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MOLECULAR PHYLOGENY OF *MACARANGA*, *MALLOTUS*, AND RELATED GENERA (EUPHORBIACEAE S.S.): INSIGHTS FROM PLASTID AND NUCLEAR DNA SEQUENCE DATA[1]

KRISTO K. M. KULJU,[2,4] SORAYA E. C. SIERRA,[2] STEFANO G. A. DRAISMA,[2] ROSABELLE SAMUEL,[3] AND PETER C. VAN WELZEN[2]

[2]Nationaal Herbarium Nederland, Leiden University branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands; and
[3]Department of Systematic and Evolutionary Botany, Universität Wien, Rennweg 14, A-1030 Vienna, Austria

Macaranga and *Mallotu*s (Euphorbiaceae s.s.) are two closely related, large paleo(sub)tropical genera. To investigate the phylogenetic relationships between and within them and to determine the position of related genera belonging to the subtribe Rottlerinae, we sequenced one plastid (*trnL-F*) and three nuclear (ITS, *ncpGS*, *phyC*) markers for species representative 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 data sets. 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.

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*; Radcliffe-Smith, 2001; Whitmore, in press) 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 forest disturbance (Slik et al., 2003). Moreover, both genera have an array of interesting morphological features, most striking undoubtedly being the ant-housing adaptations 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 clarify the biogeography and taxonomic delimitations in *Mallotus* and *Macaranga*.

In the Euphorbiaceae classifications of Webster (1994) and Radcliffe-Smith (2001), *Macaranga* and *Mallotus* are placed in the tribe Acalypheae of the uniovulate subfamily Acalyphoideae. *Mallotus* is a member of the subtribe Rottlerinae, together with seven other genera (Table 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 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 (two in *Mallotus*, three or four in *Macaranga*).

The seven genera classified with *Mallotus* in the subtribe Rottlerinae (Table 1) each contain only 1–5 species. Most of them have close affinities with *Mallotus* and have been previously treated as congeneric with it. Airy Shaw (1963) 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*) closely resemble *Mallotus*. 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).

The phylogenetic relationships between *Macaranga*, *Mallo-*

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[4] Author for correspondence (e-mail: kulju@nhn.leidenuniv.nl)

TABLE 1. Distribution and some typical morphological characters for *Mallotus*, other genera in the subtribe Rottlerinae, and *Macaranga* (Webster, 1994). The number of known species per genus and the number of sampled species are given in parentheses following the generic name.

Genus (spp. total/sampled)	Distribution	Typical morphological characters
Coccoceras Miq. (4/1)	Myanmar to Borneo	Fruits indehiscent and often with horn-like processes (otherwise resembling *Mallotus*)
Cordemoya Baill. (1/1)	Mascarene Is.	Conspicuous glandular hairs absent
Deuteromallotus Pax & K.Hoffm. (3[a]/3)	Madagascar	Conspicuous glandular hairs absent
Macaranga 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
Mallotus 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
Neotrewia Pax & K.Hoffm. (1/1)	Borneo, Sulawesi, Philippines	Fruits indehiscent, 1-locular (otherwise resembling *Mallotus*)
Octospermum Airy Shaw (1/1)	New Guinea	Fruits indehiscent, 7–9-locular (otherwise resembling *Mallotus*)
Rockinghamia Airy Shaw (2/2[c])	Australia (Queensland)	Leaves pseudoverticillate, inflorescences regularly bisexual, styles often bifid
Trewia L. (2/1)	India to West Malesia	Fruits indehiscent, 3–5-locular (rarely dehiscent and 2-locular; otherwise resembling *Mallotus*)

[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 and Methods).

tus, 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 Pycnocomeae). 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 one or two species were sampled from each of the aforementioned 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 and Van Welzen, 2001a). According to this morphological phylogenetic study, *Mallotus* is paraphyletic for two reasons. First, *Mallotus* sections *Hancea* and *Oliganthae* (Table 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 three *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 to taxa ratio (76/50) and a high number of polymorphic characters (50 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 and Van Welzen, 2001a).

Macaranga, *Mallotus*, and related small genera have an intriguing distribution pattern (Tables 1 and 2): the group is Asia-centered—most species occur 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 scenarios (e.g., vicariance following the breakup of the supercontinent Gondwana or dispersal/migration from the ancestral distribution area). As the first step to resolve these biogeographical questions, the phylogenetic relationships between species on the western and eastern sides of the Indian Ocean need to be investigated.

For both *Macaranga* and *Mallotus*, infrageneric classifications exist (Table 2), but they are far from satisfactory and not based on a phylogenetic framework. In their revision of *Macaranga*, Pax and 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 he could not finish the monograph during his lifetime. However, in a manuscript being published as a prodromus (Whitmore, in press), a new subdivision of *Macaranga* is presented. Apart from three previously clearly established sections (*Pachystymon*, *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 and Hoffmann.

The first sectional delimitations of *Mallotus* were made by Müller (1865, 1866; five sections) and by Pax and 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 and Van Welzen, 2001b; Sierra and Van Welzen, 2005; Sierra et al., 2005, 2007; Van Welzen and Sierra, 2006; Van Welzen et al., 2006). 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

TABLE 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 underlined.

Genus Infrageneric group	Distribution	Species total	Species sampled
Macaranga			
"African"[a]	Africa	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
Mauritiana	Mauritius	1	1
Oblongifolia	Madagascar, Comoros	10	5
sect. *Pachystemon*	From Nicobars and Indochina to Borneo and Philippines	25	4
sect. *Pruinosae*	From Burma, Andamans, and Nicobars to Borneo and Sulawesi	9	3
sect. *Pseudorottlera*	From India to New Guinea and Australia	15	6
Tanarius	From Sulawesi to Fiji, Samoa, and Tonga	14	4
Winkleri	Borneo	2	1
Mallotus			
sect. *Axenfeldia*	Asia	≥17[b]	3
sect. *Hancea*	From southern China to New Guinea	12(+5)[c]	3(+2)[c]
sect. *Mallotus*	From India to Australia and Solomon Islands	c.10	4
sect. *Oliganthae*	From Burma to Borneo and Java	1	1
sect. *Philippinenses*	From Pakistan to Australia and New Caledonia	5(+3)[d]	4(+1)[d]
sect. *Polyadenii*	From India to Australia and Solomon Islands	8	1
sect. *Rottleropsis*	Asia, Africa (2 spp.)	≥40[b]	9
sect. *Stylanthus*	From India to Australia and Solomon Islands	6	3

[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 and van Welzen, 2001b).
[d] In parentheses species related to *M. chromocarpus*, a species excluded from sect. *Philippinenses* (Sierra et al., 2005).

into two sections only by the character of penninerved or tripli/palminerved leaves. In the morphological phylogenetic analysis (Slik and Van Welzen, 2001a), many of the sections were indicated to be nonmonophyletic, but low levels of support prevented definitive conclusions.

