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PHYLOGENY AND CLASSIFICATION OF NAUCLEEAE S.L. (RUBIACEAE) INFERRED FROM MOLECULAR (ITS, *rBCL*, AND *tRNT-F*) AND MORPHOLOGICAL DATA[1]

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Parsimony analyses of the tribe Naucleeae sensu lato (s.l.) using the noncoding internal transcribed spacer (ITS) regions of nuclear rDNA, the protein-coding *rbcL* and noncoding *trnT-F* regions of chloroplast DNA, and morphological data were performed to construct new intratribal classification, test the monophyly of previous subtribal circumscriptions, and evaluate the generic status of Naucleeae s.l. Fifty-two ITS, 45 *rbcL*, and 55 *trnT-F* new sequences are published here. Our study supports the monophyly of the subtribes Anthocephalidae, Mitragynae, Uncariae all sensu Haviland and Naucleinae sensu Ridsdale. There was no support for Cephalanthidae sensu Haviland and Adininae sensu Ridsdale. Naucleeae can be subdivided into six highly supported and morphologically distinct subtribes, Breoniinae, Cephalanthinae, Corynantheinae, Naucleinae, and Mitragyninae, Uncarinae, plus one, Adininae, which is poorly supported. The relationships among these subtribes were largely unresolved. We maintain the following 22 genera: *Adina*, *Adinauclea*, *Breonadia*, *Breonia*, *Burttdavya*, *Cephalanthus*, *Gyrostipula*, *Haldina*, *Janotia*, *Ludekia*, *Metadina*, *Mitragyna*, *Myrmeconauclea*, *Nauclea*, *Neolamarckia*, *Neonauclea*, *Ochreinauclea*, *Pausinystalia*, *Pertusadina*, *Sarcocephalus*, *Sinoadina*, and *Uncaria*. *Pseudocinchona* is reestablished. *Corynanthe* is restricted to *C. paniculata* and *Hallea* is reincluded in *Mitragyna*. Our results were inconclusive for assessing the relationships among *Adina*, *Adinauclea*, *Metadina*, and *Pertusadina* due to lack of resolution.

Key words: Breoniinae; Cinchonoideae; chloroplast DNA; Corynantheinae; Naucleeae; nuclear DNA; Rubiaceae.

Phylogenetic studies based on molecular data alone or in combination with morphological data have totally changed the view of Rubiaceae (coffee family) classifications at all taxonomic levels (subfamilial: e.g., Bremer, Andreasen, and Olsson [1995], Bremer [1996a]; tribal: e.g., Bremer and Thulin [1998], Andersson and Rova [1999], Andreasen and Bremer [2000]; generic: e.g., Nepokroeff, Bremer, and Sytsma [1999], Andersson [2001], Lantz, Andreasen, and Bremer [2002]; species: e.g., McDowell and Bremer [1998]; Persson [2000]). One of the groups of Rubiaceae that has recently drawn our attention is the tribe Naucleeae of the subfamily Cinchonoideae because of the conflicting views about its circumscriptions and generic limits (Haviland, 1897; Verdcourt, 1958; Bremekamp, 1966; Ridsdale, 1975, 1978a; Robbrecht, 1988, 1994; Bremer, Andreasen, and Olsson, 1995). The results of the phylogenetic studies by Razafimandimbison and Bremer (2002) based on molecular (internal transcribed spacer [ITS] and *rbcL*) and morphological data strongly support a broader circumscription for Naucleeae, which includes all members of Naucleeae sensu Ridsdale (1978a), *Cephalanthus*, *Hallea*, *Mitragyna*, *Uncaria* (as shown by Bremer, Andreasen, and Olsson, 1995), *Corynanthe*, and *Pausinystalia*. Naucleeae sensu Razafimandimbison and Bremer (hereafter referred to as Naucleeae sensu lato [s.l.]) as presently circumscribed consists of 26 genera and 179 species of trees, shrubs, and woody climbers. Most representatives occur in tropical Asia, mostly in Southeast Asia, with 134 species and 13 genera, followed by Madagascar with 24 species and 4 genera, Africa with 22 species and 8 genera, and Central, North, and South America together with only 5 species and 2 genera. *Neonauclea*, *Uncaria*, and *Breonia* are the most speciose genera, with 65 (Ridsdale, 1989), 34 (Ridsdale, 1978a), and 20 (Razafimandimbison, 2002) species, respectively. Nine genera (*Adinauclea*, *Breonadia*, *Burttdavya*, *Diyaminauclea*, *Haldina*, *Janotia*, *Khasiaclunea*, *Metadina*, and *Sinoadina*) are monotypic; the remaining genera contain two to four species (Tables 1 and 2). Naucleeae are a well-defined monophyletic group that can easily be recognized by numerous flowers arranged in globose inflorescences and epigynous floral nectaries deeply embedded in hypanthia (Bremer, Andreasen, and Olsson, 1995; Razafimandimbison, 2002; Razafimandimbison and Bremer, 2002).

