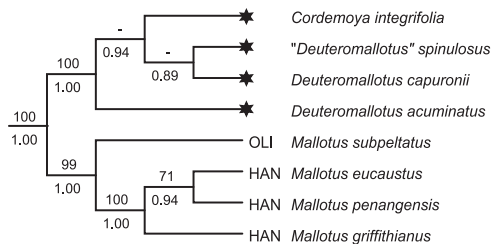


Fig. 2.3. Phylogenetic relationships inferred from the individual analysis of *Cordemoya* s.l. clade (see Fig. 2.2) with the combined dataset of four markers. A Bayesian majority consensus tree with parsimony bootstrap values shown above the branches and posterior probabilities below. -: node doesn't exist in parsimony strict consensus. Infrageneric group (see Table 2.2) indicated with three letter abbreviation. Species occurring at western side of Indian Ocean (Africa, Madagascar, Mascarenes) are indicated with stars.

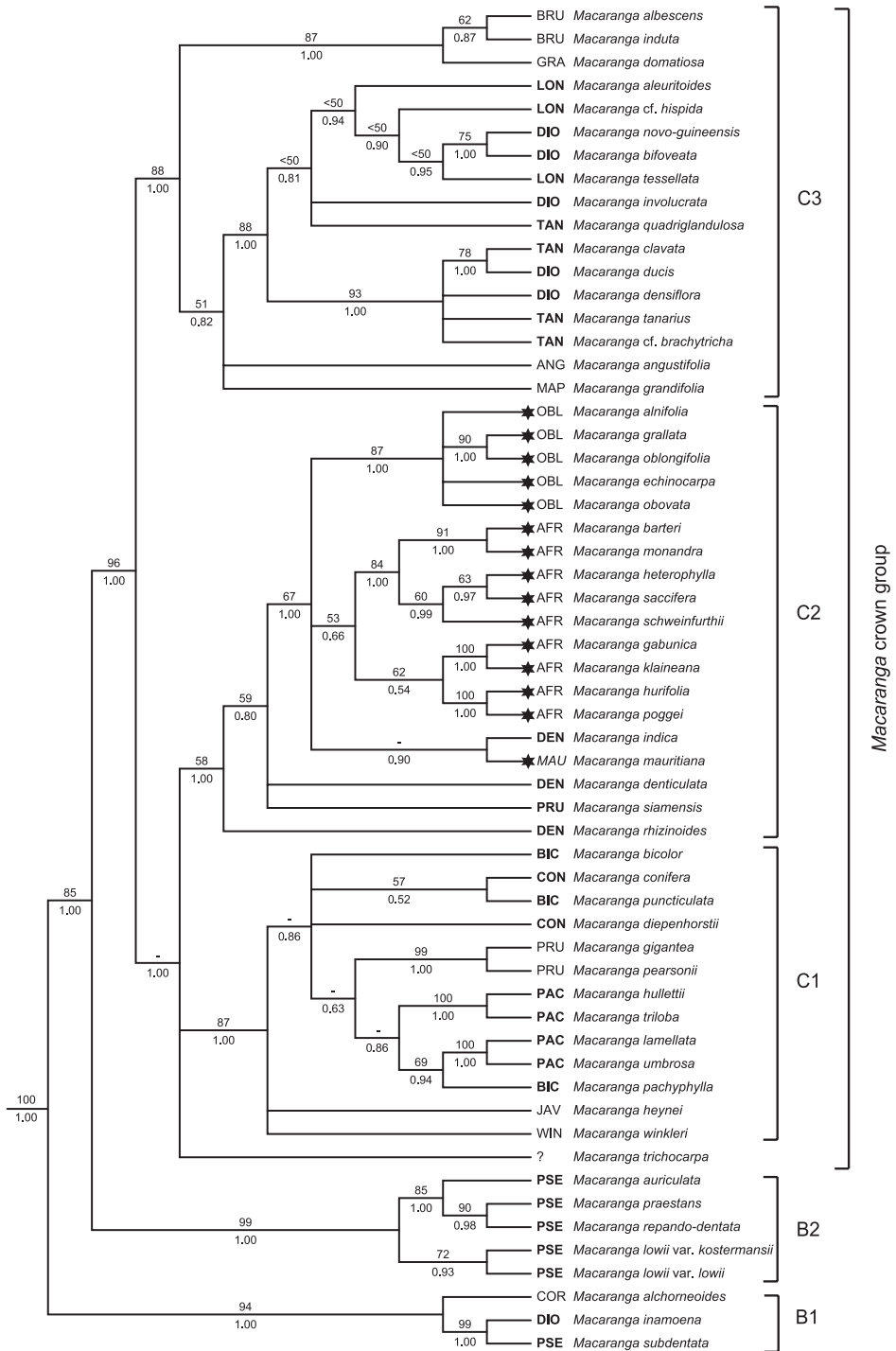
C2 and C3 (BS <50). (However, the MP analysis without indel characters gave the same result as the BI analysis; BS 59.) Second, in clade C2, the BI clade of *M. indica* and *M. mauritiana* (PP 0.90) does not exist in the MP tree, but *M. mauritiana* groups instead together with African and Madagascan *Macaranga* species (BS <50). For the other differences (concerning the placements of *M. bicolor* and *M. trichocarpa*), compare the topologies in Fig. 2.4 (BI) and in Appendix 2.7.

Mallotus s.s. clade—The ITS dataset provided the most resolved tree, whereas trees based on the three other markers have roughly the same lesser resolution. On the other hand, the number of well supported clades (BS ≥70) is almost equal in all single-marker analyses, including ITS. All datasets resemble each other also in the distribution of the supported clades: they are predominantly small, and the relationships between them are not resolved and/or supported, resulting in a large basal polytomy.

There is one hard incongruence between the datasets: in the ITS tree *M. barbatus* groups with *M. paniculatus* (BS 77), whereas in the *ncpGS* tree it forms a clade with *M. macrostachyus* and *M. tetracoccus* (BS 99) with *M. paniculatus* sister to this clade. Furthermore, two incongruences almost reach the cut-off level of BS 70. First, *phyC* groups *M. repandus* as sister to sect. *Mallotus* (BS 69), whereas *ncpGS* places *M. repandus* with *M. philippensis* (BS 98). Second, *ncpGS* groups *M. resinusus* with *M. decipiens* (BS 100), but ITS places it with *M. leucocalyx* (BS 68; *M. decipiens* being sister to the clade of these two taxa).

The combined analysis of the four markers for the *Mallotus* s.s. clade is shown in Fig. 2.5. Trees obtained by BI and MP analyses are congruent (except for one difference in the clade consisting of *M. leucocalyx*, *M. resinusus*, and *M. decipiens*), the BI tree being more resolved. The basal nodes of the MP tree are essentially not supported, whereas BI analysis gives (often strong) support for several additional nodes. ML bootstrap analysis (not shown) resulted in a topology very similar to the BI and MP analyses, but no support (BS < 50) was given to the nodes supported only by BI.

Fig. 2.4. (opposite page) Phylogenetic relationships inferred from the individual analysis of *Macaranga* clade (see Fig. 2.2) with the combined dataset of four markers. A Bayesian majority consensus tree with parsimony bootstrap values shown above the branches and posterior probabilities below. -: node does not exist in the parsimony strict consensus. Infrageneric group (see Table 2.2) indicated with three letter abbreviation (shown in boldface if the particular group is non-monophyletic). Species occurring at western side of Indian Ocean (Africa, Madagascar, Mascarenes) are indicated with stars.



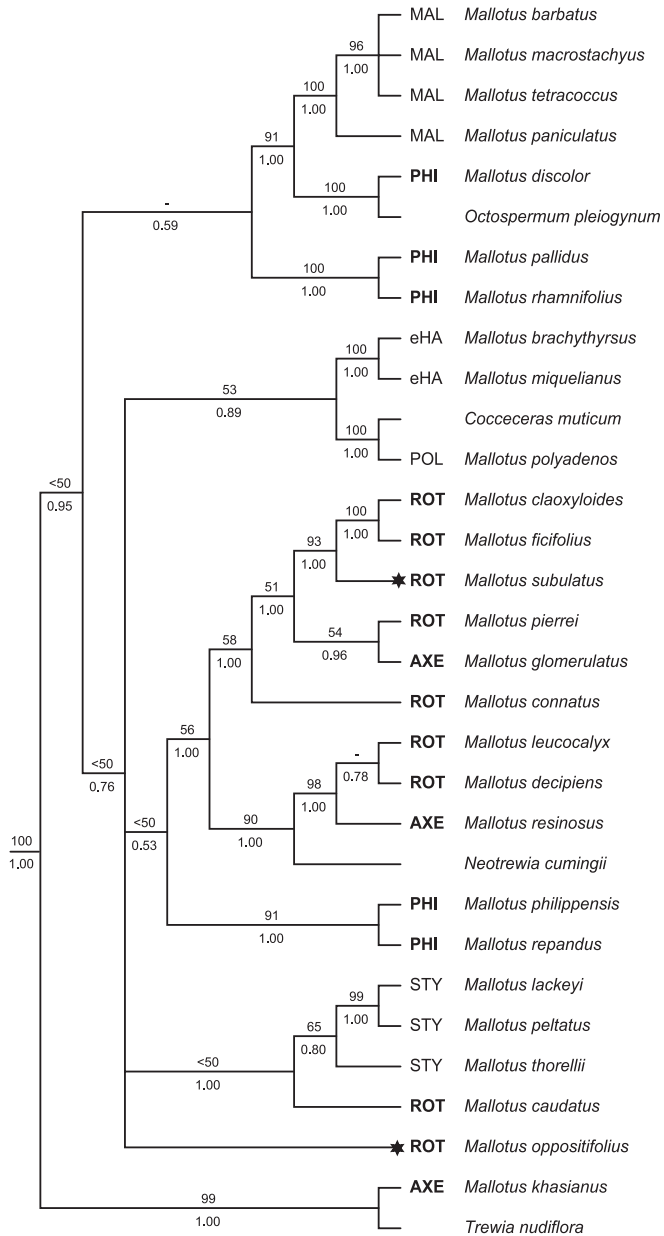


Fig. 2.5. Phylogenetic relationships inferred from the individual analysis of *Mallotus* s.s. clade (see Fig. 2.2) with the combined dataset of four markers. A Bayesian majority consensus tree with parsimony bootstrap values shown above the branches and posterior probabilities below. -: node does not exist in the parsimony strict consensus. Infrageneric group (see Table 2.2) indicated with three letter abbreviation (shown in boldface if the particular group is non-monophyletic); eHA, a group of species excluded from sect. *Hancea* (Slik and van Welzen, 2001b). Species occurring at western side of Indian Ocean (Africa, Madagascar, Mascarenes) are indicated with stars.