The aim of this study was to reconstruct the phylogeny of *Mallotus*, *Macaranga*, and related small genera to address the following questions: (1) Are *Macaranga* and *Mallotus* monophyletic, or is *Macaranga* nested within *Mallotus* as suggested by Slik and Van Welzen (2001a)? (2) Is the merging of *Coccoceras* and *Deuteromallotus* with *Mallotus* (described earlier) 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 in Africa, Madagascar, and the Mascarene Islands related to those in the Asia-Pacific, and what kind of biogeographical scenario could explain this Afro-Asian distribution pattern?

To answer these questions, we have sequenced four DNA markers, from both plastid and nuclear genomes, sampling thoroughly the study genera and the infrageneric groups of *Macaranga* and *Mallotus*. 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 (*ncpGS*) 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 had 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, 1994) are, in fact, closely related to *Macaranga* and *Mallotus*. Representatives of these genera were sequenced for the *rbcL* and/or *trnL-F* genes (data not shown). A maximum parsimony analysis with the large uniovulate Euphorbiaceae data set (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* (Appendix S1, see Supplemental Data accompanying the online version of this article). Therefore, *Rockinghamia* was excluded from subsequent analyses, and *Blumeodendron* was selected as the outgroup. Additional analyses of the individual gene data sets with more distant outgroup

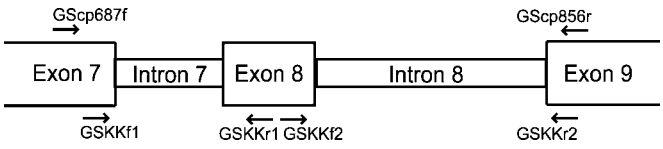

Fig. 1. Primers used in PCR and sequencing of *ncpGS*. Published primers (Emshwiller and Doyle, 1999) are above, the newly designed primers below.

taxa (e.g., *Cleidion*; data not shown) were either cumbersome because of divergent, barely alignable outgroup sequences or had 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 (see also the number of species sampled per genus or infrageneric group in Tables 1 and 2). The taxon sampling includes nearly all satellite genera (except *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 infraspecific 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 (with a prolonged lysis step with proteinase K and β-mercaptoethanol; 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×) or cleaned with PCR cleaning columns (see later in Laboratory methods) to facilitate PCR.

The marker *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 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 and Doyle, 1999). However, because these primers worked poorly with some taxa, a set of new primers was designed for the study group (Fig. 1):

GSKKf1 [5′-GGCACCAATGGAGAGGTTAT-3′],
GSKKf2 [5′-GATCACATCTGGTGTGCWAG-3′],
GSKKr1 [5′-AGCTTCAATTCCCACRCTGG-3′], and
GSKKr2 [5′-YAACACCAGCYTGTTCWGTGA-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-μL reactions using 0.2–2.0 μL of total DNA extract as a template. The reaction mixture also contained 1× PCR Buffer (Qiagen, Hilden, Germany), 20 pmol of each primer, 5 nmol dNTPs, 0.5 μg 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; 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) 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 a template in a reamplification 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, Foster City, California, USA) and Sephadex G50 AutoSeq columns (GE Healthcare, Diegem, Belgium) for reaction cleaning, or by external service (using ABI 3730xl; Applied Biosystems).

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 infraindividual 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 was coded with IUPAC ambiguity codes.

PCR products were cloned to facilitate the sequencing of a few difficult samples and to further determine infraindividual polymorphisms 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 from insert-containing colonies were directly used as a template for standard PCR with M13 forward and reverse primers. The resulting products were size-selected using agarose gel electrophoresis. Three clones per individual were sequenced as described earlier.

Sequence alignment and indel characters—The sequences were aligned either completely by eye using MacClade version 4.08 (Maddison and Maddison, 2001) and Bioedit version 7.0.5.2 (Hall, 1999), or with the multiple sequence alignment algorithm of ClustalW version 1.81 (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 be 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, and possible substitutions within, were considered to have uncertain homologies. Therefore, the variation in mononucleotide repeats of 6 bp or longer 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, then this information was used to place the gap. (4) Because undetected inverted repeats can bias phylogenetic analysis (Quandt et al., 2003), we specifically looked for them in the alignment. (5) In the cases of overlapping gaps, the gaps were placed in a way to minimize the total number of indel events. (6) Sometimes a gap could be reasonably placed in two or more positions. If the choice could affect the phylogenetic analyses, question marks or ambiguity codes were introduced in the data matrix to account for this uncertainty while still preserving as much phylogenetic information as possible. (6) Ambiguously alignable regions with uncertain homologies were excluded.

The indel information from the 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. Simple indel coding (SIC; Simmons and 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 an alternative model-based phylogeny method.

For MP analyses, PAUP* version 4.0b10 (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 tree-bisection-reconnection (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. Because only a moderate exploration of tree space is necessary for estimating

TABLE 3. Summary of alignment properties.

DNA region	No. taxa sampled	Sequence length	No. polymorphic sites/ sequence (average)	Nucleotide characters			Indel characters	
				Alignment length	No. excluded	No. informative	No. total	No. informative
trnL-F	99	897–1160	0	1343	179	108 (9.3%)	53	28
ITS (+5.8S)	96	712–747	0–10 (1.3)	812	85	241 (33.2%)	54	22
ncpGS	94	320–910	0–6 (0.4)	1000	38	161 (16.7%)	56	32
phyC	91	632–644	0–14 (0.9)	644	0	119 (18.5 %)	2	0

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 a support measure was done with MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The model of molecular evolution for each gene data set was selected using the Akaike information criterion (AIC) as implemented in MrModel test version 2.2 (Nylander, 2004); for advantages of AIC over hierarchical likelihood ratio test (hLRT), see Posada and 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 Results), 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 three heated and one cold chain with default temperature (0.2). Markov chains were sampled every 100th generation. Analyses were run until the average standard deviation of the 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 data set, 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 the combined data set of the *Mallotus* s.s. clade (see Results) was conducted with PHYML v.2.4.4 (Guindon and Gascuel, 2003); the GTR+G+I model with four rate categories and 500 pseudoreplicates was used. Model parameters were estimated from data for the whole concatenated data set (because PHYML does not allow splitting a data set 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 the outgroup. Second, each of the three main clades found in the analyses of all taxa (see Results) were analyzed individually, with one or two species selected from two other clades to serve as an outgroup (indicated in the Appendix). In both of these cases, four markers were first analyzed separately, and the results were screened for hard incongruences (i.e., incongruences with bootstrap support >70%; Hillis and Bull, 1993) before the combined analysis of all four data sets. 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 and Lecointre, 2002; see also Hipp et al., 2004). Moreover, because combining incongruent data sets can sometimes lead to a more robust phylogeny (Sullivan, 1996; Flynn and 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): data sets with hard incongruences can be combined, but parts of the resulting tree that are in strongly supported conflict between data sets should be regarded as questionable.

The internal conflict within each data set was inspected with the consensus network approach (Holland and Moulton, 2003; Holland et al., 2005). This tree-based method visualizes 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 data set. For this analysis, SplitsTree version 4.3 was used (Huson and Bryant, 2006), with threshold proportion $x = 0.1$.

