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Source: Systematic Botany, 39(1) : 304-315
Published By: American Society of Plant Taxonomists
URL: https://doi.org/10.1600/036364414X678116
Phylogeny and Generic Delimitations in the Sister Tribes Hymenodictyeae and Naucleae (Rubiaceae)

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Communicating Editor: Andrea Weeks

Abstract—The Hymenodictyeae-Naucleeae clade is a predominantly Paleotropical group with 220 species in 28 genera. The phylogenetic relationships and generic limits within Naucleae have previously been assessed using combined molecular-morphological data, however the status of some genera remains questionable. The evolutionary relationships within Hymenodictyeae have never been investigated before. We performed phylogenetic analyses of the Hymenodictyeae-Naucleeae clade using nuclear [nrETS; nrITS] and chloroplast [ndhF; rbcL; rps16; trnT-F] data and a large sampling of both tribes. Our study supports the monophyly of the tribes, all subtribes of Naucleeae (Adininae, Breoniinae, Cephalanthinae, Corynantheinae, Mitragyninae, Naucleinae, and Uncarinae), and the Hymenodictyeae genera Hymenodictyon and Paracorynanthe. In Naucleeae, the monotypic genera Adnauclea, Metadina, and Pertusadina are nested within Adina, Mitragyna within Floroga, Ludwia, Myrmeconauclea, and Ochreinauclea within Neonauclea, and Burdiaoq and Sarcocephalus within Nauclea. Corynanthe and Pausinystalia are mutually paraphyletic. We provisionally maintain the current generic status of Neonauclea and its allied genera, pending further study. In sum, we recognize 17 genera in Naucleeae: Adina s. 1., Breonia, Breonia, Cephalanthus, Corynanthe s. l., Diqamnuclea, Gyrostipula, Iaota, Khusiinauclea, Ludwia, Mitragyna s. l., Myrmeconauclea, Nauclea s. l., Neolamarckia, Neonauclea, Ochreinauclea, and Uncaria. Five new combinations were made: Adina eryncha, Adina malaccensis, Corynanthe lane-poolei subsp. iturens, Corynanthe talbotii, and Nauclea myasica.

Keywords—Bayesian analyses, chloroplast DNA, Cinchonoideae, nuclear DNA, parsimony, taxonomy.

The sister tribes Hymenodictyeae sensu Razafimandimbison and Bremer (2006) and Naucleae sensu Razafimandimbison and Bremer (2001, 2002) are positioned as an early branch in the mostly Neotropical subfamily Cinchonoideae of the family Rubiaceae ( Andersson and Antonelli 2005; Manns and Bremer 2010; Manns et al. 2012). This group has been circumscribed to include 220 species in 28 genera.

Hymenodictyeae includes two genera, Hymenodictyon Wall. with 24 species and Paracorynanthe Capuron with two species (Razafimandimbison and Bremer 2006). The tribe is restricted to the Paleotropics with the highest species diversity found in Madagascar. Hymenodictyeae species are typically medium-sized to tall trees, and often grow on rocky substrates; however, Hymenodictyon epiphyticum Razaf. & B. Bremer is an epiphyte, and Hymenodictyon biuranum Hiern and Hymenodictyon flaccidum Wall. are facultative epiphytes. The members of the tribe can easily be distinguished from those of Naucleae by their spiriform to racemose inflorescences and lenticellate capsular fruits (Razafimandimbison and Bremer 2006). Hymenodictyeae is additionally characterized by its stipules bearing large, deciduous colleters on the margins, valvate corolla aestivation, and elongate, bilaterally flattened, and accrescent placenta. The generic status of Hymenodictyon and Paracorynanthe has never been questioned, but their monophyly and sister-group relationship have yet to be tested using molecular data.