DISCUSSION

Phylogenetic analysis methods and support values — Although unweighted Maximum Parsimony (MP) and model-based Bayesian Inference (BI) are fundamentally different methods, analyzing our datasets with them resulted to a large extent in similar topologies. Also the support for clades was measured in distinctly different ways: bootstrap analysis for MP and posterior probabilities for BI. It has become clear that these two indices are not directly comparable, and that posterior probabilities are generally higher than bootstrap values (e.g., Rannala & Yang, 1996). This trend can be observed in our results as well. Moreover, some clades without bootstrap support received high posterior probabilities, especially in the *Mallotus* s.s. clade (see below). Because recent studies have shown that posterior probabilities can overestimate the support or even give high support for incorrect nodes (Suzuki et al., 2002; Alfaro et al., 2003; Douady et al., 2003; Simmons et al., 2004), we regard these clades as dubious. However, we also recognize that the difference in these two kinds of support values can also arise from the general dissimilarities of MP and BI as phylogeny optimality criteria.

Major relationships and the monophyly of *Macaranga* and *Mallotus* — The analyses of all sequenced taxa, including a representative sample of the diversity in *Macaranga* and *Mallotus*, show that *Macaranga* is nested in the subtribe Rottlerinae, and, therefore, there is no basis for Webster's (1994b) decision to place it into a separate subtribe Macaranginae. Furthermore, three well-supported main clades are revealed, allowing the monophyly of *Macaranga* and *Mallotus* to be assessed (Fig. 2.2).

First of all, all markers agree on the monophyly of *Macaranga*, as suggested by earlier studies with limited taxon sampling (Blattner et al., 2001; Slik & Van Welzen, 2001a). The 3- or 4-locular anthers of *Macaranga* are thus a good synapomorphy for the genus, and were uniquely derived from 2-locular anthers present in the other clades and outgroup. One *Macaranga* species, *M. heudelotii* Baill. (not sampled), is reported to exceptionally have 2-locular anthers (Whitmore, in press). This species possesses spiny branches and branched staminate inflorescences and, therefore, would morphologically fit well in a deeply nested position with other African *Macaranga* species (clade C2; see Fig 2.4. and discussion on the *Macaranga* clade below). We thus regard the 2-locular condition in *M. heudelotii* as a reversal from the 3/4-locular state.

On the other hand, our results clearly show that *Mallotus*, as currently delimited, is not a monophyletic genus. All markers support the paraphyly of *Mallotus*, caused by the placement of a few *Mallotus* taxa away from the main *Mallotus* clade (= *Mallotus* s.s. clade) and forming a separate clade with *Cordemoya* and *Deuteromallotus* (= *Cordemoya* s.l. clade). These *Mallotus* segregates, namely the Asian sections *Hancea* and *Oliganthae*, were already separated from the rest of *Mallotus* in the morphological phylogeny (Slik & Van Welzen, 2001a). The species assemblage of the *Cordemoya* s.l. clade has not been suggested before, although Müller (1866) placed *Cordemoya integrifolia*, *Deuteromallotus acuminatus* and *Mallotus penangensis* (sect. *Hancea*) together in his *Mallotus* sect. *Cordemoya*.

The genus *Deuteromallotus* was originally considered to differ from *Mallotus* by characters in pistillate flowers (style/stigma very short and style scarcely papillose; Pax

& Hoffmann, 1914). Later, McPherson (1995) demonstrated that the fragile stigmas of *Deuteromallotus* break easily and when the flowers are intact they do not differ from those of *Mallotus*. We confirm this observation, but because *Deuteromallotus* falls into the *Cordemoya* s.l. clade as well, it should not be merged with *Mallotus*, as he suggested.

The *Cordemoya* s.l. clade, with the above mentioned taxon composition, is also supported by morphological characters. Most importantly, the conspicuous, spherical to disc-like glandular hairs, typical for the *Macaranga* and *Mallotus* s.s. clades, are missing in the members of the *Cordemoya* s.l. clade. The latter have capitate glandular hairs, and/or peltate-stellate hairs instead. Moreover, the pollen of the *Cordemoya* s.l. clade has areolate ornamentation instead of the perforate/microreticulate ornamentation of the *Mallotus* s.s. clade (Sierra et al., 2006).

In addition to *Cordemoya* and *Deuteromallotus* discussed above, the results presented here clarify the relationship between *Mallotus* and the other small Rottlerinae genera, revealing second cause for the paraphyly of *Mallotus*: *Neotrewia*, *Octospermum*, and *Trewia* are part of a well-supported *Mallotus* s.s. clade. Also the inclusion of *Coccoceras* into *Mallotus* (Airy Shaw, 1963; Bollendorff et al., 2000) is confirmed here (for the further discussion about these genera, see the section about *Mallotus* s.s. phylogeny below). *Rockinghamia*, a genus already considered to be distant to *Mallotus* in an earlier study (Wurdack et al., 2005), was confirmed not to be closely related. However, the position of the unsampled genus *Avellanita*, placed in the Rottlerinae by Radcliffe-Smith (2001), remains to be investigated. This genus was placed *incertae sedis* by Webster (1994b), and we regard a close relationship of it with the taxa studied here as dubious because of its discordant distribution (endemic to Chile) and inflorescence structure (bisexual cymes rather than the typical, mostly unisexual, spikes, racemes, or panicles). Preliminary molecular phylogenetic results indicate that *Avellanita* is far removed in the Acalyphoideae from our study clade (K. Wurdack, Smithsonian Institution, Washington, personal communication).

Both plastid (*trnL-F*) and nuclear (*phyC*) data strongly support the monophyly of *Mallotus* s.s. and its sister group relationship with the *Macaranga* clade, contradicting the nested placement of *Macaranga* shown by the analysis of morphological data (Slik & Van Welzen, 2001a). The same result, with moderate support for monophyly of *Mallotus* s.s. (PP 0.94) is obtained from the BI analysis of ITS. On the other hand, the result of the MP analysis of ITS, with *Macaranga* being deeply embedded in *Mallotus* s.s. and sister to a clade containing *Mallotus* sect. *Mallotus* (for a detailed tree see Appendix 2.4), clearly resembles the phylogeny inferred from the morphology.

There are, however, reasons to believe that the result of the MP analysis of ITS does not reflect the underlying organismal phylogeny. The nested position of *Macaranga* does not have bootstrap support, and the consensus network analysis of the ITS bootstrap trees (not shown) revealed a relatively strong alternative split supporting the monophyly of *Mallotus* s.s. (BS 16, whereas the split placing *Macaranga* nested in *Mallotus* s.s. had BS 24). In other words, although not visible in the strict consensus, ITS data has characters supporting *Mallotus* s.s. clade, even in the MP framework. Furthermore, the ITS dataset is highly variable, and the plot of transition vs. transversion distances shows some signs of saturation. MP analysis could therefore have failed to pick up the obscured signal supporting the separation of *Mallotus* s.s. and *Macaranga*, whereas

BI, based on molecular evolutionary models, which take multiple hits and different rates for substitution classes into account (e.g., Swofford et al., 1996), resulted in the same relationship as revealed by most of the other data. We choose therefore two monophyletic sister clades, *Mallotus* s.s. and *Macaranga*, as our phylogenetic result.

The analyses of *ncpGS* also strongly supports the monophyly of *Mallotus* s.s. On the other hand, the results of the *ncpGS* analyses deviate from those of other markers, because MP analysis of *ncpGS* data places *Macaranga* and *Cordemoya* s.l. clades together, and BI even fails to separate the members of these two clades. These deviating results are, however, weakly supported, and, because of the long gaps required to align these taxa with the *Mallotus* s.s. clade, there is only a limited number of characters available to infer the relationships between the major clades. A deviating, but unsupported, result could thus have arisen by chance. Moreover, the gaps in the *Macaranga* and *Cordemoya* s.l. clades, although occurring in the same area in the *ncpGS* intron 8, are not homologous and provide no evidence for a sister group relationship between these clades.

The phylogeny of the Cordemoya s.l. clade—Both single-marker and combined analyses of the *Cordemoya* s.l. clade reveal a strongly supported geographical signal (Fig. 2.3): one of the subclades comprises only taxa from Madagascar and the Mascarene Islands (genera *Deuteromallotus* and *Cordemoya*, respectively), whereas the other consists of the purely Asian *Mallotus* sections *Oliganthae* and *Hancea*. Furthermore, the monophyly of sect. *Hancea* as circumscribed by Slik and Van Welzen (2001b) is strongly supported (see also the discussion on the *Mallotus* s.s. clade below). The morphology of this clade and taxonomic rearrangements are further discussed in a separate paper (Sierra et al., 2006).

The phylogeny of the Macaranga clade—Combining the four *Macaranga* datasets, which show no hard incongruences, resulted in a more resolved and more highly supported phylogeny than any of the single-marker analyses (Fig. 2.4). Previous studies on the *Macaranga* phylogeny (Blattner et al., 2001; Davies et al., 2001; Bänfer et al., 2004) included mainly myrmecophytic species and their west-Malesian relatives. Therefore, this study, with all of Whitmore's (in press) *Macaranga* groups sampled, provides the first comprehensive phylogeny of the genus. In our results (Fig. 2.4) half of the 18 infrageneric groups recognized by Whitmore proved to be, although sometimes with low support, non-monophyletic. Nevertheless, taxon sampling is still limited (several large *Macaranga* groups are represented by one or a few species only) and the tree is only partially supported; caution is thus necessary when interpreting the results. In the discussion below, the information about the *Macaranga* morphology is based on Whitmore (in press) and personal observations, unless indicated otherwise.

Basal clades B1 and B2—The analysis revealed two relatively small basal lineages (Fig. 2.4: clades B1 and B2), which are separated with strong support from a large crown group. These two basal clades consist mainly of species belonging to *Macaranga* sect. *Pseudorottlera*, a section suggested to be transitional between *Mallotus* and *Macaranga* (Zollinger, 1856; Airy Shaw, 1965). Species falling into clades B1 and B2 are all shrubs or small trees growing in primary forest, and usually have small, penninerved

leaves, and 2-locular fruits. Furthermore, their staminate inflorescences are unbranched, and bear small bracteoles without disc-shaped glands (nectaries), whereas in the rest of *Macaranga* they are variously branched (often with more than two axis orders; exceptionally unbranched in very few species) and either have disc-shaped glands or not. In *Mallotus* staminate inflorescences are either unbranched or scantily branched and have small bracteoles that always lack disc-shaped glands.