RESULTS

Sequence characteristics—Properties of the sequence data sets of each marker are given in Table 3. For a few taxa, some of the nuclear markers could not be sequenced (see Appendix), mainly because of 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 fully covered the sequence contigs. In this respect, *ncpGS* was more problematic. In several cases, the chromatogram quality dropped drastically after mononucleotide repeats; consequently, parts of the *ncpGS* contigs were based on a single direction only. In these cases, the chromatograms were closely inspected, and the sequencing reaction repeated if required to obtain an unambiguous result.

As expected, the markers with both coding and noncoding parts (ITS+5.8S, *ncpGS*) had more variation in noncoding regions. Also, the exons of the protein-coding genes *ncpGS* and *phyC* were most variable at 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 the phylogenetic results later).

Sequence alignment and the indel characters—The alignments are available online as supplemental data in Appendices S2–S5. Insertion of gaps was required to align all noncoding 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 intron 8. Because of these indel events, the *ncpGS* sequences of the *Cordemoya* s.l. and *Macaranga* clades (see the phylogenetic results described later) 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. Within the three main clades (see the phylogenetic results described later), 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 data sets, but their number was generally low (Table 3), and visual inspection of the alignments revealed no clear additive patterns possibly indicating hybridization (e.g., Sang et al., 1995). Two ITS sequences with 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*, two out of five) of the putative polymorphisms (data not shown). Additionally, one 2 bp 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 near 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.

Two *ncpGS* samples were also cloned to confirm infra-individual polymorphisms, and, especially, to investigate whether sequencing problems related to mononucleotide repeats (described earlier) 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 to 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 (for detailed trees, see the online Appendices S6–S9). *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 data sets for individual analyses of main clades (without the more distant outgroup *Blumeodendron*) was easier than aligning data sets 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 in the following paragraphs (the results of the single-marker analyses are not depicted, but the reader can refer to the online Appendices S6–S9 for 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. 3) also have 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 incongruences between these data sets, and combining them (Fig. 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. 4), 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 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 (which concern the placements of *M. bicolor* and *M. trichocarpa*), the reader can compare the topologies in Fig. 4 (BI) and in the online Appendix 10S.

Mallotus s.s. clade—The ITS data set 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 data sets 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 data sets: 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. resinosus* 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. 5. Trees obtained by BI and MP analyses are congruent (except for one difference in the clade consisting of *M. leucocalyx*, *M. resinosus*, and *M. decipiens*),

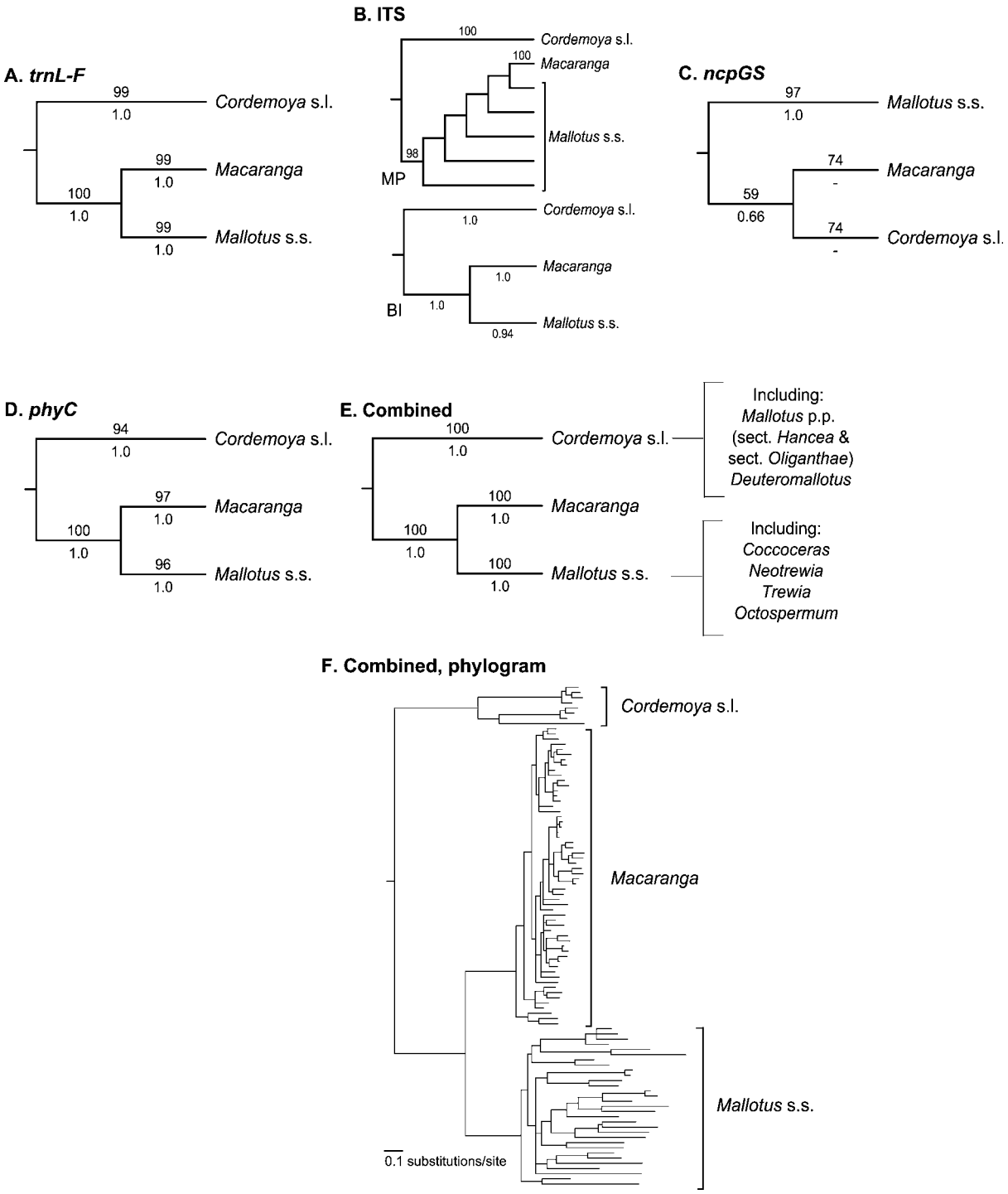

Fig. 2. Summary of the phylogenetic analyses with all sampled taxa, showing the relationships of the major clades. (A–D) Single-marker analyses. (E) Combined analysis of all four markers. (F) Bayesian phylogram from the combined analysis. MP, maximum parsimony; BI, Bayesian inference. MP bootstrap values are above the branches, BI posterior probabilities below. The outgroup (*Blumeodendron*) is not shown.

the BI tree being more resolved. The basal nodes of the MP tree are essentially not supported, whereas BI analysis gives support (often strong) 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.