Naucleae includes 26 genera and 194 species (Govaerts et al. 2013) of trees, shrubs, and lianas mostly distributed in the Paleotropics, with a few species in the Neotropics and North America (Ridsdale 1975, 1978a, 1978b; 1989; Razafimandimbison and Bremer 2002). The tribe is a well-defined monophyletic group that can easily be recognized by its spherical inflorescences; an additional synapomorphy for the tribe is epigynous floral nectaries, deeply embedded in the hypanthia (see Razafimandimbison and Bremer (2001, 2002) and Verellen et al. (2007) for more information on the morphological characters in the tribe). Naucleae has received much attention over the last 40 yr, and is known to have problematic intratribal classifications (e.g. Bremer et al. 1995; Razafimandimbison and Bremer 2001, 2002; Razafimandimbison et al. 2005; Ridsdale 1975, 1978a; Wikström et al. 2010). The broad circumscription of Naucleae, established by Razafimandimbison and Bremer (2002), subdivided the tribe in seven subtribes: Adininae, Breoniinae, Cephalanthinae, Corynantheinae, Mitragyninae, Naucleinae, and Uncarinae. However, the monophyly of Adininae and Cephalanthinae has been questioned, because the subtribes were not supported as monophyletic in their analyses and the inter-subtribal relationships were poorly supported. Razafimandimbison and Bremer (2002) proposed new generic limits within Naucleae based on their combined molecular-morphological tree. In that study, genera that contain more than one species are maintained as a genus if they 1) are monophyletic, 2) maximize nomenclatural stability, and 3) are easy to recognize in the field, criteria as outlined by Backlund and Bremer (1998). For the assessment of the monotypic genera in Naucleae, Razafimandimbison and Bremer (2002) utilized the following three criteria: 1) not nested within a well-defined genus, 2) with at least twoautopomorphic characters, and 3) with relationships to other genera that are strongly supported. Razafimandimbison and Bremer (2002) accepted a total of 24 genera based on their combined molecular-morphological tree. However, the monophyly of Corynanthe Welw., Neonauclea Merr., and Pausinystalia Pierre ex Beille, based on molecular data alone remains questionable, as the genera were shown to be non-monophyletic in the combined molecular (nrITS/rbcL/trnT-F) tree in Razafimandimbison and Bremer (2002). For example, Ludwia Ridsdale and Myrmeconauclea Merr., represented each by one species in their analyses, were nested within Neonauclea Merr, and the African genera Corynanthe and Pausinystalia, both sensu Stoffelen et al. (1996),
were mutually paraphyletic. *Nauclea* L. received low support and *Pertusadina* Ridsdale and *Cephalanthus* L. respectively were not supported as monophyletic. *Hallea* J.-F. Leroy was recognized as illegitimate because it was previously described as a fossil genus, *Hallea* G.B. Matthews (Matthews 1948). Because this is inconsistent with the priority rule of the current nomenclature code, Deng (2007) formally provided a new name for the genus, *Floroja* Y. F. Deng. More recently, Wikström et al. (2010) generated a resolved phylogeny of Naucleeae based on 31 taxa sampled from 17 genera. Inter-subtribal relationships received low support, and Adininae was potentially paraphyletic with respect to Corynantheinae. Although that study did not focus on tribe Naucleeae specifically, it raises doubts on the monophyly of *Adina* Salisb., *Breonia* A. Rich ex DC., *Neonauclea* Pausinystalia, and *Pertusadina*.

The objective of the present study is to reconstruct a robust phylogeny of the Hymenodictyeae-Naucleeae clade using a large sampling of both tribes. This will allow us to: 1) test the monophyly of the subtribes of Naucleeae, 2) test the monophyly of genera in Naucleeae and revise the classification accordingly, and 3) test the monophyly of the two genera in Hymenodictyeae.

**Materials and Methods**

**Taxon Sampling**—Taxa studied were predominantly those included in earlier phylogenetic studies of Naucleeae: 48 of the 50 taxa investigated by Razafimandimbison and Bremer (2002) were included in this study, with an addition of subsequently published *nrETS*, *nrITS*, *ndhF*, *rbcL*, and *trnT-F* sequences. A total of 77 species representing 24 of the 26 genera in Naucleeae and 20 taxa (18 species) representing both of the genera of Hymenodictyeae were analyzed (Table 1). The sampling included 30 species not previously investigated with molecular methods. Two genera from Naucleeae, *Diynaminauclea* Ridsdale and *Khasiaclunea* Ridsdale, were not included due to lack of material.

Five species from the subfamily Ixoroidae and one species from the subfamily Cinchonoideae were utilized as outgroup taxa based on Manns and Bremer (2010) and Manns et al. (2012). More information regarding the taxa investigated in this study (species names, voucher information, and sequence accession numbers) can be found in Appendix 1.