Clade B1, sister to the rest of *Macaranga*, brings two Australian species, *M. subdentata* (sect. *Pseudorottlera*) and *M. inamoena* (*Dioica* group) together with *M. alchorneoides* (*Coriacea* group, the only New Caledonian species sampled); this grouping has never been suggested before. All three species are frequently monoecious, a condition additionally present only in most of the other New Caledonian species and *M. glaberrima* (Hassk.) Airy Shaw (sect. *Pseudorottlera*, distributed from Java to New Guinea; not sampled). *M. inamoena* was placed in the *Dioica* group by Whitmore (in press), but with its unbranched staminate inflorescences it fits better in the B1 clade (staminate inflorescences are generally branched in the *Dioica* group s.s.). The next clade, B2, is sister to the *Macaranga* crown group (clades C1–3) and consists of the remaining sampled *Pseudorottlera* species.

The composition of the basal clades B1 and B2 agrees with the results from previous molecular phylogenetic studies (Blattner et al., 2001; Davies et al., 2001), which placed sect. *Pseudorottlera* as sister to the rest of *Macaranga* (other members of clades B1 and B2 were not sampled in those studies). In contrast, the morphological analysis placed a pioneer species *M. tanarius* at the base of the *Macaranga* clade (Slik and Van Welzen, 2001a). This result, together with the embedded position of *Macaranga* in a clade of pioneer *Mallotus* species, led to a conclusion that *Macaranga* originated in open vegetation, and that primary forest understorey species (e.g., sect. *Pseudorottlera*) evolved from pioneer ancestors (Slik & Van Welzen, 2001a). According to our results, the *Macaranga* ancestor could have had either ecology, depending on the results in the sister clade *Mallotus* s.s., which is unfortunately poorly resolved.

The crown group, clades C1–C3—The *Macaranga* crown group is a well-supported clade containing the majority of the species and most of the morphological diversity of the genus. It consists of 3 subclades (C1–C3 in Fig. 2.4) with varying support, and one ambiguously placed species, *M. trichocarpa* (also not placed in any of the Whitmore's groups). The relationships among the clades C1–3 are still ambiguous: BI strongly supports a clade of C1+C2, whereas MP either unites C2+C3 (indel characters included) or C1+C2 (indel characters excluded); neither of the MP groupings is supported by bootstrap. Each of these clades, especially C2 and C3, presents a high amount of morphological diversity, and no morphological synapomorphies for them are known at the moment. However, examination of the crown group clade reveals a clear geographical structure: the species from the three main centers of diversity of the genus, i.e., west-Malesia, Africa + Madagascar, and New Guinea, roughly correspond with the clades C1, C2, and C3, respectively.

The *Macaranga* clade C1 is well-supported, and comprises all taxa from the sections *Pachystemon* and *Pruinosa*, and from the *Bicolor*, *Conifera*, *Javanica* and *Winkleri* species groups, with the exception of *M. siamensis* (sect. *Pruinosa*), which is placed in clade C2. All these groups have a west-Malesian centered distribution, with some

outlier species mainly in Indochina, Sulawesi and the Philippines. This clade contains all myrmecophytic *Macaranga* species; their phylogenetic relationships and the evolution of myrmecophytism have been studied in detail elsewhere (Blattner et al., 2001; Davies et al., 2001; Bänfer et al., 2004). In our analyses, this clade is rather poorly resolved, perhaps partly due to the hybridization between myrmecophytic species revealed in a phylogeographic analysis of chloroplast haplotype data (Bänfer et al., 2006). However, our results demonstrate the close relationship of the *Bicolor* and *Conifera* groups with the myrmecophytic sections *Pachystemon* and *Pruinosa*. These two groups, together with the *Javanica* group, should, therefore, be thoroughly sampled for future studies of myrmecophytic *Macaranga*.

Clade C2 unites the mainly continental Asian *Denticulata* group, one aberrant species from the sect. *Pruinosae* (*M. siamensis*), and all species sampled from Africa, Madagascar and the Mascarenes. *Macaranga siamensis* has often been confused with *M. gigantea* (because of their enormous, similarly shaped leaves), and, although differing in several characters, was tentatively placed with it in sect. *Pruinosae* (Davies, 2001). It, however, differs from other members of the sect. *Pruinosae* in having prominent extra-floral nectaries on the apical part of leaves, disc-shaped glands on the staminate bracteoles and globose seeds (in the sect. *Pruinosae* extra-floral leaf nectaries not prominent, disc-shaped glands absent, and seeds lenticular). Considering the overall morphology and habit, the placement of *M. siamensis* among the *Denticulata* group is rather surprising, but they do share roughly the same distribution and globose-shaped seeds. Moreover, some *Denticulata* species also have staminate bracteoles with disc-shaped glands.

Most Asian species of clade C2 form a grade leading to a moderately supported clade comprising Asian *M. indica* and all species from the western side of the Indian Ocean. In the MP analysis the latter forms an unsupported clade, but in BI *M. mauritiana* (from Mauritius) groups together with *M. indica*. Nevertheless, and although denser sampling of African and Madagascan species might enhance the picture, these results demonstrate the phylogenetic affinity of all *Macaranga* species occurring in the western side of the Indian Ocean, and suggest a possible single origin of them.

The type species of *Macaranga*, *M. mauritiana*, with hollow stems, unique capitulate staminate inflorescences and bizarre fusiform fruits, was placed in a group of its own (*Mauritiana*), and was even discussed to belong to a separate genus (Whitmore, in press). Our results show that this species is clearly related to the *Denticulata* group (with which it shares the general leaf shape) and African and Madagascan *Macaranga*.

African *Macaranga* species are a diverse group of 26 species with a wide array of growth forms (including lianas), and other morphological adaptations (e.g., ant-housing stipules of *M. saccifera*). Also, many species have a spiny trunk and branches. Pax & Hoffmann (1914) classified the African species into five sections (in one case even together with Asian species), whereas Whitmore left them ungrouped. In our analysis all nine sampled African species (belonging to three different sections of Pax and Hoffmann), form one monophyletic, but poorly supported, group. Therefore, our data suggest that all African *Macaranga* species originated from a single common ancestor. Also, all species endemic to Madagascar (5 out of 10 sampled) form a single clade, a result supporting Whitmore's decision to unite them in the *Oblongifolia* group (classified in four sections by Pax and Hoffmann).

C3 is a well-supported clade with all taxa sampled from the groups *Angustifolia*, *Brunneofloccosa*, *Dioica*, *Gracilis*, *Longistipulata*, *Mappa* and *Tanarius* (except *M. inamoena* of the *Dioica* group, which belongs to clade B1). Together, these groups comprise almost 120 species, and display huge morphological variation, from montane species with delicate leaves (*Gracilis* group) to large-leaved species (*Mappa* group). Most of these groups are clearly New Guinea centered, with a few species occurring in the neighboring areas, such as the Moluccas, Sulawesi, the Philippines, Australia and the west Pacific Islands. The *Mappa* group (only one species sampled) has a markedly west Pacific distribution, with some species reaching Micronesia and Polynesia. Only one species from C3 clade, the widespread *M. tanarius*, occurs also in west-Malesia and continental Asia.

The fact that members of both the *Dioica* group and sect. *Pseudorottlera* have fruits subtended by leafy bracts led Whitmore (Whitmore, 1980, in press) to suggest a close affinity between them. The present study indicates, however, that the *Dioica* group, as a member of clade C3 (except the misplaced *M. inamoena*, see above), is phylogenetically distant to sect. *Pseudorottlera* (clades B1 and B2).

Our results group the *Brunneofloccosa* and *Gracilis* groups together; species in both groups are restricted to montane forests (except two *Brunneofloccosa* species). Furthermore, taxa from the *Dioica*, *Longifolia* and *Tanarius* groups form a well-supported clade. However, none of these groups appears to be monophyletic. A study with denser taxon sampling is needed to clarify the phylogeny of this clade.

The phylogeny of the Mallotus s.s. clade—Single-marker analyses of the *Mallotus* s.s. clade produced largely polytomous trees, and, in contrast to the effects of combining data in the *Macaranga* clade, the combined *Mallotus* s.s. analyses yielded only a limited amount of additional supported clades (Fig. 2.5), especially in the MP analysis. The topologies of the MP and BI trees are largely the same, and both analyses gave strong support to small terminal clades. On the other hand these analyses differ greatly in the support given to the basal and inner nodes: many of them are highly supported (PP 0.95–1.00) by BI, but do not receive any BS support in the MP analysis.

Apart from the general tendency of BI to overestimate support, it has been shown to be especially prone to give high confidence to very short internodes (Alfaro et al., 2003). Although it is not obvious what should be considered as a short internode, the *Mallotus* s.s. internodes supported only by PP are on average clearly shorter than those supported by both PP and BS (result not shown). Further evidence that BI overestimated the support for these nodes comes from the ML bootstrap analysis conducted for this dataset. The ML bootstrap results in high support for nodes supported by both MP and BI, but the nodes supported only by BI receive ML bootstrap values of less than 50. In PHYML different models cannot be used for different partitions, and, therefore, the results of BI and ML analyses might not be directly comparable. However, this result strengthens the hypothesis that the high support of BI for the basal nodes unsupported by MP is not because of general methodological differences between unweighted MP and model-based BI, but because of the tendency of BI to overestimate support in some circumstances. Therefore, we regard the backbone of combined *Mallotus* s.s. phylogeny to be essentially unresolved.

The failure of all four gene regions, each with different properties (e.g., plastid vs.

nuclear genomes, exons vs. introns, and different levels of variation), to reliably resolve the *Mallotus* s.s. phylogeny is a puzzling phenomenon. The sequence divergence is in an acceptable range: the divergence in the *Mallotus* s.s. datasets is similar or higher than in the *Macaranga* clade, but the transition vs. transversion distance plots (result not shown) revealed no signs of saturation for the *Mallotus* s.s. clade.

Additional insights into datasets were gained with consensus networks produced from MP bootstrap pseudoreplicate trees. The consensus networks from all single-marker *Mallotus* s.s. datasets have a similar structure: small groups (corresponding to the highly supported terminal clades) are connected to a central reticulation, indicating that datasets have internal conflicts (i.e., homoplasy) in the relationship between these groups. Moreover, studying the incompatible splits causing the central reticulations revealed no common pattern among the four datasets, i.e., the conflicting splits involve different taxa in different datasets.