Intratribal classifications of Naucleeae have been controversial because previous researchers have applied inconsistent subtribal and generic concepts (Table 2). Haviland (1897) recognized four subtribes: Anthocephalidae (containing *Neolamarckia* and *Nauclea*); Cephalanthidae (containing *Adina*, *Breonia*, *Cephalanthus*, and *Neonauclea*); Mitragynae (forming *Mitragyna*); and Uncariae (containing *Uncaria*). Haviland (1897) tentatively placed the Malagasy genus *Paracephaelis* in Naucleeae, but its position within the tribe Pavetteae (sub-

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TABLE 1. List of genera currently placed in Naucleeae s.l. (Razafimandimbison and Bremer, 2002).

Genera[a]	Number of species	Geographic distributions
1. ***Adina***	3	Asia
2. ***Adinauclea***	1	Celebes, Mollucas (Indonesia)
3. ***Breonadia***	1	Afro-Madagascar
4. ***Breonia***[b]	20	Madagascar
5. ***Burttdavya***	1	East Africa
6. *Cephalanthus*[c]	6	Pan- and subtropical
7. *Corynanthe*[d]	3	Central and West Africa
8. ***Diyaminauclea***	1	Sri Lanka
9. ***Gyrostipula***[e]	2	Comoro Islands and Madagascar
10. ***Haldina***	1	Asia
11. *Hallea*[f]	3	Africa (except northern Africa)
12. ***Janotia***[e]	1	Madagascar
13. ***Khasiaclunea***	1	India
14. ***Ludekia***	2	Southeast Asia
15. ***Metadina***	1	Southeast Asia
16. *Mitragyna*[g]	6	Afro-Asia
17. ***Myrmeconauclea***	3	Southeast Asia
18. ***Nauclea***	9	Afro-Asia
19. ***Neolamarckia***	1	India eastwards to New Guinea
20. ***Neonauclea***[h]	65	Southeast Asia
21. ***Ochreinauclea***	2	India and Borneo
22. *Pausinystalia*[d]	5	Central Africa
23. ***Pertusadina***	4	Southeast Asia
24. ***Sarcocephalus***	2	Africa (except northern Africa)
25. ***Sinoadina***	1	Asia
26. *Uncaria*[g]	34	Pantropical
Total	179	

[a] Genera in boldface: genera accepted by Ridsdale (1975 and 1978a).
[b] Razafimandimbison (2002).
[c] Ridsdale (1976).
[d] Stoffelen, Robbrecht, and Smets (1996).
[e] Leroy (1975a).
[f] Leroy (1975b).
[g] Ridsdale (1978b).
[h] Ridsdale (1989).

family Ixoroideae) has now widely been accepted (e.g., Bridson and Robbrecht, 1985; Robbrecht, 1988, 1994). Ridsdale (1978a) divided Anthocephalidae sensu Haviland into two subtribes: Anthocephalinae, currently known as Neolamarckiinae (Robbrecht, 1988) (comprising one genus *Neolamarckia*), and Naucleinae (including *Burttdavya*, *Nauclea* sensu stricto (s.s.) (*Ochreinauclea* and *Sarcocephalus*). Ridsdale further recognized a third subtribe Adininae (comprising *Adina*, *Breonia*, and *Neonauclea* all sensu Haviland).