**Laboratory Procedures**—DNA extraction, amplification, and sequencing followed the procedures outlined in Kjærked and Bremer (2007). The primers used for amplification are listed in Table 2. The raw sequencing data were assembled using the Staden package version 2.0 (Staden 1996). Insertion/deletion events (indels) were visually inferred, following the alignment criteria outlined in Oxelman et al. (1997). One inversion of six base pairs was found in some of the *rps16* sequences in Naucleeae (position circa 585–591). The inversion was treated as a separate indel to prevent false sister group relationships forming. The aligned DNA matrices are available on TreeBASE (Study number TB2:S14215).

**Phylogenetic Analyses**—Sequence data for each individual marker was analysed using Bayesian inference. The chloroplast *ndhF*, *rbcL*, *rps16*, and *trnT-F* datasets were combined in one partition (hereafter referred to as “cp”; Table 3), and the nuclear [ETS and ITS] datasets in another partition (hereafter referred to as “nr”; Table 3). We performed visual comparisons of the cp and nr trees to detect any taxa with conflicting positions. A combined Bayesian analysis, based on these two partitions (hereafter referred to as “cp/nr”; Table 3) was conducted. Gaps were treated as missing data.

For each single marker data set, as well as the cp and nr data sets, the best-performing evolutionary model was identified using MrAIC version 1.4.4 (Nylander 2004) under the AICc criterion (Posada and Buckley 2004). Bayesian analyses were performed in MrBayes version 3.2 (Ronquist et al. 2012). The cp and nr matrices were analysed unpartitioned, and the cp/nr analysis in two unlinked partitions, nr and cp. The following settings were applied in all Bayesian analyses: two parallel runs of 10,000,000 generations in four chains each, a sample frequency of 1,000 and the temperature set to 0.10. Convergence of the Monte Carlo Markov Chains was assumed when the standard deviation of split frequencies for the parallel runs was below 0.01 (Ronquist et al. 2011). In addition, the minimum Estimated Sample Size was well above 100.

**Table 1.** Distribution of taxa included in the study. Genus and species counts retrieved from Govaerts et al. (2013). *Hymenodictyon parvifolium* is represented by three individual specimens. One undescribed species of *Hymenodictyon* is included.

**Table 2.** Primers used for PCR and sequencing. F = Forward; R = Reverse.

**Table 3.** Parsimony data, percentage of characters scored as missing data, and evolutionary model selection results.
Fig. 1. Bayesian 50% Majority rule consensus cladogram of the combined chloroplast and nuclear data set. Bayesian posterior probabilities (PP) and parsimony bootstrap values (BS) are included to the right of the nodes, separated by a semi-colon (PP; BS). BS < 50 is indicated by a hyphen (-). Tribes and subtribes are marked with arrows and subclades discussed in the text are delimited with vertical bars. In the lower left corner of the figure a small phylogram is included to illustrate the branch lengths.
In total, 276 new sequences were generated for this study: 44 nrETS, 39 nrITS, 59 ndhF, 42 rbcL, 61 rps16, and 31 trnT-F. There was no conflict between the phylogenies resulting from the Bayesian analyses of the individual chloroplast (ndhF, rbcL, rps16, and trnT-F) or nuclear (nrETS and nrITS) markers. Posterior probability (PP) values of nodes are further discussed as follows: nodes with PP between 0.98 and 1 are considered to be strongly supported, those with PP between 0.95 and 0.97 are supported, and those with PP below 0.95 are not supported.

The cp and nr trees (TreeBASE study numbers TB2:Tr63483 and TB2:Tr63484) both identify two major lineages (Hymenodictyeae and Naucleaeae) and five of the seven subtribes of Naucleaeae (Mitragyninae, Breoniinae, Corynantheinae, Uncariinae, and Naucleinae). Visual inspections of the cp and nr trees reveal that nine taxa, namely Adina pilulifera, Neonauclea clemensiae, Paracorynanthe, Ludekia (Roxb.) Bakh.f. & Ridsdale, and Neonauclea pseudocalycina are resolved as sister to the remaining Hymenodictyeae (PP = 1) and Naucleinae and Uncariinae form a strongly supported clade (PP = 1).