The *Mallotus* s.s. analyses also revealed incongruences between single-marker datasets: there are one hard (i.e., BS>70) and two almost hard (BS 68–69) incongruences. We do not consider them to contribute significantly to the lack of resolution and support observed in the combined analysis, because excluding the taxa causing the incongruences did not improve the results. Also, the incongruences are unlikely to cause the basal polytomy, because most of them are localized, i.e., occur between closely related species.

The present study is a first molecular phylogenetic analysis of the genus *Mallotus*. Despite the fact that all analyses resulted in poorly resolved phylogenetic trees, several conclusions can be drawn from it. Apart from the placement of certain *Mallotus* species in the *Cordemoya* s.l. clade (discussed above), our results confirm the inclusion of the genus *Coccoceras* with *Mallotus* sect. *Polyadenii* (Bollendorff et al., 2000) and demonstrate the close relationship of *Neotrewia*, *Octospermum*, and *Trewia* with *Mallotus*. All four genera are placed in the *Mallotus* s.s. clade and all of them form strongly supported clades with morphologically similar *Mallotus* species. The indehiscent or tardily dehiscent fruits are probably independently derived in each of these genera from the typical dehiscent capsules of *Mallotus* (two unsampled *Mallotus* species, *M. blumeanus* and *M. sphaerocarpus* also have indehiscent fruits, in this case with thick fleshy layer surrounding the seeds). A similar phenomenon can be observed in several other euphorb groups (Esser, 2003; Wurdack et al., 2005). Below we discuss *Neotrewia*, *Octospermum*, and *Trewia* briefly; more detailed morphological descriptions and the taxonomic rearrangements are provided in a separate paper (Kulju et al., 2007, Chapter 3).

The Malesian genus *Neotrewia* (1 sp.) is characterised by unilocular (or rarely bilocular) indehiscent fruits. This contrasts with the mainly dehiscent, and typically 3-locular (sometimes 2–5-locular) fruits of *Mallotus* (however, unilocular fruits are common in *Macaranga*). Because no other morphological differences from *Mallotus* exist, it is no surprise to see this genus deeply embedded in the *Mallotus* s.s. clade, and as sister to a group of quite similar species.

New Guinean *Octospermum* (1 sp.) with 7–9-locular and indehiscent fruits groups with *M. discolor* (Australia). This result is supported by morphology as well: *M. discolor* together with *M. chromocarpus* Airy Shaw and *M. nesophilus* Müll.Arg. (both not sampled) forms a small group of New Guinean and Australian species, which shares

several characters with *Octospermum*: stipules absent, anther connectives broadened and fruits indehiscent. These *Mallotus* species have traditionally been placed in sect. *Philippinenses* (alternate leaves and unarmed fruits), but Sierra et al. (2005) noticed (considering only Malesian taxa) the morphological similarity of *M. chromocarpus* with *Octospermum pleiogynum* and excluded the former from sect. *Philippinenses*. This result is supported by our data, although the exact relationship of *Octospermum* and allies with this section needs further investigation.

Trewia nudiflora, the type species of Asian genus *Trewia* (2 spp.), differs from *Mallotus* primarily in its indehiscent, hard, somewhat fleshy, and often thick-walled fruits, which have no match in *Mallotus* and are possibly an adaptation to megafaunal dispersal (Dinerstein & Wemmer, 1988). Here we see a strongly supported sister group relationship of *Trewia nudiflora* with *M. khasianus*, a species whose distribution partially overlaps with *Trewia* (both occurring from India to Thailand). Although *M. khasianus* has the dehiscent, thin-walled capsules typical for *Mallotus*, these two species share an often deciduous habit and very long staminate inflorescences.

Neotrewia and *Octospermum* are clearly embedded in the *Mallotus* s.s. clade, and, because both *N. cumingii* and *O. pleiogynum* were originally described as *Mallotus* species, they can readily be merged with it again. The case of *Trewia* is less straightforward, as the clade of *Trewia nudiflora* and *M. khasianus* is placed as the sister to the rest of *Mallotus* s.s. clade. This raises the possibility of transferring *M. khasianus* to *Trewia* instead of merging the two genera (*Trewia* L. is the older generic name and has priority over *Mallotus*!). However, this position of the *Trewia-M.khasianus* clade does not appear in any of the individual gene analyses (whether analyzed by MP or BI) and is only supported by the combined BI analysis. More research is thus needed to clarify the issue.

Our *Mallotus* s.s. phylogeny allows only a limited assessment of the monophyly of the *Mallotus* sections (as defined by Airy Shaw, 1968). Sections *Mallotus*, *Polyadenii* and *Stylanthus* seem to be monophyletic, although this might change with further sampling. On the other hand, section *Philippinenses* is polyphyletic, even after the removal of *M. discolor* (see discussion above under *Octospermum*), although the polyphyly is not supported by the MP bootstrap.

Most of the *Mallotus* species belong to two sections with truly opposite leaves, *Axenfeldia* and *Rottleropsis*. These sections differ from each other by having either an penninerved or tripli/palminerved leaf venation, respectively. Our analyses with limited taxon sampling suggest that the venation character is homoplastic, and also that these two sections together do not form a clade. Members of *Mallotus* sect. *Hancea* (*Cordemoya* s.l. clade) also have opposite leaves, but in this case one leaf of a pair is stipuliform. Slik & Van Welzen (2001b) and Van Welzen et al. (2006) excluded five species from this section on the basis that one leaf of a pair is reduced but not stipuliform (one species excepted) and/or other characters, and suggested instead an affinity with sections *Axenfeldia* and *Rottleropsis*. As two sampled former *Hancea* species fall into the *Mallotus* s.s. clade, their separation from the sect. *Hancea* is confirmed here, although their relationship with the above-mentioned sections is still unclear.

Afro-Asian biogeography—Both *Macaranga* and *Mallotus* s.s. have disjunct distributions across the Indian Ocean basin. These patterns could be explained by vicariance

caused by the Gondwanan breakup, only if these genera date from the Cretaceous (the last major separation of Gondwanan elements, between Madagascar and Seychelles-Indian block, happened c. 95–84 million years ago; McLoughlin, 2001). We consider this unlikely because the *Acalyphoideae* s.l. clade (the clade in which *Macaranga*, *Mallotus* and related genera are nested, see Wurdack et al., 2005) has been estimated to have a crown group age of c. 70 Ma (Davis et al., 2005); the *Macaranga* and *Mallotus* s.s. clades are probably considerably younger (the inferred dates are, however, minimum ages based on fossil calibration points, and therefore an older, even Gondwanan, age for these clades cannot be completely ruled out). Moreover, instead of a basal split between the two geographical areas, the African and Madagascan taxa are in both cases clearly nested inside Asian (and Australasian) clades (Figs. 2.4 and 2.5). Therefore *Macaranga* and *Mallotus* s.s. clades should have had largely diversified already in Gondwana before the breakup in order to make the Gondwanan vicariance hypothesis possible.

The observed pattern of African and Madagascan taxa being nested in Asian clades is, therefore, more compatible with dispersal and/or migration from Asia to Africa and Madagascar. Three different scenarios can be hypothesized for these events; 1) a direct, and perhaps relatively recent, long-distance dispersal; 2) an Eocene-Oligocene dispersal from India to Madagascar through “Lemurian stepping stones” in the western Indian Ocean (Schatz, 1996); or 3) an overland migration from Asia through SW Asia and NE Africa before the Miocene aridification of the climate and subsequent disappearance of (sub)tropical forests in these areas (Zohary, 1973; Raven & Axelrod, 1974). Several leaf fossils from the Eocene onwards have been assigned to *Macaranga* and *Mallotus* (e.g., Akhmetiev & Vikulin, 1995; Horiuchi & Takimoto, 2001), but their identification is based on the leaf shape and venation pattern, and can therefore be questioned. However, a leaf fossil that can be confidently placed in the *Macaranga-Mallotus* clade was recently found in an Oligocenic stratum in NW Ethiopia (27.36 Ma; J.L. Garcia Massini, Southern Methodist University, Dallas, personal communication). This fossil shows two typical features for these genera: extra-floral nectaries and globose to disc-shaped glandular hairs. The leaf shape and long petioles suggest that it belongs to *Macaranga* (e.g., similar to extant East African *M. kilimandscharica* Pax, although *Mallotus* cannot be completely ruled out as an identification). This fossil suggests that African *Macaranga* are old enough to have reached the continent through the “Lemurian stepping stones” or by overland migration. A carefully conducted molecular dating analysis of *Macaranga*, *Mallotus* and relatives with multiple calibration points and smoothing methods might shed more light on this issue.

Although the exact scenario of dispersal or migration in *Macaranga* and *Mallotus* s.s. is unclear, the direction, from Asia to Africa and Madagascar, can be deduced from the phylogeny. Similar results have been found in biogeographical studies in rodents (Jansa et al., 1999) and in some Melastomataceae genera (Renner, 2004), but, on the other hand, many of the Afro-Asian biogeographical studies, both with plants and animals, have demonstrated an opposite direction of dispersal (Dayanandan et al., 1999; Raxworthy et al., 2002; Austin et al., 2004; Alejandro et al., 2005; Yuan et al., 2005). More studies are thus needed to find out whether one direction has been prevailing in dispersal between Asia and Africa and Madagascar; several other paleo- or pantropical euphorb genera, like *Acalypha*, *Claoxylon*, *Cleidion*, and *Croton*, could work as feasible study systems (for initial results in *Croton* see Berry et al., 2005).

Another remarkable result of our study is that the only two African species in the *Mallotus* s.s. clade, *Mallotus oppositifolius* and *M. subulatus* (the widespread former species occurs in Madagascar as well), are clearly not each other's closest relatives (Fig. 2.5; a result also supported by morphology; Sierra et al., 2007). This implies two separate introductions to Africa, a result contrasting with that of the *Macaranga* clade, where all 14 sampled (out of 36) African and Madagascan species form a distinct clade (although in the Bayesian analysis together with Indian *Macaranga indica*; see Fig. 2.4 and supplement S9).

Conclusions—In this study we used DNA sequence data to investigate the phylogeny of *Macaranga*, *Mallotus* and related genera. The results clarified the relationships among these genera and the question of the monophyly of *Macaranga* and *Mallotus*. *Macaranga* is a monophyletic genus, whereas *Mallotus*, as currently delimited, is distinctly paraphyletic. A new monophyletic *Mallotus* s.s. can be obtained by excluding sections *Hancea* and *Oliganthae*, which group together with the genera *Cordemoya* and *Deuteromallotus* in a separate basal clade, and including the genera *Coccoceras*, *Neotrewia*, *Octospermum* and (probably) *Trewia*. These taxonomic rearrangements, together with morphological treatments, are published in separate papers (Sierra et al., 2006; Kulju et al., 2007, Chapter 3). Insights into the infrageneric phylogeny of *Macaranga* were also gained and five mostly well-supported main clades were identified. On the other hand, the phylogeny of the *Mallotus* s.s. clade is still poorly known; additional studies of this group with new datasets and denser taxon sampling might help to resolve the issue. Biogeographically, the results of this study suggest that migrations or dispersals from Asia to Africa and Madagascar have happened both in *Macaranga* and *Mallotus* s.s. clades.