Generic limits in Naucleeae have also long been controversial and unsettled (see Table 2). Haviland's (1897) first worldwide revision of Naucleeae consisted of nine genera: *Adina*, *Breonia*, *Cephalanthus*, *Mitragyna*, *Nauclea*, *Neolamarckia*, *Neonauclea*, *Paracephaelis*, and *Uncaria*. While the generic limits of *Cephalanthus*, *Neolamarckia*, and *Uncaria* have never been disputed, the delimitations of *Adina*, *Breonia*, *Mitragyna*, *Nauclea*, and *Neonauclea* have been a source of disagreement (Bremekamp, 1966; Leroy, 1975a; Ridsdale, 1975, 1978a, b, 1989; Razafimandimbison, 2002). All genera described after Haviland's revision, except *Burttdavya* and *Neobreonia*, included only species previously described in *Adina*, *Nauclea*, and *Neonauclea*. These three genera were considered by Ridsdale (1975, 1978a) to be heterogeneous. He adopted new generic circumscriptions, leading to recognition of several small genera (Table 2).

Razafimandimbison and Bremer's (2002) studies on Naucleeae s.l. show that the combined morphological and molecular data sets yield the best resolution at different areas of the cladograms. Reviews of arguments for combining different data sets are discussed thoroughly by several authors (e.g., de Queiroz, Donoghue, and Kim, 1995; Bremer, 1996b; Nixon and Carpenter, 1997) and will not be repeated here.

The present study was intended to include a much larger taxon sampling than Razafimandimbison and Bremer (2002) and one additional data set from *trnT-F*. It strengthened our previous conclusions on the circumscriptions of Naucleeae and also allowed us to elucidate its highly controversial intratribal classifications. The main objective of this study is to produce, with combined molecular and morphological data, robust phylogenies for Naucleeae s.l. The results of this phylogenetic study will subsequently be used to (1) substantiate new intratribal classification; (2) test the monophyly of previous subtribal circumscriptions; (3) and evaluate the status of all formally described genera currently placed in Naucleeae s.l.

MATERIALS AND METHODS

Taxon sampling—We investigated 55 taxa representing 24 of the 26 genera currently placed in Naucleeae s.l. (Table 2). We were unable to obtain material from the two monotypic Asian genera, *Diyaminauclea* and *Khasiaclunea* (Table 1). Of these 55 taxa, 44 were previously studied by Razafimandimbison and Bremer (2002) and 11 were added to this study (http://ajbsupp.botany.org/v89/). The genus *Luculia*, which has been shown to be basal in Rubiaceae (e.g., Bremer et al., 1999), was used to root the trees. *Cinchona pubescens* and *Exostema lineatum*, used as ougroup taxa in Razafimandimbison and Bremer's (2002) study, were also included here in order to test the monophyly of Naucleeae s.l.

Molecular data—Freshly collected, dried silica-gel leaves (Chase and Hills, 1991) and/or herbarium specimen leaves were used for extracting DNA following the mini-prep procedure of Doyle and Doyle (1987). Three molecular data sets, ITS (regions of nrDNA), *rbcL* (a protein coding gene of cpDNA), and *trnT-F* (mainly noncoding regions of cpDNA), in combination with morphological data, were used in this study. Both amplification and sequencing of ITS and *rbcL* were done following the protocols described in Razafimandimbison and Bremer (2002).

The *trnT-F* consists of four regions: an intergenic spacer between the *trnT* (UGU) and *trnL* (UAA) 5′ exon, the *trnL* 5′ exon, a *trnL* (UAA) intron, and another intergenic spacer between the *trnL* (UAA) 3′ exon and *trnF* (GAA) (Taberlet et al., 1991). The primers used to amplify and sequence these four regions are given in Table 3. The primer pair *trnT-F*_aF or *trnT-F*_a1F/*trnT-F*_dR amplifies the region between the *trnT* and *trnL* exon 3′; the primer pair *trnT-F*_cF/*trnT-F*_fR amplifies the region between the *trnL* intron and *trnF*. The internal primers, *trnT-F*_bR, *trnT-F*_eF, *trnT-F*_jF, *trnT-F*_iR, and *trnT-F*_rF, were also used to produce complete sequences of the entire regions of the *trnT-F*, with at least partial sequence overlap. The four regions of the *trnT-F* were amplified separately for the taxa with degraded DNA. The positions of the primers used in *Nicotiana tabacum* and their directions are shown in Table 3. Polymerase chain reactions (PCR) for the *trnT-F* were 50-μL reactions including 28.25 μL sterilized H_2O, 5 μL reaction buffer, 5 μL $MgCL_2$, 5 μL TMACL (Chevet LeMaître, and Katinka, 1995), 4 μL, 0.25 μL Taq (5U/μL), 0.5 μL 5′ primer, 0.5 μL 3′ primer, 0.5 μL BSA 1%, and 1 μL of DNA templates. The PCR amplifications, performed in a Eppendorf Mastercycle gradient (Bergman & Beving Instrument, Stockholm, Sweden), began with initial melting for 1 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min 30 s at 55°C, and 1 min 30 s at 72°C, and ended with a final extension phase of 7 min at 72°C.