Subtribe Cephalanthinae (PP = 0.98) is strongly supported as monophyletic in our analysis (Fig. 1). The African and Asian species of Mitragyna Korth. form a monophyletic group (PP = 1) in our analysis (Fig. 1), while Fleroya is paraphyletic with respect to Mitragyna sensu Ridsdale (1978b). Within subtribe Adininae (PP = 0.98), two strongly supported clades are formed: the Adina clade (PP = 1), containing the genera Adina, Adinauclea Ridsdale, Haldinia Ridsdale, Metadina Bakh.f., Pertusadina, and Sinoadina Ridsdale, and the Neonauclea clade (PP = 1), consisting of the genera Ludekia, Myrmeconuclea, Neonauclea, and Ochreinauclea Ridsdale & Bakh.f. (Fig. 1). Adinauclea, Metadina and Pertusadina nested within Adina, Ludekia, Myrmeconuclea, and Ochreinauclea are nested within Neonauclea. Within subtribe Breoniinae (PP = 1), Breonadia Ridsdale is resolved as sister (PP = 1) to a clade containing three strongly supported subclades (Fig. 1): the Gyrostipula J.-F. Leroy-Janota clade A (PP = 1), the Breonia clade B (PP = 1), and the Breonia clade C (PP = 1). Within the subtribe Corynantheinae (PP = 1) Pausinystalia and Corynanthe are mutually paraphyletic (Fig. 1). Uncariinae (PP = 1) is resolved in two lineages (Fig. 1): one Asian clade (PP = 1) and one Afro-Neotropical clade (PP = 1). Within Naucleaeae (PP = 1) Neolamarckia Bosser is resolved as sister to a clade resolved two distinct lineages: one subclade is formed by the African Nauclea species, Sarcoccephalus Afzel. ex R.Br., and Burttdanya Hoyle (PP = 0.98) and the other subclade consists of the Asian Nauclea species (PP = 1). The monotypic Burttdanya and the monophyletic Sarcoccephalus are both nested within Nauclea with strong support (Fig. 1).

**Results**

We discuss the phylogenetic relationships within the Hymenodictyeae-Naucleaeae clade and re-evaluate the generic limits of Hymenodictyeae and Naucleaeae based on the cp/nr tree (Fig. 1), as it is the best-supported hypothesis. The cp and nr partitions were utilized because the individual chloroplast and nuclear markers, respectively, resolved in congruent phylogenies, and the combination of genetic sequences in partitions is proved to increase the phylogenetic accuracy of the resulting species tree (e.g. Gadagkar et al. 2005; Nylander et al. 2004).

It is worth noting that the sampling in this study is considerably larger than that of earlier phylogenetic studies of Naucleaeae (e.g. Razafianambison and Bremer 2001, 2002; Manns and Bremer 2010; Wikström et al. 2010).
Hymenodictyeae as defined by Razafimandimbison and Bremer (2006) and the broadly circumscribed Naucleeae as proposed by Razafimandimbison and Bremer (2001, 2002). The tribes are sisters, also consistent with the results of previous phylogenetic studies (e.g. Bremer and Eriksson 2009; Manns and Bremer 2010; Razafimandimbison and Bremer 2001; Robbrecht and Manen 2006). This sister group relationship is supported by the presence of fiber tracheids (Koek-Noorman 1970) and chromosome data (x = 11, 2x-4x-6x series of polyploids, Kiehn 1986) in both tribes. Moreover, the Hymenodictyeae-Naucleeae clade is a predominantly Paleotropical group in the otherwise mainly Neotropical Cinchonioideae.

Within Naucleeae, all seven subtribes (Adininae, Breoniniinae, Cephalanthinae, Corynantheinae, Mitragyninae, Naucleinae, and Uncarinae) are strongly supported as monophyletic. The monophyly of Adininae was neither supported in Razafimandimbison and Bremer (2002), nor Wikström et al. (2010). The most likely explanation is our larger sampling size and data set. The inter-subtribal relationships in Naucleeae remain largely unsupported, with the exceptions previously mentioned (Fig. 1). The sister relationship between Naucleinae and Uncarinae was moderately supported by the combined molecular tree in Razafimandimbison and Bremer (2002), but not supported by their combined molecular-morphological tree. They postulated “a rapid early diversification of the subtribes” as the likely cause of the relatively low number of informative characters resolving the phylogeny of the tribe.