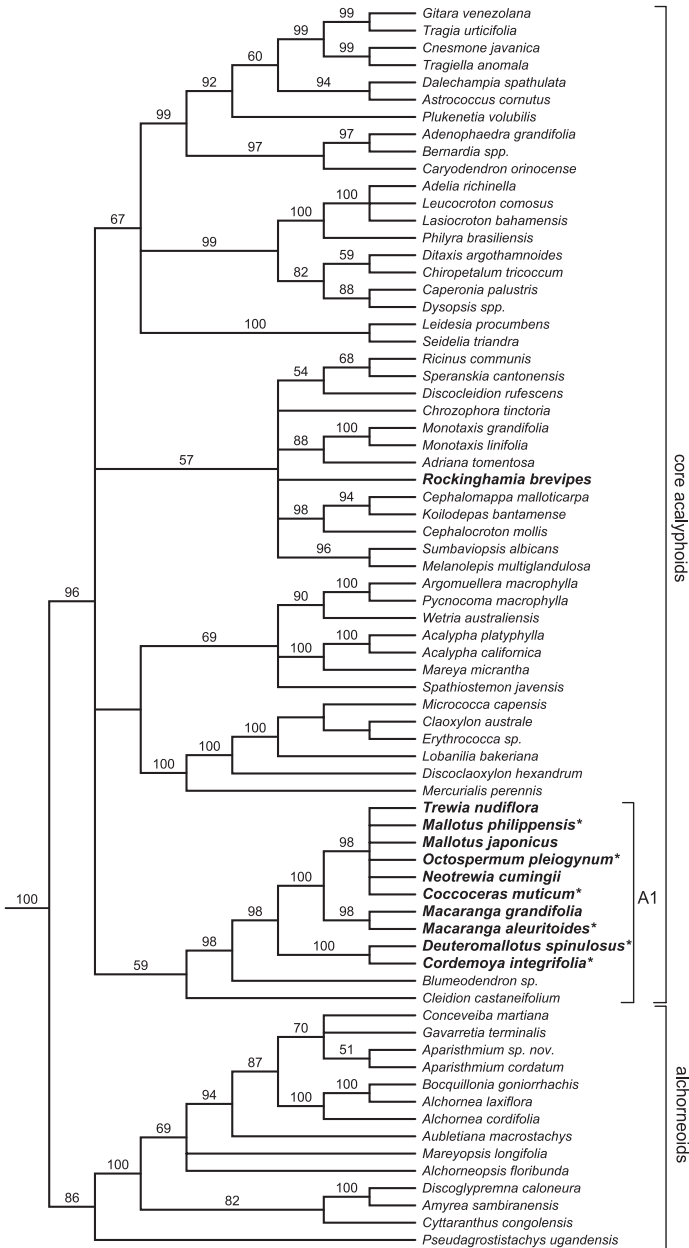
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APPENDICES

- Appendix 2.1 — The results of the pilot study.
- Appendix 2.2 — Voucher information.
- Appendix 2.3 — *trnL-F* tree, full taxan sampling.
- Appendix 2.4 — ITS tree, full taxan sampling.
- Appendix 2.5 — *ncpGS* tree, full taxan sampling.
- Appendix 2.6 — *phyC* tree, full taxan sampling.
- Appendix 2.7 — Maximum parsimony tree of the *Macaranga* clade.

Appendix 2.1—The results of the pilot study. Strict consensus of the parsimony analysis of the combined *trnL-F* and *rbcL* data in the Acalyphoideae s.s. (from the uniovulate euphorb dataset of Wurdack et al., 2005) with newly sequenced *Coccoceras*, *Cordemoya*, *Deuteromallotus*, *Neotrewia*, *Octospermum* and *Rockinghamia* (taxa marked with asterisk are sequenced for *trnL-F* only). Bold denotes taxa belonging to subtribes Rottlerinae and Macaranginae. Outgroups (the acalyphoid grade) omitted from the tree. Bootstrap values shown above branches. Clade names follow Wurdack et al. (2005).



Appendix 2.2—Voucher information. Voucher information and GenBank accession numbers for samples used in this study. A dash indicates the region was not sampled. In the cases of several samples per species, the first one listed was used in the analyses presented. Generic circumscriptions follow Webster (1994b). ^(a) indicates five *Macaranga* samples for which the actual voucher is missing; the voucher information given is for a specimen collected from the same locality, and confirmed to belong to the same species by the collector well familiar with the plant group. ^(b) indicates outgroup specimens for the individual analyses of the three main clades. ^(c) *Mallotus spinulosus* McPherson is clearly close to the two *Deuteromallotus* species, but has never been treated as part of the genus. Here we informally consider it as “*Deuteromallotus spinulosus*” for clarity.

Taxon— Genbank accessions: *trnL-F*, ITS, *ncpGS*, *phyC*; Origin; Voucher.

Blumeodendron calophyllum Airy Shaw — DQ899180, —, DQ901956, DQ767726; Indonesia, East Kalimantan, ITCI-concession; *Slik 2826 (L)*. *Blumeodendron kurzii* J.J.Sm. — DQ899181, DQ866525, DQ901957, DQ767727; Indonesia, Java, Bogor Botanic Garden IX.C.144; *Gravendeel et al. 521 (L)*.

Coccoceras muticum Müll.Arg. — DQ899183, DQ866527, DQ901959, DQ767729; Indonesia, East Kalimantan, Labanan; *Slik M1234 (L)*. *Coccoceras muticum* Müll.Arg. — DQ899182, DQ866526, DQ901958, DQ767728; Indonesia, East Kalimantan, ITCI-concession; *Slik M901 (L)*. *Cordemoya integrifolia* (Willd.) Baill. — DQ899184, DQ866528, DQ901960, DQ767730; Reunion, Mare Longue; *Coode 4958 (L)*. *Cordemoya integrifolia* (Willd.) Baill. — DQ899185, DQ866529, DQ901961, —; Mauritius, Piton de Milieu; *Lorenze 2231 (K)*.

Deuteromallotus baillonianus (Baill.) Pax & K.Hoffm. — DQ899186, DQ866530, DQ901962, DQ767731; Madagascar, Toamasina Prov., Soanierana Ivongo; *Ralimanana et al. 387 (K)*. *Deuteromallotus capuronii* Leandri — DQ899187, DQ866531, DQ901963, DQ767732; Madagascar, Fianarantsoa; *Rabenantoandro et al. 739 (MO)*. “*Deuteromallotus spinulosus*” (= *Mallotus spinulosus* McPherson) — DQ899188, DQ866532, DQ901964, DQ767733; Madagascar, Fianarantsoa; *Rabenantoandro & McPherson 681 (MO)*.

Macaranga albescens Perry — DQ899189, DQ866533, DQ901965, DQ767734; Papua New Guinea, Chimbu valley; *Sterly 80-350 (L)*. *Macaranga alchorneoides* Pax & Lingelsheim — DQ899190, DQ866534, DQ901966, DQ767735; New Caledonia, Province du Nord; *McPherson & Lowry 18526 (MO)*. *Macaranga aleuritoides* ^a F.Muell. — DQ899191, DQ866535, DQ901967, DQ767736; Papua New Guinea, Madang; *Weiblen 2049 (MIN)*. *Macaranga alnifolia* Baker — DQ899192, DQ866536, DQ901968, DQ767737; Madagascar, Toliara Prov.; *Hoffmann et al. 191 (K)*. *Macaranga angustifolia* K.Schum. & Lauterb. — DQ899193, DQ866537, DQ901969, DQ767738; Papua New Guinea, Morobe Prov., near Bubia; *Takeuchi & Ama 15542 (L)*. *Macaranga auriculata* (Merr.) Airy Shaw — DQ899194, DQ866538, —, DQ767739; Indonesia, East Kalimantan, ITCI-concession; *Slik M958 (L)*. *Macaranga barteri* Müll.Arg. — DQ899195, DQ866539, DQ901970, DQ767740; Ghana, Brong-Ahafo; *Jongkind & Nieuwenhuis 1604 (WAG)*. *Macaranga bicolor* Müll.Arg. — DQ899196, DQ866540, DQ901971, DQ767741; Philippines, Luzon, Los Baños, Mt. Makiling; *Fernando 1736 (L)*. *Macaranga bifoveata* J.J.Sm. — DQ899197, —, DQ901972, —; Papua New Guinea, Madang Prov., Ohu; *Novotny & Molem EUP258 (L)*. *Macaranga cf. brachytricha* Airy Shaw — DQ899198, DQ866541, DQ901973, —; Papua New Guinea, Madang Prov., Wannang; *Weiblen 1713 (L)*. *Macaranga clavata* Warb. — DQ899200, DQ866543, DQ901975, DQ767743; Papua New Guinea, East Sepik Province, April River; *Stancik 5122 (LAE)*. *Macaranga conifera* (Reichb.f. & Zoll.) Müll.Arg. — DQ899201, DQ866544, DQ901976, DQ767744; Indonesia, East Kalimantan, Bukit Bangkirai; *Slik M628 (L)*. *Macaranga densiflora* ^a Warb. — DQ899202, DQ866545, DQ901977, DQ767745; Papua New Guinea, Madang; *Weiblen 2022 (MIN)*. *Macaranga denticulata* (Blume) Müll.Arg. — DQ899203, DQ866546, DQ901978, DQ767746; Thailand, Eastern Floristic District, Nakhon Ratchasima Prov., Khao Yai National Park; *van Welzen 2003-20 (L)*. *Macaranga diepenhorstii* (Miq.) Müll.Arg. — DQ899204, DQ866547, DQ901979, DQ767747; Thailand, Peninsular Floristic Region, Thung Khai; *Chamchumroon 2017 (L)*. *Macaranga domatiosa* Airy Shaw — DQ899205, DQ866548, DQ901980, DQ767748; Papua New Guinea, Morobe Prov., Wau-area, Mt. Kaindi; *van Valkenburg 281 (L)*. *Macaranga ducis* ^a Whitmore — DQ899206, DQ866549, DQ901981,