DISCUSSION

Phylogenetic analysis methods and support values—Although unweighted Maximum Parsimony (MP) and model-based Bayesian Inference (BI) are fundamentally different methods, analyzing our data sets with them resulted to a large

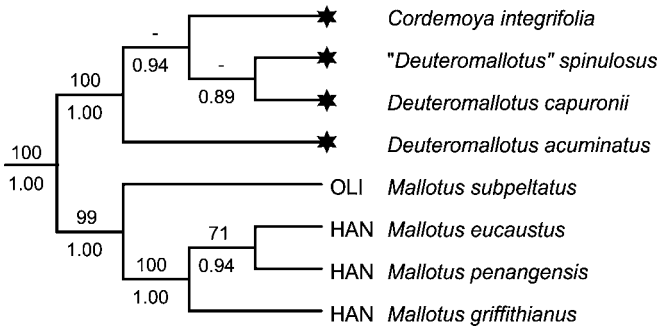

Fig. 3. Phylogenetic relationships inferred from the individual analysis of *Cordemoya* s.l. clade (see Fig. 2) with the combined data set of four markers. A Bayesian majority consensus tree is shown with parsimony bootstrap values above the branches and posterior probabilities below. A hyphen (-) indicates that the node does not exist in parsimony strict consensus. Infrageneric group (see Table 2) indicated with three letter abbreviation. Species occurring at western side of Indian Ocean (Africa, Madagascar, Mascarenes) are indicated with stars.

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 and 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 (discussed later in detail). 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 optimality criteria for phylogeny reconstruction.

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 (1994) 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).

First, all markers agree on the monophyly of *Macaranga*, as suggested by earlier studies with limited taxon sampling (Blattner et al., 2001; Slik and 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. As an exception, one *Macaranga* species, *M. heudelotii* Baill. (not sampled), is reported to 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 discussion on the *Macaranga* clade and Fig. 4). 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 and 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 and 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 disagree with his suggestion to merge *Deuteromallotus* with *Mallotus* because in our results it falls into the *Cordemoya* s.l. clade as well.

The *Cordemoya* s.l. clade, with the taxon composition described in the previous paragraphs, is also supported by morphological characters. Most importantly, the conspicuous, spherical to disclike 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 earlier, 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 following section about *Mallotus* s.s. phylogeny). *Rockinghamia*, a genus already considered to be distant to *Mallotus* in an earlier study (Wurdack et al., 2005), was confirmed to be not 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 (1994), and we regard its having a close relationship 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 and 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

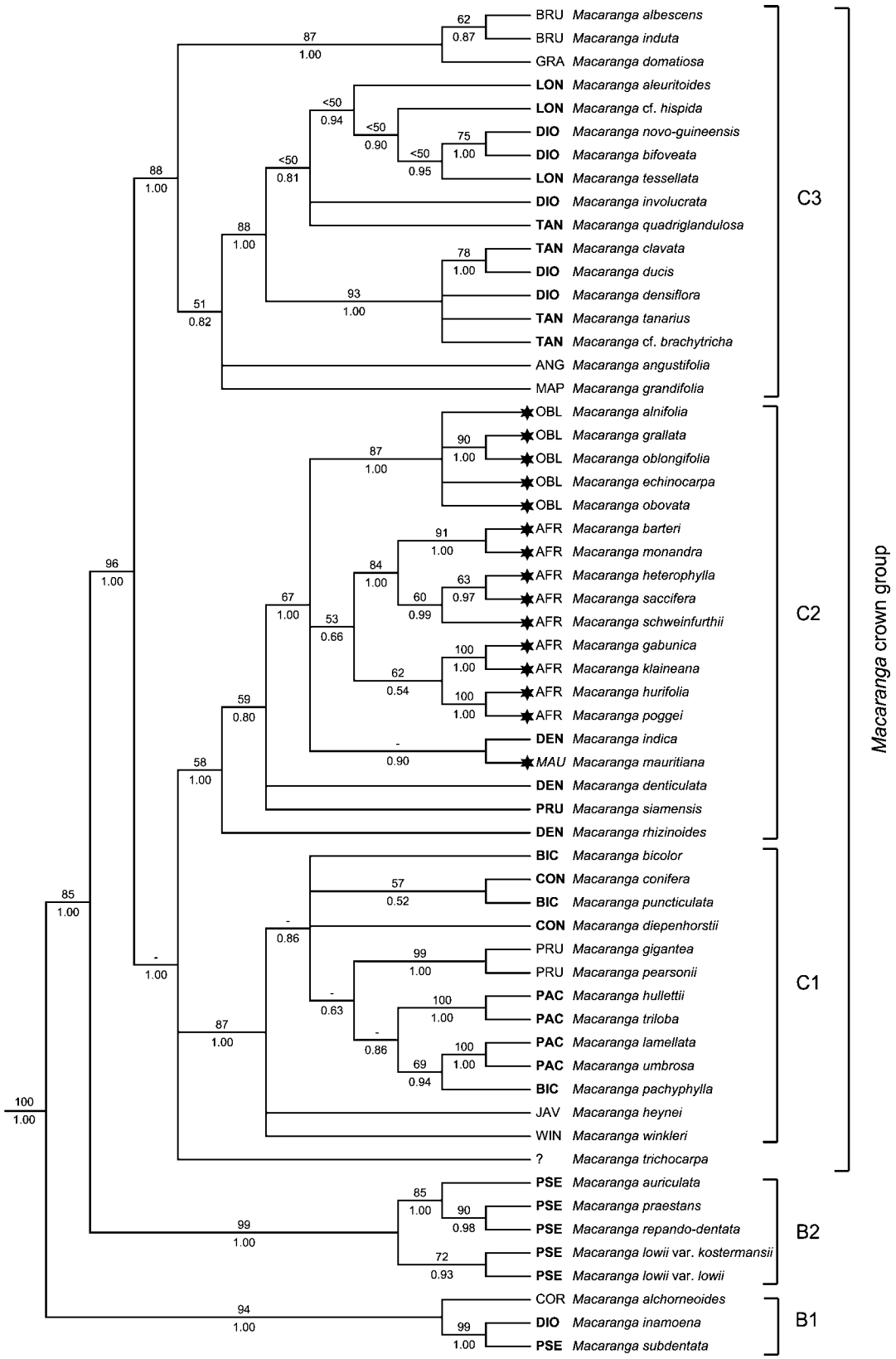

Fig. 4. Phylogenetic relationships inferred from the individual analysis of *Macaranga* clade (see Fig. 2) with the combined data set of four markers. A Bayesian majority consensus tree is shown with parsimony bootstrap values above the branches, posterior probabilities below. A hyphen (-) indicates that the node does not exist in the parsimony strict consensus. Infrageneric group (see Table 2) indicated with three letter abbreviation (shown in boldface if the particular group is nonmonophyletic). Species occurring at western side of Indian Ocean (Africa, Madagascar, Mascarenes) are indicated with stars.