**Phylogenetic Relationships Within Hymenodictyeae**—This study supports the sister group relationship between the Malagasy genus Paracorynanthe and the Palaeotropical genus Hymenodictyon, as reported by Razafimandimbison and Bremer (2006). The monophyly of the genera is also supported, which is inconsistent with the results of Razafimandimbison and Bremer (2001) and Manns and Bremer (2010). The previously uncertain monophyly of Hymenodictyon with respect to Paracorynanthe could be explained by the low variability of the markers used in these two studies; the rbcL sequences of the genera are almost identical, and the rps16, ndhF, and nrETS sequences display very low variation.

Paracorynanthe is morphologically distinct from Hymenodictyon by its thin, plated bark, corolla lobes prolonged by conspicuous appendages, bilaterally flattened fruits, and oblanceolate, angular placentae, as opposed to thick, nonplated bark, corolla lobes without appendages, ellipsoid fruits, and fusiform, bilaterally flattened placentae in the latter genus (Razafimandimbison and Bremer 2006). Hymenodictyon is resolved in an Asian clade, a Malagasy clade, and the African species, that do not group in one clade. The African species Hymenodictyon parvifolium is non-monophyletic, as the two subspecies do not form a monophyletic group in our analyses (Fig. 1). No new combinations are needed, because all three genera differ from Hymenodictyon in having large colleters in the sinuses between the corolla lobes and arillate seeds. Hymenodictyon parvifolium is not investigated in this study due to lack of material.

**Mitragyninae**—The subtribe, as defined by Razafimandimbison and Bremer (2002), comprises two genera, Fleroya and Mitragyna. Our analyses reaffirm the paraphyly of Fleroya (= Hallea sensu Leroy 1975), as shown by Razafimandimbison and Bremer (2002) and Manns and Bremer (2010). Accordingly, the generic status of Fleroya is untenable, and we formally put Hallea J.-F. Leroy and Fleroya Y. F. Deng as synonyms of Mitragyna (see Taxonomic Treatment). No new combinations are needed, because all three species of Fleroya were originally described as Mitragyna (Ridsdale 1978b). The broadly delimited Mitragyninae is distinct in Naucleeae by having mitriform stigmas, and is additionally distinguished by three-zonocolporate pollen with H-shaped endoapertures (Huysmans et al. 1994) and numerous, basally attached ovules per locule (Razafimandimbison and Bremer 2002).

**Adininae**—The subtribe is resolved in two strongly supported subclasses: the Adina clade (PP = 1) and the Neonauclea clade (PP = 1). The Adina clade corresponds largely to Adina as defined by Haviland (1897), containing Adina cordifolia (Roxb.) Ridsdale (= Haldina, Ridsdale 1978a), Adina multifolia (Havil.) Ridsdale and Adina rubescens Hemsl. (= Pertusadina, Ridsdale 1978a), Adina oligocephala Havil. (= Khasiaclunea (Havil.) Ridsdale, Ridsdale 1978a), Adina polycephyla Wall. (= Metadina, Bakhuizen van den Brink 1970), Adina racemosa (Siebold & Zucc.) Miq. (= Sinoadina, Ridsdale 1978a), Adina pilitifera (Lam.) Franch. ex Drake, and Adina rubella Hance (= Adina sensu Ridsdale 1978a). The only exception is Haviland’s Adina microcephala (Delile) Hiern (= Breonadia, Ridsdale 1975), which belongs to Breoniniinae. The Adina clade is restricted to tropical Asia and characterized by the presence of interfloral bracteoles, capsular fruits with the calyx remnants falling off together with the central axes, and winged seeds. Breonadia also has interfloral bracteoles and capsular fruits, but differs in having unwinged seeds. Adinauclea, Haldina, Khasiaclunea, Metadina, and Sinoadina are all monotypic. In our analyses (Fig. 1), Sinoadina and Haldina form a basal grade in the Adina clade...
and are distinct from the rest of the Adina clade by their cordate (sometimes obtuse in Sinoadina) leaves and capsular fruits dehiscing first septicidally, then loculicidally into two valves (in contrast to two or four valves in the other genera of the Adina clade). The two genera can easily be distinguished from each other by their inflorescences: terminal in Sinoadina and axillary in Haldina. However, all other genera of Adininae bear terminal inflorescences so Sinoadina does not differ from the rest of the subtribe in this feature. Moreover, Adinauclea forms a clade with Adina rubella and A. pilifera, while Pertusadina is paraphyletic with respect to Metadina and Adina pubicostata. These genera are only separated by a few small characters (Ridsdale 1975), which is incongruent with our criterion 2. Based on these findings, we here accept a broad circumscription of Adina, including Adinauclea, Haldina, Metadina, Pertusadina, and Sinoadina. The monotypic Khalioclunea is also a likely candidate for inclusion in our broadly delimited Adina, but it was not included in our analyses and thus, we refrain from making the taxonomic change, pending further study.