DQ767749; Papua New Guinea, Madang; *Weiblen 2025 (MIN)*. *Macaranga echinocarpa* Baker—DQ899207, DQ866550, DQ901982, DQ767750; Madagascar, Toamasina; *Miller et al. 8765 (MO)*. *Macaranga gabunica* Prain—DQ899208, DQ866551, DQ901983, DQ767751; Gabon, Nyanga, chantier SFN, Igotchi; *van Valkenburg et al. 2611 (WAG)*. *Macaranga gigantea* (Reichb.f.& Zoll.) Müll.Arg.—DQ899209, DQ866552, DQ901984, DQ767752; Indonesia, East Kalimantan, Sungai Wain; *Slik M91 (L)*. *Macaranga grallata* McPherson—DQ899210, DQ866553, DQ901985, DQ767753; Madagascar, Toamasina Prov., Soanierana Ivongo; *Ralimanana et al. 408 (K)*. *Macaranga grandifolia* (Blanco) Merr.—DQ899211, DQ866554, DQ901986, DQ767754; Philippines, Luzon, Los Baños, Mt. Makiling; *Fernando 1737 (L)*. *Macaranga heterophylla* (Müll.Arg.) Müll.Arg.—DQ899212, DQ866555, DQ901987, DQ767755; Liberia, Grand Cape Mount; *Jongkind et al. 6135 (WAG)*. *Macaranga heynei* I.M.Johnson—DQ899214, DQ866556, DQ901989, DQ767756; Malaysia, W.Malaysia, Salangor/Pahaus, Genting Highlands; *Moog 98-011 (KAS)*. *Macaranga heynei* I.M.Johnson—DQ899213, —, DQ901988, —; Malaysia, W-Malaysia, road to Tanah Rata; *Moog 01-032 (L)*. *Macaranga cf. hispida* Müll.Arg.—DQ899199, DQ866542, DQ901974, DQ767742; Papua New Guinea, East Sepik, near Yuat river; *Weiblen 1831 (L)*. *Macaranga hullettii* King ex Hook.f.—DQ899215, DQ866557, DQ901990, DQ767757; Indonesia, East Kalimantan, Sungai Wain; *Slik M132 (L)*. *Macaranga hurifolia* Beille—DQ899216, DQ866558, DQ901991, DQ767758; Liberia, Sino, Sapu NP; *Jongkind et al. 5549 (WAG)*. *Macaranga inamoena*^b F.Muell. ex Benth.—DQ899217, DQ866559, DQ901992, DQ767759; Australia, Queensland, Cook district; *Forster 29763 (BRI, L)*. *Macaranga indica* Wight—DQ899218, DQ866560, DQ901993, DQ767760; India, Dindigul Dist., Kodaikanal, Palmi Hills; *Steward & Balcar RHT 55221 (L, RHT)*. *Macaranga induta* Perry—DQ899219, DQ866561, DQ901994, DQ767761; Papua New Guinea, Madang Prov., nr. Kaironk; *Weiblen et al. 1064 (L)*. *Macaranga involucrata* (Roxb.) Baill.—DQ899220, DQ866562, DQ901995, DQ767762; Australia, Queensland, Cook district; *Forster PIF29768 (BRI, L)*. *Macaranga klaineana* Pierre ex Prain—DQ899221, DQ866563, DQ901996, DQ767763; Gabon, Estuaire; *Breteler et al. 14289 (WAG)*. *Macaranga lamellata* Whitmore—DQ899222, DQ866564, DQ901997, DQ767764; Indonesia, East Kalimantan, ITCI-concession; *Slik M1060 (L)*. *Macaranga lowii* King ex Hook.f. *var. kostermansii* Airy Shaw—DQ899223, DQ866565, DQ901998, DQ767765; Indonesia, East Kalimantan, Bukit Bangkirai; *Slik M208 (L)*. *Macaranga lowii* King ex Hook.f. *var. lowii*—DQ899224, DQ866566, DQ901999, DQ767766; Indonesia, East Kalimantan, Sungai Wain; *Slik M56 (L)*. *Macaranga mauritiana* Bojer ex Müll.Arg.—DQ899225, DQ866567, DQ902000, —; Mauritius, Mt. Cocotte; *Lorence 2349 (K)*. *Macaranga monandra* Müll.Arg.—DQ899226, DQ866568, DQ902001, DQ767767; Gabon, Nyanga, Concession SFN; *van Valkenburg et al. 2531 (WAG)*. *Macaranga novo-guineensis*^a J.J.Sm.—DQ899227, DQ866569, DQ902002, DQ767768; Papua New Guinea, Madang; *Weiblen 1803 (MIN)*. *Macaranga oblongifolia* Baill.—DQ899228, DQ866570, DQ902003, DQ767769; Madagascar, Fianarantsoa; *McPherson & Rabenantoandro 18350 (MO)*. *Macaranga obovata* Baill.—DQ899229, DQ866571, DQ902004, DQ767770; Madagascar, Fianarantsoa; *McPherson & Rabenantoandro 18279 (MO)*. *Macaranga pachyphylla* Müll.Arg.—DQ899230, DQ866572, DQ902005, DQ767771; Thailand, Peninsular Floristic Region, Thung Khai; *Chamchumroon 2016 (L)*. *Macaranga pearsonii* Merr.—DQ899231, DQ866573, DQ902006, DQ767772; Indonesia, East Kalimantan, Sungai Wain; *Slik M104 (L)*. *Macaranga poggei* Pax—DQ899232, DQ866574, DQ902007, DQ767773; Gabon, Woleu-Ntem, Tchimbele; *Tabak & Feijen 10 (WAG)*. *Macaranga praestans* Airy Shaw—DQ899233, DQ866575, DQ902008, DQ767774; Brunai, Belait; *Challen et al. 31 (K)*. *Macaranga puncticulata* Gage—DQ899234, DQ866576, DQ902009, DQ767775; Indonesia, East Kalimantan, Labanan; *Slik M1179 (L)*. *Macaranga quadriglandulosa*^a Warb.—DQ899235, DQ866577, DQ902010, DQ767776; Papua New Guinea, Madang; *Weiblen 1853 (MIN)*. *Macaranga repando-dentata* Airy Shaw—DQ899236, DQ866578, DQ902011, DQ767777; Indonesia, East Kalimantan, ITCI-concession; *Slik M994 (L)*. *Macaranga rhizinoides* (Blume) Müll.Arg.—DQ899237, DQ866579, DQ902012, DQ767778; Indonesia, Java, Cibodas Botanic Gardens VII.C.64-64a; *Gravendeel et al. 676 (L)*. *Macaranga saccifera* Pax—DQ899238, DQ866580, DQ902013, —; Gabon, Ogooue-Ivindo; *Wieringa et al. 3565 (WAG)*. *Macaranga schweinfurthii* Pax—DQ899239, DQ866581, DQ902014, DQ767779; Gabon, Ogooue-Lolo; *Wieringa et al. 4088 (WAG)*. *Macaranga siamensis* S.J.Davies—DQ899240, DQ866582, DQ902015, DQ767780; Thailand, Eastern Floristic District, Nakhon Ratchasima Prov., Khao Yai National Park;