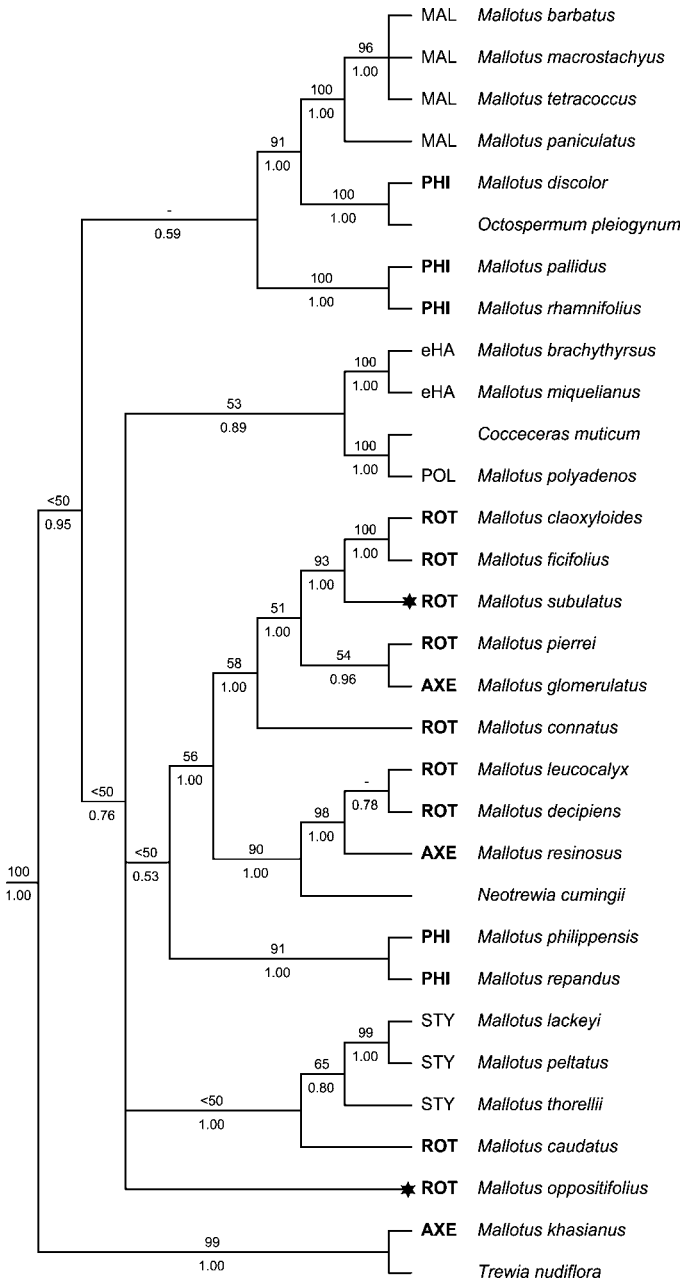

Fig. 5. Phylogenetic relationships inferred from the individual analysis of *Mallotus* s.s. clade (see Fig. 2) with the combined data set of four markers. A Bayesian majority consensus tree is shown with parsimony bootstrap values above the branches, posterior probabilities below. A hyphen (-) indicates that the node does not exist in the parsimony strict consensus. Infrageneric group (see Table 2) indicated with three letter abbreviation (shown in boldface if the particular group is nonmonophyletic); eHA, a group of species excluded from sect. *Hancea* (Slik and van Welzen, 2001b). Species occuring at western side of Indian Ocean (Africa, Madagascar, Mascarenes) are indicated with stars.

result of the MP analysis of ITS, with *Macaranga* deeply embedded in *Mallotus* s.s. and sister to a clade containing *Mallotus* sect. *Mallotus* (for a detailed tree, see Appendix S7 in Supplemental Data with the online version of this article), 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 data set is highly variable, and the plot of transition vs. transversion distances has some signs of saturation. MP analysis could therefore have failed to detect the obscured signal supporting the separation of *Mallotus* s.s. and *Macaranga*, whereas the BI, based on molecular evolutionary models, which accounts for multiple hits and different rates for substitution classes (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 support the monophyly of *Mallotus* s.s. On the other hand, the results of the *ncpGS* analyses deviate from those of other markers, because the MP analysis of *ncpGS* data places *Macaranga and Cordemoya* s.l. clades together, and the BI fails even 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. 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*. Futhermore, the monophyly of sect. *Hancea* as circumscribed by Slik and Van Welzen (2001b) is strongly supported (see also the following discussion on the *Mallotus* s.s. clade). 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* data sets, which have no hard incongruences, resulted in a more resolved and more highly supported phylogeny than any of the single-marker analyses (Fig. 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 samples from all of Whitmore's (in press) *Macaranga* groups, provides the first comprehensive phylogeny of the genus. In our results (Fig. 4), half of the 18 infrageneric groups recognized by Whitmore proved to be, although sometimes with low support, nonmonophyletic. 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 following discussion, 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. 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 that grow in primary forest and that 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 staminate inflorescences in the rest of *Macaranga* are variously branched (often with more than two axis orders, but 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). *Macaranga 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–C3) 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 and 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 three subclades (C1–C3 in Fig. 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–C3 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 level of morphological diversity, and no morphological synapomorphies are known for them 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 all taxa 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 extrafloral nectaries on the apical part of leaves, disc-shaped glands on the staminate bracteoles, and globose seeds (in the sect. *Pruinosae*, extrafloral leaf nectaries are not prominent, disc-shaped glands are absent, and seeds are 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 as belonging 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 and 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 (five 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 occuring 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.

That members of both the *Dioica* group and sect. *Pseudorottlera* have fruits subtended by leafy bracts led 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 the discussion on basal clades earlier), 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. 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 its general tendency to overestimate support, BI is 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 data set. 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 puzzling. The sequence divergence is in an acceptable range: the divergence in the *Mallotus* s.s. data sets 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 data sets were gained with consensus networks produced from MP bootstrap pseudoreplicate trees. The consensus networks from all single-marker *Mallotus* s.s. data sets have a similar structure: small groups (corresponding to the highly supported terminal clades) are connected to a central reticulation, indicating that data sets 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 data sets, i.e., the conflicting splits involve different taxa in different data sets.

The *Mallotus* s.s. analyses also revealed incongruences between single-marker data sets: one incongruence is hard (i.e., BS $>$ 70) and two are almost hard (BS 68–69). 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., they occur between closely related species.

The present study is a first molecular phylogenetic analysis of the genus *Mallotus*. Even though all analyses resulted in poorly resolved phylogenetic trees, several conclusions can be drawn. Apart from the placement of certain *Mallotus* species in the *Cordemoya* s.l. clade (discussed earlier), 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 but with a thick,

fleshy layer surrounding the seeds). A similar phenomenon can be observed in several other euphorb groups (Esser, 2003; Wurdack et al., 2005). In the following paragraphs 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).