The Neonauclea clade comprises the four genera, Ludekia, Neonauclea, Myrmeconauclea, and Ochreinauclea. Based on morphology, Diagaminauclea Ridsdale (Ridsdale 1978a) probably also belongs to the clade. The lineage is characterized by well-developed, deciduous calyx lobes and the absence of interfloral bracteoles (although these are present in a few Neonauclea species). Myrmeconauclea and Ochreinauclea are distinct from Ludekia and Neonauclea in having pseudomultiple fruits. Myrmeconauclea additionally have seeds with long, ventral wings (Ridsdale 1978a) and Ochreinauclea has spindle-shaped stigmas; both characters are unique in the clade. Ludekia is unique in the clade in having globose stigmatic lobes with seven to nine prominent, longitudinal ridges. Neonauclea sensu Ridsdale (1989) is characterized by its unusually well-developed or appendaged calyx lobes. The four genera all have free (sometimes pseudomultiple), capsular fruits and winged seeds (Ridsdale 1978a). In our analyses (Fig. 1) both Myrmeconauclea and Ludekia are monophyletic, but the genera are nested in Neonauclea, along with Ochreinauclea maingayi (Hook.f.) Ridsdale; in other words, Neonauclea is paraphyletic with respect to Ludekia, Myrmeconauclea, and Ochreinauclea. Razafimandimbison et al. (2005) found Neonauclea as delimited by Ridsdale (1989) to be monophyletic, based on nrETS and nrITS datasets of 28 Neonauclea species, two Myrmeconauclea species, and one Ludekia species. It is worth noting that Neonauclea brassii S.Moore, the sister of the rest of the Neonauclea clade in this study was not investigated by Razafimandimbison et al. (2005). Therefore, a larger sampling of Neonauclea is needed to evaluate the monophyly of the genus. Additionally, the inclusion of Ochreinauclea within the Neonauclea clade is inconsistent with Ridsdale (1978a), who considered the genus to be closely related to Nauclea, based on its spindle-shaped stigmas. We provisionally maintain the genera Diagaminauclea, Ludekia, Myrmeconauclea, Neonauclea, and Ochreinauclea, pending further study.

Breonia—The subtribe, as defined by Razafimandimbison (2002) and Razafimandimbison and Bremer (2002) comprises four genera, the Afro-Malagasy Breonadia, the Malagasy Breonia and Janotia, and the Malagasy-Comorian Gyrostipula. Both Breonadia and Janotia are monotypic, while Breonia contains 20 species (Razafimandimbison 2002), and Gyrostipula contains three species (Emanuelsson and Razafimandimbison 2007). Breonadia is clearly distinct from the other genera by its verticillate leaves, intrapetiolar stipules, interfloral bracteoles, and wingless seeds. Breonia has multiple fruits, as opposed to capsular fruits in the other three genera. Gyrostipula is distinguished from the other genera by its long, red, and convolute stipules and long, filiform calyx lobes. Janotia is easily identified by its very large leaves and persistent, foliaceous stipules.

In our analyses (Fig. 1), Breoniinae is resolved in four lineages: Breonadia, sister to the rest of Breoniinae, the Breonia clades A and B, and the Gyrostipula-Janotia clade, making Breonia potentially non-monophyletic. These results are consistent with those of Wikström et al. (2010), but inconsistent with those of Razafimandimbison and Bremer (2002), which support a monophyletic Breonia. We suspect that the findings of Razafimandimbison and Bremer (2002) are due to a smaller sampling than in the present study; Breonia clade A (Fig. 1) was only represented by Breonia perrieri Homolle in their analysis, and it grouped with the members of our present Breonia clade B. In contrast, four species (Breonia perrieri, Breonia fragifera Capuron ex Razafim., Breonia capuronii Razafim., and Breonia sphaeranthua (Bail.) Homolle ex Ridsdale) of the Breonia clade A were analyzed in this study. Additionally, we investigated seven species of the Breonia clade B in this study; only four were included in Razafimandimbison and Bremer (2002). Our analyses suggest that Breonia is potentially paraphyletic with respect to Janotia and Gyrostipula, but we refrain from making any taxonomic changes, pending further study.