Sequences of nucleotides of the investigated taxa were determined using an ABI Prism 377 DNA automated sequencer (Applied Biosystems, Stockholm, Sweden) and/or a MegaBace 1000 (Amerham Pharmacia Biotech, Uppsala, Sweden). In total, our studies included 53 ITS sequences (of which 52

TABLE 2. Circumscriptions of Naucleeae.

Haviland (1897)	Bremekamp (1966)	Ridsdale (1978a)	Razafimandimbison and Bremer (2002)	Razafimandimbison and Bremer (this study)
Subtribe Anthocephalidae		Subtribe Anthocephalinae		Subtribe Naucleinae
Neolamarckia	—	*Neolamarckia*	*Neolamarckia*	*Neolamarckia*
		Subtribe Naucleinae		
Nauclea	*Nauclea*	*Nauclea* s.s	*Nauclea* s.s	*Nauclea* s.s
		Burttdavya	*Burttdavya*	*Burttdavya*
		Ochreinauclea	*Ochreinauclea*	*Ochreinauclea*
	Sarcocephalus	*Sarcocephalus*	*Sarcocephalus*	*Sarcocephalus*
Subtribe Cephalanthidae		Subtribe Adininae		Subtribe Adininae
Adina	—	*Adina* s.s.	*Adina* s.s.	*Adina* s.s.
		Adinauclea	*Adinauclea*	*Adinauclea*
		Haldina	*Haldina*	*Haldina*
		Metadina	*Metadina*	*Metadina*
		Pertusadina	*Pertusadina*	*Pertusadina*
		Sinoadina	*Sinoadina*	*Sinoadina*
Neonauclea	—	*Neonauclea* s.s.	*Neonauclea* s.s.	*Neonauclea* s.s.
		Diyaminauclea	*Diyaminauclea*	?[b]
		Khasiaclunea	*Khasiaclunea*	?[b]
		Ludekia	*Ludekia*	*Ludekia*
		Myrmeconauclea	*Myrmeconauclea*	*Myrmeconauclea*
				Subtribe Breoniinae
		Breonadia	*Breonadia*	*Breonadia*
		Gyrostipula	*Gyrostipula*	*Gyrostipula*
		Janotia	*Janotia*	*Janotia*
Breonia	—	*Breonia* s.s.	*Breonia* s.l.	*Breonia* s.l.
		Neobreonia	—	—
				Subtribe Cephalanthinae
Cephalanthus	—	—	*Cephalanthus*	*Cephalanthus*
Subtribe Mitragynae				Subtribe Mitragyninae
Mitragyna	—	—	*Mitragyna* s.s.	*Mitragyna* s.l.
			Hallea	—
Subtribe Uncarinae				Subtribe Uncarinae
Uncaria	—	—	*Uncaria*	*Uncaria*
				Subtribe Corynantheinae
			Corynanthe	*Corynanthe* s.s.
			Pausinystalia	*Pausinystalia*
				Pseudocinchona
Paracephaelis	—	—	—	—

[a] Haviland tentatively placed *Paracephaelis* in Naucleeae.
[b] Question mark means genera not included in this study.

TABLE 3. Primers used for new *trnT-F* sequences in this study. Positions of primer corresponding to chloroplast DNA of tobacco (Shinozaki et al., 1986). Primers marked with A and B are designed by Taberlet et al. (1991) and at the Department of Systematic Botany, Uppsala University, respectively.

Marker and primer names	Primer sequences from the 5′ end	Primer positions in tobacco (GenBank GBAN-Z00044)[a]
Forward		
*trnT-F*_aF	CAT TAC AAA TGC GAT GCT CT	(48546–48565)[A]
*trnT-F*_a1F	ACA AAT GCG ATG CTC TAA CC	(48550–48469)[B]
*trnT-F*_cF	CGA AAT CGG TAG ACG CTA CG	(49306–49325)[A]
*trnT-F*_rF	GTT ATA ACT AAT GAG ACA TTC	(48953–48974)[B]
*trnT-F*_eF	GGT TCA AGT CCC TCT ATC CC	(49862–49881)[A]
*trnT-F*_jF	GTT CTA ACA AAT GGA GTT GG	(49493–49512)[B]
Reverse		
*trnT-F*_bR	TCT ACC GAT TTC GCC ATA TC	(49318–49299)[A]
*trnT-F*_dR	GGG GAT AGA GGG ACT TGA AC	(49882–49863)[A]
*trnT-F*_fR	ATT TGA ACT GGT GAC ACG AG	(50299–50280)[A]
*trnT-F*_iR	CCA ACT CCA TTT GTT AGA AC	(49508–49495)[B]