The Malesian genus *Neotrewia* (one sp.) is characterized 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* (one 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) form 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 in considering only Malesian taxa, Sierra et al. (2005) noticed 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* (two 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 and Wemmer, 1988). Here we see a strongly supported sister group relationship of *T. 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 of *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 because the clade of *T. 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 previous discussion 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 a 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 and 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, suggesting instead an affinity with sections *Axenfeldia* and *Rottleropsis*. As two former *Hancea* species sampled in this study fall into the *Mallotus* s.s. clade, their separation from the sect. *Hancea* is confirmed here, although their relationship with the aforementioned 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 Ma; 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 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. 4 and 5). Therefore, *Macaranga* and *Mallotus* s.s. clades should already have largely diversified in Gondwana before the breakup 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 and Axelrod, 1974). Several leaf fossils from the Eocene onwards have been assigned to *Macaranga* and *Mallotus* (e.g., Akhmetiev and Vikulin, 1995; Horiuchi and 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, personal communication). This fossil shows two typical features for these genera: extrafloral nectaries and globose to disc-shaped glandular hairs. Although *Mallotus* cannot be ruled out as an identification, the leaf shape and long petioles strongly suggest that it belongs to *Macaranga* (e.g., similar to extant East African *M. kilimandscharica* Pax).

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 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. 5; a result also supported by morphology; Sierra et al., 2007). This indicates two separate introductions to Africa, a result contrasting with that of the *Macaranga* clade, where all the 14 sampled African and Madagascan species (of 36 total) form a distinct clade (although in the Baysian analysis Indian *Macaranga indica* is included in this clade; see Fig. 4 and online Appendix 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 by 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). 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 data sets 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 occurred in both the *Macaranga* and *Mallotus* s.s. clades.

LITERATURE CITED

AIRY SHAW, H. K. 1963. Notes on Malaysian and other Asiatic Euphorbiaceae. *Kew Bulletin* 16: 341–372.

AIRY SHAW, H. K. 1965. Notes on Malaysian and other Asiatic Euphorbiaceae. *Kew Bulletin* 19: 299–328.

AIRY SHAW, H. K. 1968. Malesian and other Asiatic Euphorbiaceae. *Kew Bulletin* 21: 379–400.

AIRY SHAW, H. K. 1969. Notes on Malesian and other Asiatic Euphorbiaceae. *Kew Bulletin* 23: 1–131.

AIRY SHAW, H. K. 1971. Notes on Malesian and other Asiatic Euphorbiaceae. *Kew Bulletin* 25: 473–553.

AKHMETIEV, M. A., AND S. V. VIKULIN. 1995. Peltate leaves *Macaranga* Thouin (Euphorbiaceae) in the Cenozoic of Eastern Sikhote-Alin' [in Russian]. *Paleontological Journal* 1995(2): 151–156.

ALEJANDRO, G. D., S. G. RAZAFIMANDIMBISON, AND S. LIEDE-SCHUMANN. 2005. Polyphyly of *Mussaenda* inferred from ITS and *trnT-F* data and its implications for generic limits in Mussaendeae (Rubiaceae). *American Journal of Botany* 92: 544–557.

ALFARO, M. E., S. ZOLLER, AND F. LUTZONI. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* 20: 255–266.

AUSTIN, J. J., E. N. ARNOLD, AND C. G. JONES. 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene Islands. *Molecular Phylogenetics and Evolution* 31: 109–122.

BÄNFER, G., B. FIALA, AND K. WEISING. 2004. AFLP analysis of phylogenetic relationships among myrmecophytic species of *Macaranga* (Euphorbiaceae) and their allies. *Plant Systematics and Evolution* 249: 213–231.

BÄNFER, G., U. MOOG, B. FIALA, M. MOHAMED, K. WEISING, AND F. R. BLATTNER. 2006. A chloroplast genealogy of myrmecophytic *Macaranga* species (Euphorbiaceae) in Southeast Asia reveals hybridisation, vicariance and long-distance dispersal. *Molecular Ecology* 15: 4409–4424.

BERRY, P. E., A. L. HIPP, K. J. WURDACK, B. VAN EE, AND R. RIINA. 2005. Molecular phylogenetics of the giant genus *Croton* and tribe Crotoneae (Euphorbiaceae sensu stricto) using ITS and *trnL-trnF* DNA sequence data. *American Journal of Botany* 92: 1520–1534.

BLATTNER, F. R., K. WEISING, G. BÄNFER, U. MASCHWITZ, AND B. FIALA. 2001. Molecular analysis of phylogenetic relationships among myrmecophytic *Macaranga* species (Euphorbiaceae). *Molecular Phylogenetics and Evolution* 19: 331–344.

BOLLENDORFF, S. M., P. C. VAN WELZEN, AND J. W. F. SLIK. 2000. A taxonomic revision of *Mallotus* section *Polyadenii* (Euphorbiaceae). *Blumea* 45: 319–340.

DARLU, P., AND G. LECOINTRE. 2002. When does the incongruence length difference test fail? *Molecular Biology and Evolution* 19: 432–437.

DAVIES, S. J. 2001. Systematics of *Macaranga* sects. *Pachystemon* and *Pruinosae* (Euphorbiaceae). *Harvard Papers in Botany* 6: 371–448.

DAVIES, S. J., S. K. Y. LUM, R. CHAN, AND L. K. WANG. 2001. Evolution of myrmecophytism in western Malesian *Macaranga* (Euphorbiaceae). *Evolution* 55: 1542–1559.

DAVIS, C. C., O. W. CAMPBELL, K. J. WURDACK, C. A. JARAMILLO, AND M. J. DONOGHUE. 2005. Explosive radiation of Malphigiales supports a Mid-Cretaceous origin of modern tropical rain forests. *American Naturalist* 165: E36–E65.

DAYANANDAN, S., P. S. ASHTON, S. M. WILLIAMS, AND R. B. PRIMACK. 1999. Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast *rbcL* gene. *American Journal of Botany* 86: 1182–1190.

DINERSTEIN, E., AND C. M. WEMMER. 1988. Fruits rhinoceros eat: dispersal of *Trewia nudiflora* (Euphorbiaceae) in lowland Nepal. *Ecology* 69: 1768–1774.

DOLPHIN, K., R. BELSHAW, C. D. L. ORME, AND D. L. J. QUICKE. 2000. Noise and incongruence: interpreting results of the incongruence length difference test. *Molecular Phylogenetics and Evolution* 17: 401–406.

DOUADY, C. J., F. DELSUC, Y. BOUCHER, W. F. DOOLITTLE, AND E. J. P. DOUZERY. 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molecular Biology and Evolution* 20: 248–254.

EMSHWILLER, E., AND J. J. DOYLE. 1999. Chloroplast-expressed glutamine synthetase (*ncpGS*): potential utility for phylogenetic studies with an

example from *Oxalis* (Oxalidaceae). *Molecular Phylogenetics and Evolution* 12: 310–319.

EMSHWILLER, E., AND J. J. DOYLE. 2002. Origins of domestication and polyploidy in oca (*Oxalis tuberosa*: Oxalidaceae). 2. Chloroplast-expressed glutamine synthetase data. *American Journal of Botany* 89: 1042–1056.