Corynantheinae—The subtribe contains two African genera: Corynanthe and Pausinystalia. Corynanthe is distinct by its infundibular corolla tubes, exerted styles and anthers, spherical, unidi-vided stigmas, and mainly loculicidal capsules. Pausinystalia is characterized by its corolla being differentiated into a basal narrow and cylindrical part, ending apically in a bladder-shaped part (resembling a wine glass), inserted styles and anthers, bilobed stigmas, and mainly septicidal capsules (Stoffelen et al. 1996). Chevalier (1909) described the genus Pseudocinchona A.Chevalier as morphologically distinct from Corynanthe by its four-merous flowers, exerted styles and anthers, and largely septicidal capsules, as opposed to five-merous flowers, exerted styles and anthers, and loculicidal capsules in Corynanthe s.s. In Razafimandimbison and Bremer (2002), Corynanthe and Pausinystalia, both sensu Stoffelen et al. (1996), were mutually paraphyletic in the combined molecular tree. In their combined molecular-morphological tree, Pausinystalia became monophyletic and Corynanthe paniculata Welw. (includes the type of the genus), was resolved as sister to the rest of Corynantheinae, but Corynanthe remained paraphyletic. Based on their combined molecular-morphological tree, Razafimandimbison and Bremer (2002) tentatively resurrected the genus Pseudocinchona, restricted Corynanthe to include only the type of the genus, and retained Pausinystalia as defined by Stoffelen et al. (1996).

The results of our analyses (Fig. 1) support the paraphyly of Corynanthe and Pausinystalia, as suggested by the combined molecular tree of Razafimandimbison and Bremer (2002). Pseudocinchona sensu Chevalier (1909), represented in our analyses by Corynanthe myumbensii (R. D. Good) N. Hallé and Corynanthe pachyceras K.Schum, form a monophyletic group that is nested in a paraphyletic Pausinystalia. Accordingly, we merge both Pausinystalia sensu
Stoffelen et al. (1996) and *Pseudocinchona* sensu Chevalier (1909) in *Corynanthe* in order to make the latter monophyletic. The broadly delimited *Corynanthe* is characterized by valvate corolla lobes, prolonged by glabrous, well-developed appendages, numerous basally attached and ascendingly imbricate ovules, and capsular fruits. This taxonomic adjustment requires two new combinations.

**Uncarinae**—The monogenic subtribe has a pantropical distribution, most species rich in Asia (36 species), with only two species in the Neotropics and two species in Africa (Ridsdale 1978b). The subtribe is strongly supported as monophyletic and is resolved in two sister lineages: an Asian clade and an Afro-Neotropical clade. However, we cannot draw any major conclusions, because only five of the Asian *Uncaria* species are represented in this study. *Uncaria* is easily recognized by the lianescent growth habit and the presence of paired hooks (modified peduncles), both characters are unique in Naucleae.

**Naucleinae**—The Palaeotropical subtribe *Naucleinae* is composed of four genera: the African *Burtttadia*, the Afro-Asian *Nauclea*, the Asian *Neolamarckia*, and the African *Sarcocephalus*. *Burtttadia*, *Nauclea*, and *Sarcocephalus*, all sensu Ridsdale (1975), can be distinguished based on their placen- cias, stipule shapes, and fruit type. The placen- cias in *Burtttadia* and *Sarcocephalus* are attached to the middle of the sepal, while they are attached to the upper third in *Nauclea* (Ridsdale 1975, 1978a). *Burtttadia* has linear-oblong to slightly bilobed placen- cias, while they are discoidal in *Sarcocephalus*, and Y-shaped in *Nauclea*. Both *Nauclea* and *Sarcocephalus* have multiple fruits, but *Sarcocephalus* can be distinguished by the deltoid or short stipules, while *Nauclea* has ovate, elliptic, or obovate stipules. *Sarcocephalus* is also distinct by having obtuse to emarginate or shortly bilobed stipule apices and calyx lobes prolonged by small appendages. The third scenario is to merge *Sarcocephalus* and *Burtttadia* in *Nauclea*. This broadly delimited *Nauclea* in easily distinguished from *Neolamarckia* by flattened terminal buds, multiple fruits (this latter absent in *Burtttadia*), and the lack of the false septa in their locules. Accordingly we here include *Burtttadia* and *Sarcocephalus* in a broadly delimited *Nauclea*. This requires one new combination.