van Welzen 2003-16 (L). *Macaranga subdentata* Benth. — DQ899241, DQ866583, DQ902016, DQ767781; Australia, Queensland, near Wyvuri Swamp; *Forster et al. 24032* (L). *Macaranga tanarius* (L.) Müll.Arg. — DQ899242, DQ866584, DQ902017, DQ767783; Indonesia, East Kalimantan, Bukit Bangkirai; *Slik M705* (L). *Macaranga tanarius* (L.) Müll.Arg. — DQ899243, DQ866585, DQ902018, DQ767782; Australia, Queensland, Port Curtis District; *Forster 29660* (BRI, L). *Macaranga tessellata* Gage — DQ899244, DQ866586, DQ902019, —; Indonesia, Papua, Mt. Jaya, Mimika Regency; *Utteridge 329* (L). *Macaranga trichocarpa*^b (Reichb.f. & Zoll.) Müll.Arg. — DQ899245, DQ866587, DQ902020, DQ767784; Indonesia, East Kalimantan, Bukit Bangkirai; *Slik M398* (L). *Macaranga triloba* (Thunb.) Müll.Arg. — DQ899246, DQ866588, DQ902021, DQ767785; Indonesia, Java, Jawa Barat, Halimun National Park; *Gravendeel et al. 619* (L). *Macaranga umbrosa* S.J.Davies — DQ899247, DQ866589, DQ902022, DQ767786; Brunai, Tutong, Tasek Merimbun Heritage Park; *Challen et al. 41* (K). *Macaranga winkleri* Pax & K.Hoffm. — DQ899248, DQ866590, DQ902023, DQ767787; Indonesia, East Kalimantan, Bukit Bangkirai; *Slik M678* (L). *Mallotus barbatus* Müll.Arg. — DQ899249, DQ866591, DQ902024, DQ767788; Leiden Botanic Garden, acc. 920695; *Kulju 90* (L). *Mallotus brachythyrus* Merr. — DQ899250, DQ866592, DQ902025, DQ767789; Indonesia, East Kalimantan, ITCI-concession; *Slik M900* (L). *Mallotus caudatus* Merr. — DQ899251, DQ866593, DQ902026, DQ767790; Indonesia, East Kalimantan, Labanan; *Slik M1243* (L). *Mallotus claoxyloides*^b (F.Muell.) Müll.Arg. — DQ899252, DQ866594, DQ902027, DQ767791; Australia, Queensland, Port Curtis District; *Forster 29663* (BRI, L). *Mallotus connatus* M. Aparicio — DQ899279, DQ866620, DQ902050, DQ767816; Indonesia, Java, Bogor Botanic Gardens, IX.C.; *Gravendeel et al. 522* (L). *Mallotus decipiens* Müll.Arg. — DQ899253, DQ866595, DQ902028, DQ767792; Thailand, Southwestern, Prachuap Khiri Khan, Kaeng Kra Chan National Park; *Middleton et al. 1104* (L). *Mallotus decipiens* Müll.Arg. — DQ899254, DQ866596, DQ902029, DQ767793; Thailand, Southwestern, Prachuap Khiri Khan, Kaeng Kra Chan National Park; *Middleton et al. 1065* (L). *Mallotus discolor* F.Muell. ex Benth. — DQ899255, DQ866597, DQ902030, DQ767794; Australia, Queensland, Port Curtis District; *Forster 29659* (BRI, L). *Mallotus eucaustus* Airy Shaw — DQ899256, DQ866598, DQ902031, DQ767795; Indonesia, East Kalimantan, Labanan; *Slik M1085* (L). *Mallotus ficifolius* (Baill.) Pax & K.Hoffm. — DQ899257, DQ866599, DQ902032, DQ767796; Australia, Queensland, Cook district; *Forster PIF29782* (BRI, L). *Mallotus glomerulatus* Welzen — DQ899258, —, —, —; Thailand, Northeastern, Nakhon Phanom Prov., Phu Langka National Park; *Koonkhanthod et al. 517* (L). *Mallotus griffithianus*^b (Müll.Arg.) Hook.f. — DQ899259, DQ866600, DQ902033, DQ767797; Indonesia, East Kalimantan, Labanan; *Slik M1076* (L). *Mallotus khasianus* Hook.f. — DQ899260, DQ866601, DQ902034, DQ767798; Thailand, Northern, Nan province, Doi Phu Ka National Park; *Kessler PK3276* (L). *Mallotus lackeyi* Elmer — DQ899261, DQ866602, DQ902035, DQ767799; Indonesia, East Kalimantan, ITCI-concession; *Slik M912* (L). *Mallotus leucocalyx* Müll.Arg. — DQ899262, DQ866603, —, DQ767800; Thailand, Surat Thani Prov., Phanom Dist., Khlongphanom National Park; *Middleton et al. 2122* (L). *Mallotus macrostachyus* (Miq.) Müll.Arg. — DQ899263, DQ866604, DQ902036, DQ767801; Indonesia, East Kalimantan, Bukit Bangkirai; *Slik M262* (L). *Mallotus miquelianus* (Scheff.) Boerl. — DQ899264, DQ866605, DQ902037, DQ767802; Indonesia, East Kalimantan, ITCI-concession; *Slik M879* (L). *Mallotus oppositifolius* (Geisel.) Müll.Arg. — DQ899265, DQ866606, DQ902038, DQ767803; Gabon, Ngounié, Sindara; *Wieringa et al. 4384* (WAG). *Mallotus pallidus* (Airy Shaw) Airy Shaw — DQ899266, DQ866607, DQ902039, DQ767804; Thailand, Southwestern, Prachuap Khiri Khan, Khao Sam Roi Yot National Park; *Middleton et al. 1136* (L). *Mallotus paniculatus*^b (Lam.) Müll.Arg. — DQ899267, DQ866608, DQ902040, DQ767806; Indonesia, East Kalimantan, Sungai Wain; *Slik M144* (L). *Mallotus paniculatus* (Lam.) Müll.Arg. — DQ899268, DQ866609, DQ902041, DQ767805; Australia, Queensland, Cook district; *Forster 29762* (BRI, L). *Mallotus peltatus* (Geisel.) Müll.Arg. — DQ899269, DQ866610, DQ902042, DQ767807; Indonesia, East Kalimantan, ITCI-concession; *Slik M896* (L). *Mallotus penangensis* Müll.Arg. — DQ899270, DQ866611, DQ902043, DQ767808; Indonesia, East Kalimantan, Gunung Meratus; *Slik M845* (L). *Mallotus philippensis* (Lam.) Müll.Arg. — DQ899272, DQ866613, DQ902045, DQ767809; Australia, Queensland, Port Curtis District; *Forster 29664* (BRI, L). *Mallotus philippensis* (Lam.) Müll.Arg. — DQ899271, DQ866612, DQ902044, DQ767810; Indonesia, Java, Bogor Botanic Gardens, IX.C.23; *Gravendeel 504* (L). *Mallotus philippensis* (Lam.) Müll.Arg. — DQ899273, DQ866614, —, DQ767811; Sri

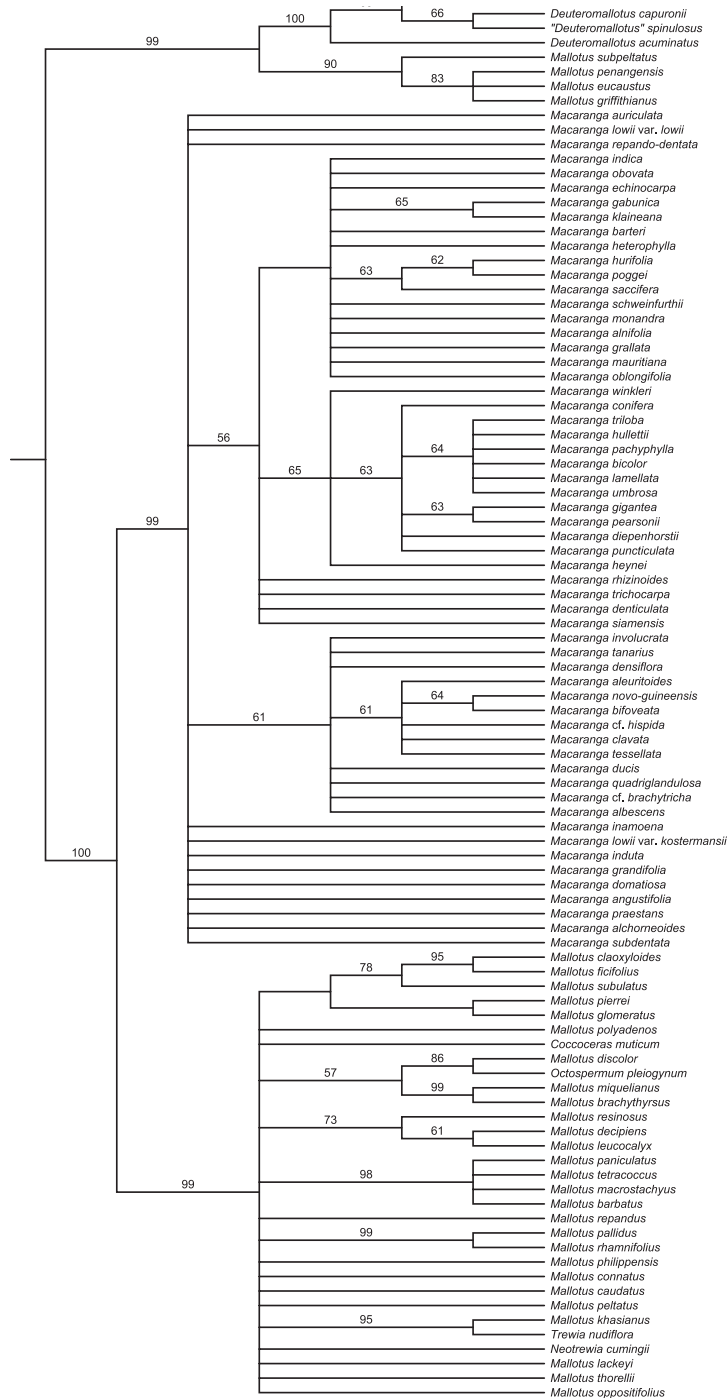
Lanka, Matale Distr., Illukkumbura; *Kathriarachchi et al. 64 (K, WU)*. *Mallotus pierrei* (Gagnep.) Airy Shaw— DQ899274, DQ866615, DQ902046, —; Thailand, Southwestern, Prachuap Khiri Khan, Huay Yang National Park; *Middleton et al. 1320 (L)*. *Mallotus polyadenos* F.Muell. — DQ899275, DQ866616, DQ902047, DQ767812; Australia, Queensland, Port Curtis District; *Forster 29780 (BRI, L)*. *Mallotus repandus* (Rottler) Müll.Arg. — DQ899276, DQ866617, DQ902048, DQ767813; Indonesia, Java, Bogor Botanic Gardens XV.C.20-20a; *Gravendeel et al. 515 (L)*. *Mallotus resinosus* (Blanco) Merr. — DQ899277, DQ866618, DQ902049, DQ767814; Sri Lanka, Kurunegala distr., Weuda; *Kathriarachchi et al. 67 (K, WU)*. *Mallotus rhamniifolius* (Willd.) Müll.Arg. — DQ899278, DQ866619, —, DQ767815; Sri Lanka, Ratnapura Distr., Puwakgahawala; *Kathriarachchi et al. 38 (K, WU)*. *Mallotus subpeltatus* (Blume) Müll.Arg. — DQ899280, DQ866621, DQ902051, DQ767817; Thailand, Peninsular, Krabi, Khao Phanom Bencha National Park; *Middleton et al. 494 (L)*. *Mallotus subulatus* Müll.Arg. — DQ899281, DQ866622, —, —; Cameroon, South West Prov., Fako, Buea; *Wheatley 16 (K)*. *Mallotus tetracoccus* Kurz— DQ899282, DQ866623, DQ902052, DQ767818; Sri Lanka, Matale Distr., Knuckles; *Kathriarachchi et al. 2 (K, WU)*. *Mallotus thorellii* Gagnep. — DQ899283, DQ866624, DQ902053, DQ767819; Cambodia, Kampong Speu Prov., Chabarmon dist.; *Huq et al. 10865 (L)*.