[a] The prefix GBAN- has been added to this Genbank accession but is not part of the actual accession number.

are newly published here, while the last one, *Cinchona pubescens*, was published by Andreasen, Baldwin, and Bremer [1999]), 53 *rbcL* sequences (of which 45 are newly published here, while eight were previously published by the second author alone or with her co-authors [Bremer, Andreasen, and Olsson, 1995; Bremer, 1996a, b; Bremer and Thulin, 1998; Bremer et al., 1999]), and 55 *trnT-F* sequences, all newly published here.

Morphological data—All 49 morphological characters investigated in this study were from Razafimandimbison and Bremer (2002). They represented variation in gross morphology, karyology, and phytochemical data.

Data analyses—*Molecular analysis*—Sequence data sets of each marker were aligned using CLUSTAL X (Thompson et al., 1997) to produce an initial alignment; this was followed by manual alignment using Se-Al (Rambaut, 1995). The sequence data were subsequently analyzed using the Sequencher (Gene Codes Corporation, Stockholm, Sweden) software package. All new sequences were submitted to EMBL. All accession numbers may be found at the *American Journal of Botany*'s supplemental data site (http://ajbsupp.botany.org/v89/). The sequenced species were used as terminals.

Morphological analysis—Using species as terminals in morphological analyses provides a potential test for monophyly of highly variable genera (e.g., Wiens, 2000). However, both our previous (Razafimandimbison and Bremer, 2002) and present molecular analyses demonstrate that most Naucleeae genera with at least two species are monophyletic. In our morphological analysis, genera and species (sequenced species) were used as terminals for the monophyletic and paraphyletic (*Cephalanthus*, *Neonauclea*, and *Pertusadina*) genera, respectively.

Combined analysis—The morphological character states of the monophyletic genera were used to represent that of their sequenced species in the combined molecular-morphological analysis.

Search strategies—Parsimony analyses of the ITS, *rbcL*, and *trnT-F* matrices (excluding uninformative characters) were performed with PAUP* version 4.0b6 (Swofford, 2000) using heuristic searches, with the MULTREES option off, nearest neighbor interchanges (NNI) branch swapping, and 10 000 random additions. The characters were unordered (i.e., using Fitch parsimony) and equally weighted. The shortest tree was saved for each replicate regardless of its length. All trees retained were submitted to a second round of tree bisection-reconnection (TBR) branch swapping with MULTREES on. Parsimony analyses of the combined molecular (ITS-*rbcL*-*trnT-F*) and molecular-morphological data sets were subsequently carried out using heuristic search, unordered and unweighted characters, MULTREES option on, TBR branch swapping, and 10 000 random addition sequences to search for multiple islands of most-parsimonious trees (Maddison, 1991). In all analyses indels were treated as missing data and all potential phylogenetically informative indels were rescored as binary characters (0 and 1). The consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989) were calculated to estimate homoplasy. Bootstrap (BS; Felsenstein, 1985) and jackknife (JK; Farris et al., 1996) values using heuristic searches, with the MULTREES option off, NNI branch swapping, five random additions, and 10 000 replicates were performed to assess relative support for the identified clades.

RESULTS

The ITS, *rbcL*, and morphological data sets used in this study produced trees similar to those in Razafimandimbison and Bremer's (2002) studies. Accordingly, we present only the tree from our new *trnT-F* analysis as well as the strict consensus trees from the combined molecular and molecular-morphological analyses. All formally published generic names currently placed in Naucleeae s.l. (except *Diyaminauclea* and *Khasiaclunea*) are used in the trees (Figs. 1–3), although no single author accepts all these genera.

trnT-F analysis—The nonaligned *trnT-F* sequences ranged from 1707 (*Luculia grandifolia*) to 1785 base pairs (bp) (*Cinchona pubescens* with a long insertion of 53 bp). Within Naucleeae s.l. the length ranged from 1711 bp (*Sarcocephalus pobeguinii*) to 1758 bp (*Cephalanthus occidentalis*). The *trnT-F* alignment of 55 taxa consisted of 1988 positions and contained 131 phylogenetically informative characters, 111 substitutions, and 20 phylogenetically informative indels (Table 4). Of these 131 informative characters, 71 were from the *trnT-L* spacer, 31 from the *trnL* intron, and 31 from the *trnL-F* spacer. The total GC content of the *trnT-F* ranged from 30.95% (*Luculia grandifolia*) to 32.62% (*Gyrostipula foveolata*) and its average was 32.02%.