ESSER, H.-J. 2003. Fruit characters in Malesian Euphorbiaceae. *Telopea* 10: 169–177.

FARRIS, J. S., V. A. ALBERT, M. KALLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

FARRIS, J. S., M. KALLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

FELSENSTEIN, J. 1985. Confidence-limits on phylogenies—an approach using the bootstrap. *Evolution* 39: 783–791.

FLYNN, J. J., AND M. A. NEDBAL. 1998. Phylogeny of the Carnivora (Mammalia): congruence vs. incompatibility among multiple data sets. *Molecular Phylogenetics and Evolution* 9: 414–426.

FREUDENSTEIN, J. V., C. VAN DEN BERG, D. H. GOLDMAN, P. J. KORES, M. MOLVRAY, AND M. W. CHASE. 2004. An expanded plastid DNA phylogeny of Orchidaceae and analysis of jackknife branch support strategy. *American Journal of Botany* 91: 149–157.

GUINDON, S., AND O. GASCUEL. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

HILLIS, D. M., AND J. J. BULL. 1993. An empirical-test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.

HIPP, A. L., J. C. HALL, AND K. J. SYTSMA. 2004. Congruence versus phylogenetic accuracy: revisiting the incongruence length difference test. *Systematic Biology* 53: 81–89.

HOLLAND, B., AND V. MOULTON. 2003. Consensus networks: a method for visualising incompatibilities in collections of trees. *In* G. Benson and R. Page [eds.], Algorithms in bioinformatics, WABI 2003. Springer-Verlag, Berlin, Germany.

HOLLAND, B. R., F. DELSUC, AND V. MOULTON. 2005. Visualizing conflicting evolutionary hypotheses in large collections of trees: using consensus networks to study the origins of placentals and hexapods. *Systematic Biology* 54: 66–76.

HORIUCHI, J., AND H. TAKIMOTO. 2001. Plant mega-fossils from the Late Early to Early Middle Miocene Asakawa formation at Inuboe Pass, Ibaraki Prefecture, Kanto District, Japan. *Bulletin of Ibaraki Nature Museum* 4: 1–32.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

HUSON, D. H., AND D. BRYANT. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.

JANSA, S. A., S. M. GOODMAN, AND P. K. TUCKER. 1999. Molecular phylogeny and biogeography of the native rodents of Madagascar (Muridae: Nesomyinae): a test of the single-origin hypothesis. *Cladistics* 15: 253–270.

KATHRIARACHCHI, H., R. SAMUEL, P. HOFFMANN, J. MLINAREC, K. J. WURDACK, H. RALIMANANA, T. F. STUESSY, AND M. W. CHASE. 2006. Phylogenetics of tribe Phyllantheae (Phyllanthaceae; Euphorbiaceae sensu lato) based on nrITS and plastid *matK* DNA sequence data. *American Journal of Botany* 93: 637–655.

KELCHNER, S. A. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* 87: 482–498.

KULJU, K. K. M., S. E. C. SIERRA, AND P. C. VAN WELZEN. 2007. Re-shaping *Mallotus* [part 2]: inclusion of *Neotrewia*, *Octospermum* and *Trewia* in *Mallotus* s.s. (Euphorbiaceae s.s.). *Blumea* 52: 115–136.

MADDISON, D. R., AND W. P. MADDISON. 2001. MacClade 4: analysis of phylogeny and character evolution, version 4.06. Sinauer, Sunderland, Massachusetts, USA.

MCLOUGHLIN, S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* 49: 271–300.

MCPHERSON, G. 1995. On *Mallotus* and *Deuteromallotus* (Euphorbiaceae) in Madagascar. *Bulletin Du Museum National D'Histoire Naturelle B, Adansonia* 17: 169–173.

MÜLLER, J. 1865. Euphorbiaceae. *Linnaea* 34: 184–197.

MÜLLER, J. 1866. Euphorbiaceae tribus Acalypheae. *In* A. de Candolle [ed.], Prodromus systematis naturalis regni vegetabilis, vol. 15, 2, 956–1015. Masson & Fils, Paris, France.

MÜLLER, K. 2004. PRAP-computation of Bremer support for large data sets. *Molecular Phylogenetics and Evolution* 31: 780–782.

MÜLLER, K. 2005a. SeqState: primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics* 4: 65–69.

MÜLLER, K. 2005b. The efficiency of different search strategies in estimating parsimony jackknife, bootstrap, and Bremer support. *BMC Evolutionary Biology* 5.

MÜLLER, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular Phylogenetics and Evolution* 38: 667–676.

NIXON, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.

NYLANDER, J. A. A. 2004. MrModel test, version 2. Program distributed by the author. Evolutionary Biology Center, Uppsala University.

PAX, F., AND K. HOFFMANN. 1914. Euphorbiaceae-Acalypheae-Mercurialinae. *In* A. Engler [ed.], Das Pflanzenreich, vol. IV.147.vii. Engelmann, Leipzig, Germany.

PAX, F., AND K. HOFFMANN. 1919. Euphorbiaceae-Additamentum VI. *In* A. Engler [ed.], Das Pflanzenreich, vol. IV.147.xiv. Engelmann, Leipzig, Germany.

PAX, F., AND K. HOFFMANN. 1931. Euphorbiaceae. *In* A. Engler and K. Prantl [eds.], Die Natürlichen Pflanzenfamilien, vol. 19c, 11–233. Engelmann, Leipzig, Germany.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.

QUANDT, D., K. MÜLLER, AND S. HUTTUNEN. 2003. Characterisation of the chloroplast DNA *psbT-H* region and the influence of dyad symmetrical elements on phylogenetic reconstructions. *Plant Biology* 5: 400–410.

RADCLIFFE-SMITH, A. 2001. Genera Euphorbiacearum. Royal Botanic Gardens, Kew, UK.

RANNALA, B., AND Z. H. YANG. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311.

RAVEN, P. H., AND D. I. AXELROD. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* 61: 539–673.

RAXWORTHY, C. J., M. R. J. FORSTNER, AND R. A. NUSSBAUM. 2002. Chameleon radiation by oceanic dispersal. *Nature* 415: 784–787.

RENNER, S. S. 2004. Multiple Miocene Melastomataceae dispersal between Madagascar, Africa and India. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 359: 1485–1494.

RIDLEY, H. N. 1910. Symbiosis of ants and plants. *Annals of Botany* 24: 457–483.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SAMUEL, R., W. GUTERMANN, T. F. STUESSY, C. F. RUAS, H. W. LACK, K. TREMETSBERGER, S. TALAVERA, B. HERMANOWSKI, AND F. EHRENDORFER. 2006. Molecular phylogenetics reveals *Leontodon* (Asteraceae, Lactuceae) to be diphyletic. *American Journal of Botany* 93: 1193–1205.