**Taxonomic Treatment**

Based on this study we propose new generic limits of *Naucleae*, reducing the number of genera from 26 to 17. Accordingly, we make five new combinations, two lectotypifications, and formally put the genera *Fleroya* Y. F. Deng and *Hallea* J.-F. Leroy as synonyms of *Mitragynyna* Korth. Only taxa affected by the taxonomic and nomenclatural changes are presented here; a complete list of species and synonymous taxon names can be seen in Ridsdale (1978a) for *Adina* and *Nauclea*, Ridsdale (1978a) and Deng (2007) for *Mitragynyna*, and Stoffelen et al. (1996) for *Corynanthe*.


1. **Adina cordifolia** (Roxb.) Brandis., Forest Fl. N. W. India 263. 1874. *Haldina cordifolia* (Roxb.) Ridsdale, Blumea 24: 361 (1978).—**TYPE**: INDIA. *Roxburgh* s. n. (holotype: Herb. Smith 316/5, LINN!).


**Representative Specimens Examined**—BORNEO. Teijsmann s. n. (K). SUMATRA. Teijsmann s. n. (K).


**Nomenclatural Notes**—The holotype in l was lost at sea when it was sent on a loan (Ridsdale 2007). We select
one of the isotypes currently housed at K as lectotype. A photocopy of the holotype is available at L but is inadequate as a holotype.


**Nomenclatural Notes**—The holotype at B was destroyed and we select the isotype at P as lectotype.


**Nomenclatural Notes**—The neotype at P was designated by N. Hallé, Fl. Gabon 12: 68. 1966.

3. **Corynanthe lane-poolei** Hutch., Kew Bull., 98. 1912. **Pausinystalia lane-poolei** (Hutch.) Hutch ex Lane-Poole, Trees Shrubs, Herbs & Climbers of Sierra Leone 74 1916.—**TYPE**: SIERRA LEONE. Lane-Poole 46 (holotype: K! digital image seen).


**Nomenclatural Notes**—The holotype for **Mitragyna ledermannii**, Ledermann 2402 (B), was destroyed and a neotype is needed. No other specimen was cited in the original description and no duplicates of this collection have been located. The Zenker, G. 1619 collection is a good representative of the species based on the description in the protologue. The lectotype for **Mitragyna ciliata** at P was designated by N. Hallé, Fl. Gabon 12: 37. 1966.

Nomenclatural Notes—The lectotype at K was designated by Haviland, J. Linn. Soc. Bot. 33: 73. 1897.


Burttlandya Hoyle, Hooker’s, Icon. Pl. 3318. 1939, syn. nov.—TYPE: Burttlandya nyasica Hoyle = Nauclea nyasica (Hoyle) Å. Krüger & Löför.


Nomenclatural Notes—The lectotype at BM is an illustration based on the Smeathman s. n. (BM) collection. We have not been able to determine who designated the lectotype.

2. Nauclea nyasica (Hoyle) Å. Krüger & Löför. comb. nov. Burttlandya nyasica Hoyle, Hooker’s Icon. Pl. 34: t. 3318. 1936.—TYPE: MALAWI. Toensend 23 (holotype: K!).


Acknowledgments. We thank the curators of the herbaria A, ABD, BR, K, L, LBV, MO, NY, P, PTBG, PRE, S, TAN, TEF, UPS, and WAG for access to herbarium material, Anbar Khodabandeh for help with sequencing, and the reviewers for valuable comments on an earlier version of the paper. We also thank DGF (Direction Générale des Forêts) and MNP (Madagascar National Parks) in Madagascar for issuing collecting permits for S. G. R., Missouri Botanical Program, Madagascar, for logistical support, Parc Botanique et Zoologique de Tsimbazaza, Lalao Andriamahelo and Faranirina Lantoniaina (MBG program, Madagascar) for arranging collecting permits for S. G. R., and Per-Ola Karis for help with the nomenclatural issues. This study was funded by grants from the Knut and Alice Wallenberg Foundation and the Swedish Research Council to B. B.

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