The tree search could not be completed due to computional limitations. The *trnT-F* data were then analyzed by parsimony jackknifing (Farris et al., 1996) using the following settings: a deletion frequency of 37%, emulate ''jac'' resampling option on, 10 000 replicates, the MULTREES option off, NNI branch swapping, and five random addition sequences. All investigated taxa of Naucleeae s.l. were resolved with high support (JK = 100) as a monophyletic group (Fig. 1). The following clades, also revealed by the morphological, ITS and *rbcL* trees (results not presented), were retained: Clade A (consisting of *Cephalanthus glabratus*, *C. occidentalis*, and *C. salicifolius*; JK = 100); Clade B (consisting of *Adina* sensu Ridsdale, *Adinauclea*, *Haldina*, *Metadina*, and *Pertusadina*; JK = 56); Clade C (formed by *Myrmeconauclea*, *Neonauclea*, and *Ochreinauclea*; JK = 72); Clade D (including *Breonadia*, *Breonia*, *Gyrostipula*, and *Janotia*; JK = 96); Clade E (consisting of *Hallea* and *Mitragyna*, JK = 71); Clade F (containing *Corynanthe* and *Pausinystalia*; JK = 99); Clade G (formed by *Uncaria africana* and *Uncaria* sp.; JK = 67); Clade H (including *Uncaria guianensis* and *U. rhynchophylla*, JK = 82); Clade I (consisting *Nauclea diderrichii* and *N. xanthoxylon*; JK = 71); and Clade J (formed by *Nauclea orientalis* and *N. subdita*; JK = 89).

Combined analyses—The partition of homogeneity tests performed between the ITS, *rbcL*, and morphological data sets of Naucleeae s.l. in Razafimandimbison and Bremer (2002) showed that these three data sets were combinable. The visual inspection shows that the jackknife consensus *trnT-F* tree (Fig. 1) yields almost identical topologies as the strict consensus ITS, *rbcL*, and morphological trees from this study (results not shown). Therefore, we feel justified in merging all data sets in a large matrix for conducting combined analyses. We were not able to obtain ITS and *trnT-L* (corresponding to A–E regions, approximately 1200 bp) sequences from *Sinoadina racemosa* and *Ludekia borneensis*, respectively, mainly due to difficulties with amplification of their DNA templates from herbarium material. These two taxa were included in the combined analyses by inserting question marks (?) for their missing nucleotides. Fusion of the three separate molecular data sets of 53 taxa in one matrix yielded 4044 positions and 381 parsimony-informative characters (Table 4). A parsimony analysis resulted in three islands containing 15 most equally parsimonious trees each 1181 steps long, with CI of 0.466 (excluding uninformative characters) and RI of 0.651. The addition of 49 morphological characters to the combined molecular matrix, which contained 4093 characters, of which 426 were parsimony-informative (Table 4), resulted in 38 most parsimonious trees in four islands each 1429 steps long, with CI of 0.467 (excluding uninformative characters), and RI of

0.685. Both combined trees (Figs. 2 and 3) supported the monophyly of Naucleeae s.l. and diagnosed the same monophyletic groups, which we recognize here as subtribes: Cephalanthinae, Mitragyninae, Uncarinae, Corynantheinae, Naucleinae, Breoniinae (all highly supported), and ''Adininae'' (poorly supported). Within the ''Adininae,'' the *Neonauclea* clade received moderate and high support in the combined molecular (JK = 79, BS = 73) and combined molecular-morphological trees (JK = 94, BS = 89), respectively. The relationships among these seven subtribes were largely unresolved.