SAMUEL, R., H. KATHRIARACHCHI, P. HOFFMANN, M. H. J. BARFUSS, K. J. WURDACK, C. C. DAVIS, AND M. W. CHASE. 2005. Molecular phylogenetics of Phyllanthaceae: evidence from plastid *MatK* and nuclear *phyC* sequences. *American Journal of Botany* 92: 132–141.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1995. Documentation of

reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences, USA* 92: 6813–6817.

SCHATZ, G. E. 1996. Malagasy/Indo-Australo-Malesian phytogeographic connections. *In* W. R. Lourenco [ed.], Biogeography of Madagascar, 73–83. Editions de l'ORSTOM, Paris, France.

SIERRA, S. E. C., M. APARICIO, M. J. H. GEBRAAD, K. K. M. KULJU, AND P. C. VAN WELZEN. 2007. The morphological range in *Mallotus* and a taxonomic revision of its section *Rottleropsis* (including *Axenfeldia*) in Malesia, Thailand and Africa. *Blumea* 52: 21–113.

SIERRA, S. E. C., M. APARICIO, K. K. M. KULJU, Ž. FIŠER, P. C. VAN WELZEN, AND R. W. J. M. VAN DER HAM. 2006. Re-shaping Mallotus [part 1]: expanded circumscription and revision of the genus Cordemoya (Euphorbiaceae). *Blumea* 51: 519–540.

SIERRA, S. E. C., AND P. C. VAN WELZEN. 2005. A taxonomic revision of *Mallotus* section *Mallotus* (Euphorbiaceae) in Malesia. *Blumea* 50: 249–274.

SIERRA, S. E. C., P. C. VAN WELZEN, AND J. W. F. SLIK. 2005. A taxonomic revision of *Mallotus* section *Philippinenses* (former section *Rottlera*-Euphorbiaceae) in Malesia and Thailand. *Blumea* 50: 221–248.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

SIMMONS, M. P., K. M. PICKETT, AND M. MIYA. 2004. How meaningful are Bayesian support values? *Molecular Biology and Evolution* 21: 188–199.

SLIK, J. W. F., P. J. A. KEßLER, AND P. C. VAN WELZEN. 2003. *Macaranga* and *Mallotus* species (Euphorbiaceae) as indicators for disturbance in the mixed lowland dipterocarp forest of East Kalimantan (Indonesia). *Ecological Indicators* 2: 311–324.

SLIK, J. W. F., AND P. C. VAN WELZEN. 2001a. A phylogeny of *Mallotus* (Euphorbiaceae) based on morphology: indications for a pioneer origin of *Macaranga*. *Systematic Botany* 26: 786–796.

SLIK, J. W. F., AND P. C. VAN WELZEN. 2001b. A taxonomic revision of *Mallotus* sections *Hancea* and *Stylanthus* (Euphorbiaceae). *Blumea* 46: 3–66.

SULLIVAN, J. 1996. Combining data with different distributions of among-site rate variation. *Systematic Biology* 45: 375–380.

SUZUKI, Y., G. V. GLAZKO, AND M. NEI. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences, USA* 99: 16138–16143.

SWOFFORD, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic inference. *In* D. M. Hillis, C. Moritz, and B. Mable [eds.], Molecular systematics, 407–514. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.

VAN WELZEN, P. C., AND S. E. C. SIERRA. 2006. The *Mallotus wrayi* complex (Euphorbiaceae). *Blumea* 51: 373–388.

VAN WELZEN, P. C., S. E. C. SIERRA, M. J. H. GEBRAAD, AND K. K. M. KULJU. 2006. The distinction of five *Mallotus* species formerly in section *Hancea* (Euphorbiaceae). *Blumea* 51: 367–372.

WEBSTER, G. L. 1994. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* 81: 33–144.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols. A guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

WHITMORE, T. C. 1965. Studies in *Macaranga*, an easy genus of Malayan wayside trees. *Malayan Nature Journal* 20: 89–99.

WHITMORE, T. C. 1969. Studies in *Macaranga*, III. First thoughts on species evolution in Malayan *Macaranga*. *Biological Journal of the Linnean Society* 1: 223–231.

WHITMORE, T. C. 1980. Studies in *Macaranga*, VIII. *Macaranga* in New Guinea and the Bismarck Archipelago. *Kew Bulletin* 34: 599–606.

WHITMORE, T. C. In press. Prodromus of *Macaranga* (Euphorbiaceae). M. J. E. Coode and P. Hoffmann [eds.]. Royal Botanic Gardens, Kew, UK.

WIENS, J. J. 1998. Combining data sets with different phylogenetic histories. *Systematic Biology* 47: 568–581.

WURDACK, K. J., P. HOFFMANN, AND M. W. CHASE. 2005. Molecular phylogenetic analysis of uniovulate Euphorbiaceae (Euphorbiaceae sensu stricto) using plastid *rbcL* and *trnL-F* DNA sequences. *American Journal of Botany* 92: 1397–1420.

WURDACK, K. J., P. HOFFMANN, R. SAMUEL, A. DE BRUIJN, M. VAN DER BANK, AND M. W. CHASE. 2004. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *RbcL* DNA sequences. *American Journal of Botany* 91: 1882–1900.

YODER, A. D., J. A. IRWIN, AND B. A. PAYSEUR. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* 50: 408–424.

YUAN, Y.-M., S. WOHLHAUSER, M. MÖLLER, J. KLACKENBERG, M. W. CALLMANDER, AND P. KÜPFER. 2005. Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian Ocean basin resulted from long-distance dispersal and extentive radiation. *Systematic Biology* 54: 21–34.

ZOHARY, M. 1973. Geobotanical foundations of the Middle East. Gustav Fischer-Verlag, Stuttgart, Germany.

ZOLLINGER, H. 1856. Über die Rottlera-Arten. *Linnaea* 28: 299–332.

APPENDIX. Voucher information and GenBank accession numbers for samples used in this study. A dash indicates the region was not sampled. Where there are several samples per species, the first one listed was used in the analyses presented. Generic circumscriptions follow Webster (1994). ([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 (Herbarium).*

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; *Lorence 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***[c] (= *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 Distr., 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, Morobo 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, Sapo NP; *Jongkind et al. 5549 (WAG)*. ***Macaranga inamoena***[b] F.Muell. ex Benth.—DQ899217, DQ866559, DQ901992, DQ767759; Australia, Queensland, Cook Distr.; *Forster 29763 (BRI, L)*. ***Macaranga indica*** Wight—DQ899218, DQ866560, DQ901993, DQ767760; India, Dindigul Distr., Kodaikanal, Palni Hilss; *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 Distr.; *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 Distr., 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 Distr.; *Forster 29660 (BRI, L)*. ***Macaranga tessellata*** Gage—DQ899244, DQ866586, DQ902019, —; Indonesia, Papua, Mt. Jaya, Mimika Regency; *Utterridge 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 brachythyrsus*** 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 Distr.; *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 Distr.; *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 Distr.; *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 Prov., 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 Distr., 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 Distr.; *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 Distr.; *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 Distr.; *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 rhamnifolius*** (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 Distr.; *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)*.