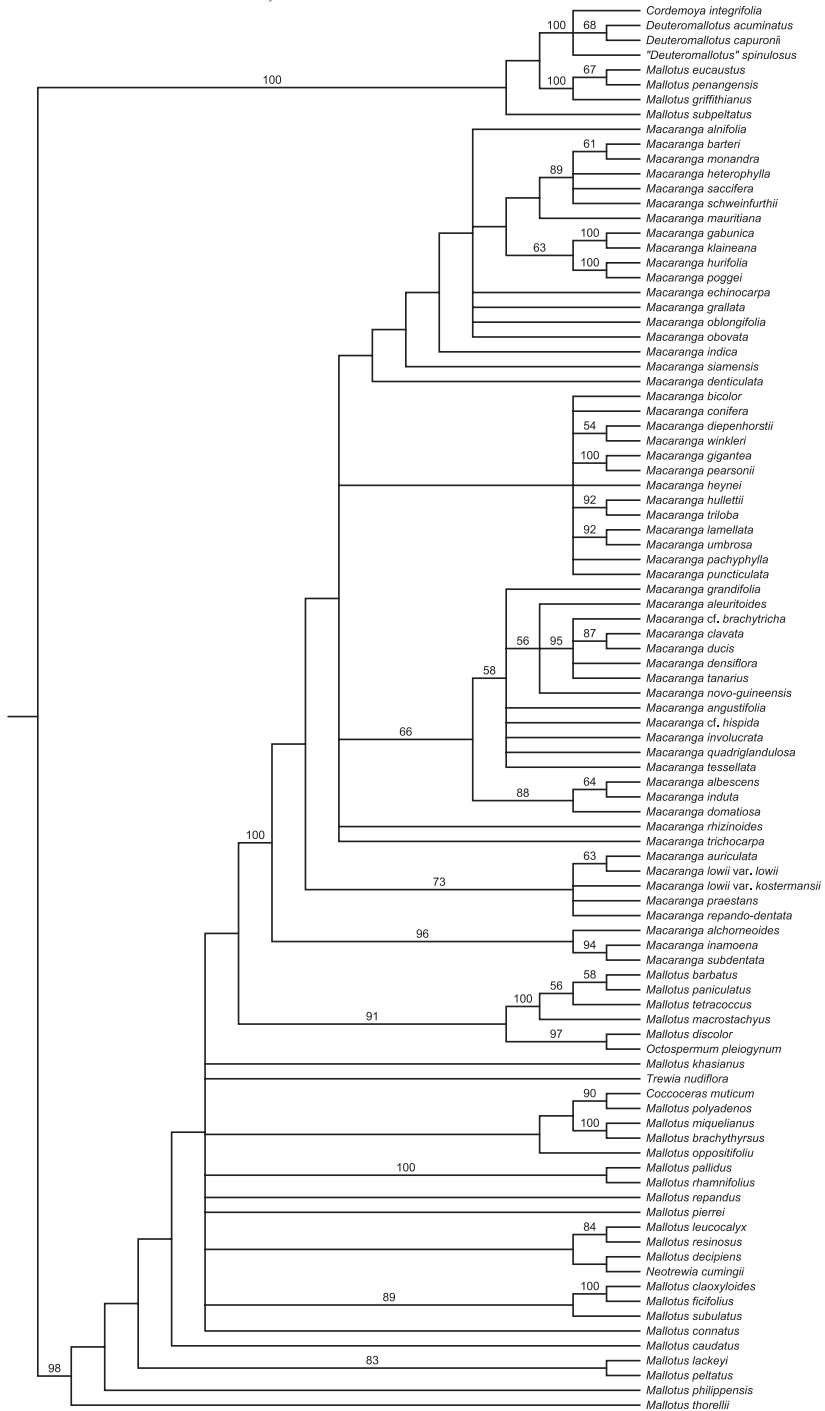
Neotrewia cumingii (Müll.Arg.) Pax & K.Hoffm. — DQ899284, DQ866625, DQ902054, DQ767820; Philippines, Luzon, Los Baños, Mt. Makiling; *Fernando 1735 (L)*.

Octospermum pleiogynum (Pax & K.Hoffm.) Airy Shaw— DQ899285, DQ866626, DQ902055, DQ767821; Indonesia, Irian Jaya, Bird's Head Peninsula; *Polak NT11610 (L)*.

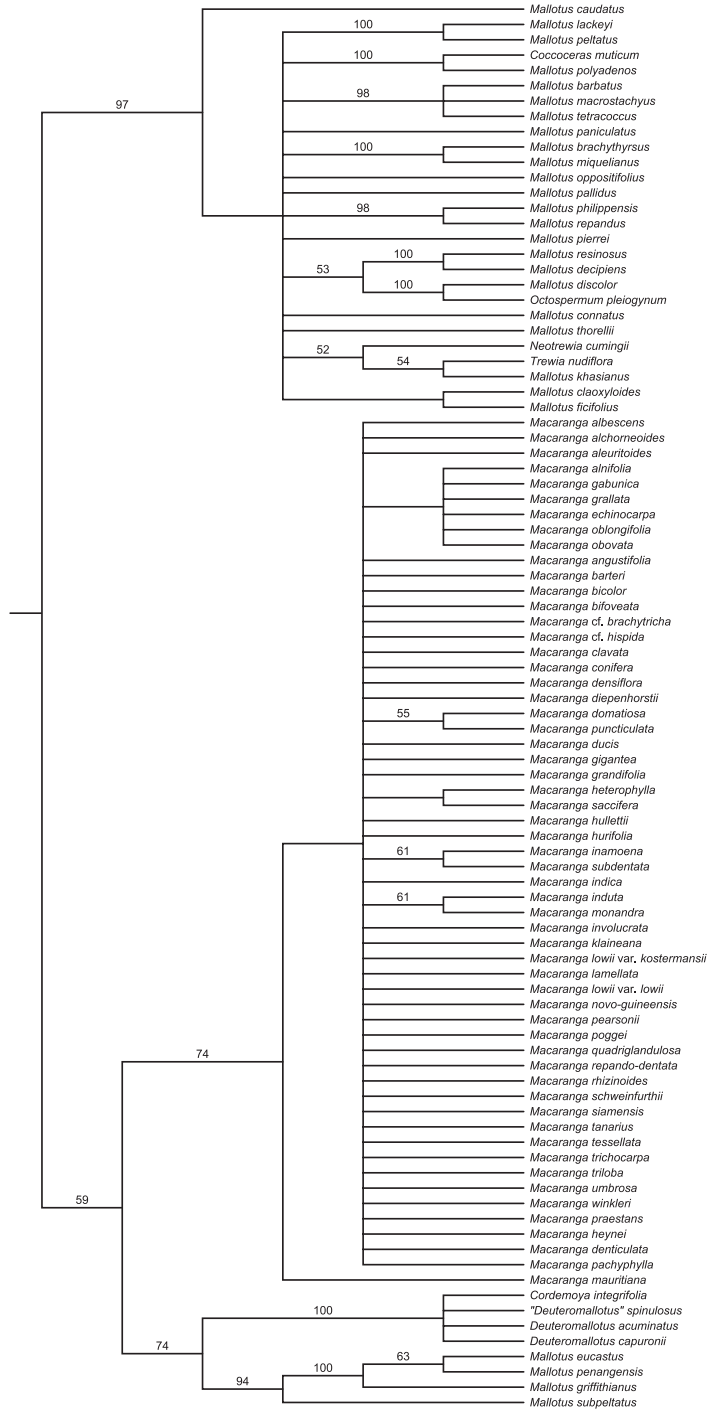
Trewia nudiflora L. — DQ899286, DQ866627, DQ902056, DQ767823; Thailand, Central Floristic District, Saraburi Prov., Phu Khae Botanical Garden; *van Welzen 2003-5 (L)*. *Trewia nudiflora* L. — DQ899287, DQ866628, DQ902057, DQ767822; India, Tiruchi Dist., Srirangam; *Perianayagam RHT 74579 (L, RHT)*.

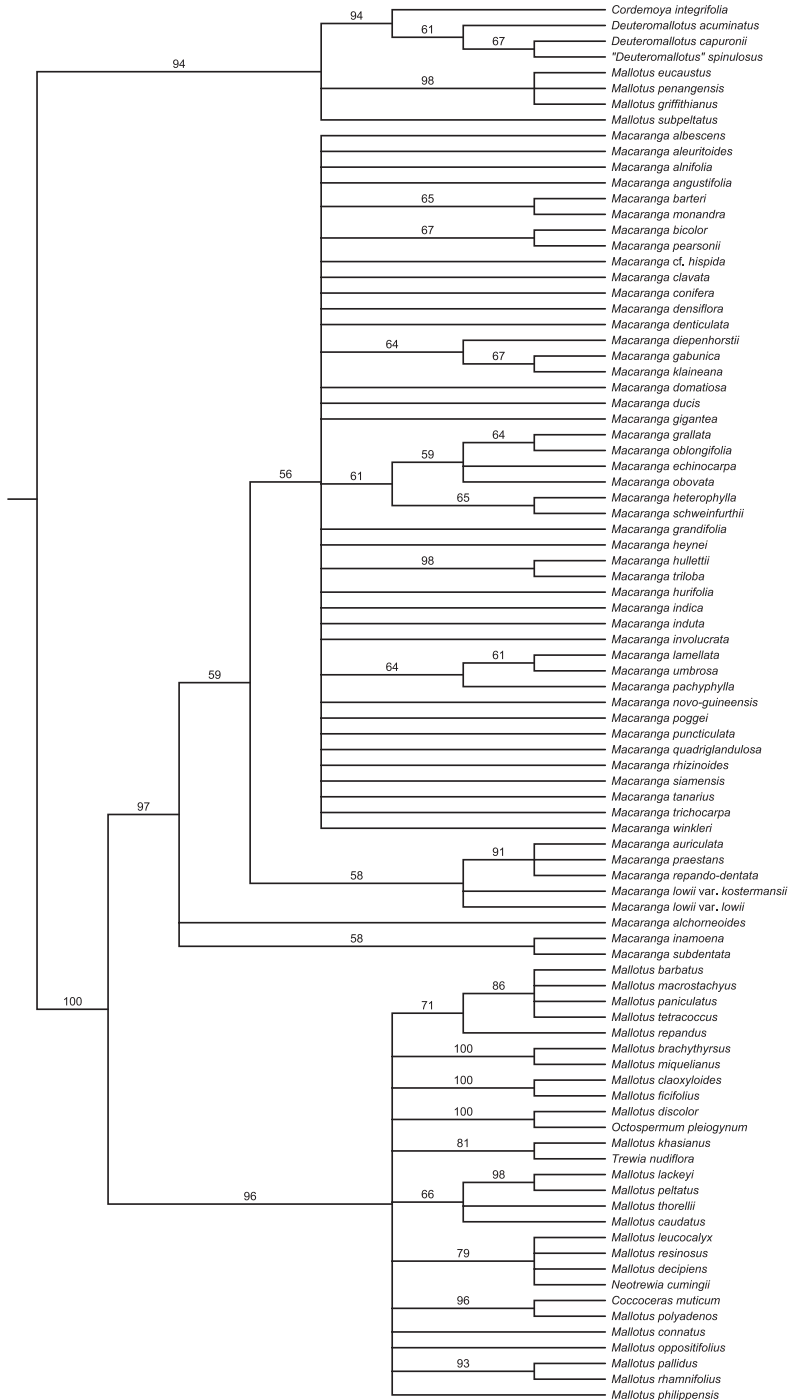
Appendix 2.3—*TrnL-F* tree, full taxon sampling (maximum parsimony strict consensus, bootstrap values >50 shown above branches).



Appendix 2.4—ITS tree, full taxon sampling (maximum parsimony strict consensus, bootstrap values >50 shown above branches).

Appendix 2.5—*nepGS* tree, full taxon sampling (maximum parsimony strict consensus, bootstrap values >50 shown above branches).



Appendix 2.6—*phyC* tree, full taxon sampling (maximum parsimony strict consensus, bootstrap values >50 shown above branches).

Appendix 2.7—Maximum parsimony tree of the *Macaranga* clade, combined dataset of four markers (*trnL-F*, ITS, *ncpGS*, *phyC*). Strict consensus with bootstrap values >50 shown above branches.