Comparisons between the two combined trees (Figs. 2 and 3), however, revealed slight differences in resolution and support. In the combined molecular-morphological tree (Fig. 3), *Haldina* and *Sinoadina* were left unresolved as separate lineages, whereas in the combined molecular tree (Fig. 2), they were resolved with poor support as members of ''Adininae.'' *Adina* sensu Ridsdale (1978a), represented by *A. pilulifera* and *A. rubella* here, was resolved with weak (JK = 58, BS < 50%) and strong support (JK = 94, BS = 88) as monophyletic in the combined molecular and combined molecular-morphological trees, respectively. Similarly, Cephalanthinae (including *C. natalensis*) had no support in the combined molecular tree, but received high support (JK = 95, BS = 93) in the combined molecular-morphological tree. Also, some differences were perceived within Corynantheinae: in the combined molecular tree (Fig. 2), *Corynanthe paniculata* was resolved with high support as sister to *Pausinystalia macroceras* and nested between this latter and *P. johimbe*; *P. lane-poolei* subsp. *ituriense* was resolved as sister to the rest of the members of Corynantheinae. However, in the combined molecular-morphological tree (Fig. 3), the three species of *Pausinystalia* formed a strongly monophyletic group and *C. paniculata* was resolved as sister to the remaining members of Corynantheinae.

Classification—We propose here a new subtribal classification of Naucleeae s.l. based on the combined molecular-morphological tree (Fig. 3) and supplemented by comprehensive information from literature. We provisionally recognize the subtribe Adininae to accommodate *Adina*, *Adinauclea*, *Haldina*, *Metadina*, *Pertusadina*, *Sinoadina*, and the members of *Neonauclea* clade (*Lukedia*, *Myrmeconauclea*, and *Neonauclea*).

Subtribe Cephalanthinae DC., Prodromus 4: 538 (1830) (as Cephalantheae)
—Cephalantheae H. B. K. [Nov. Gen. Sp. 3: 379 (1818), nom. Prov. (as Sectio) ex Kunth, Synop. Pl. Aequinoct. 4: 37 (1824) (as Sectio); Cham. & Schlecht. Linnea 4: 147 (1829) (as Sectio); Lindl., Intr. Nat. Syst. Bot., p. 204 (1830) (as Cephalantheae); Endl., Gen. Pl.: 530 (1838); Ench. Bot.: 271 (1841) (as Cephalantheae).
—Cephalanthidae Haviland. J. Linn. Soc. Bot. 33: 21 (1897)
Useful recent study: Ridsdale (1976)
Erect shrubs or trees. Stipules small, entire. Young inflorescences not surrounded by calyptra-like bracts. Inflorescences terminal. Flowers homostylous. Corolla lobes imbricate in bud, with large colleters in sinuses between corolla lobes (absent in *Cephalanthus natalensis*). Ovary bicarpellate, with one ovule in each locule, pendulous. Infructescences constituted by free, indehiscent fruits. Seeds unwinged, arillate (except *C. natalensis*).
Genus included: *Cephalanthus*

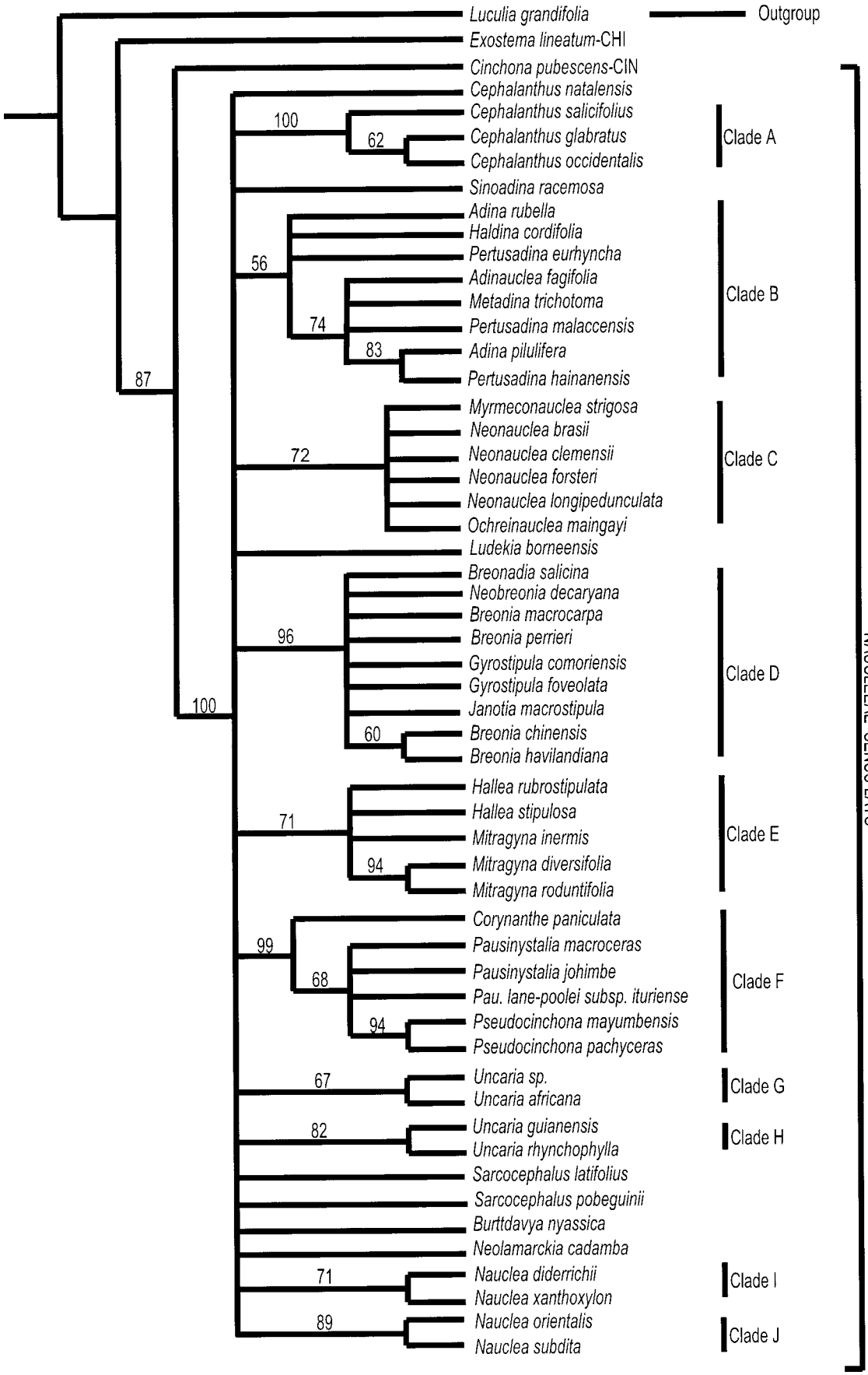
Subtribe Mitragyninae Haviland, J. Linn. Soc. Bot. 33: 21 (1897) (as Mitragyneae)
Useful recent studies: Leroy (1975a); Ridsdale (1978b)
Trees or shrubs. Stipules large, entire. Young inflorescences not surrounded by calyptra-like bracts. Inflorescences terminal. Flowers homostylous. Corolla lobe valvate in bud. Stigmas mitriform; ovary bicarpellate, with numerous ovules in each locule, basally attached, and ascendingly imbricate. Pollens 3-zonocolporate with H-shaped endoapertures. Infructescences formed by free, capsular fruits. Seeds winged.
Genus included: *Mitragyna* s.l.

Subtribe Uncarinae Haviland, J. Linn. Soc. Bot. 33: 21 (1897)
Useful recent study: Ridsdale (1978b)
Woody lianas. Stipules large, entire or shallowly to deeply bifid. Young inflorescences not surrounded by calyptra-like bracts. Paired fang hooks (modified inflorescence peduncles) always present. Inflorescences terminal or axillary. Flowers homostylous. Corolla lobe valvate in bud. Ovary bicarpellate, with numerous ovules in each locule, basally attached, and ascendingly imbricate. Infructescences constituted by free, capsular fruits. Seeds winged.
Genus included: *Uncaria*

Subtribe Corynantheinae Razafimandimbison and B. Bremer, subtrib. nov.
Type genus: *Corynanthe*
Useful recent studies: Stoffelen, Robbrecht, and Smets (1996)
Subtribus a subtribubus aliis differt appendicibus glabris prominentibus e corollae lobis prolongatis.
Trees. Stipules entire. Young inflorescences not surrounded by calyptra-like bracts. Inflorescences terminal or axillary or both. Flowers homostylous. Corolla lobes valvate in bud, prolonged by glabrous, well-developed appendages. Ovary bicarpellate, with numerous ovules, basally attached, and ascendingly imbricate. Infructescences constituted by free, capsular fruits. Seeds winged.
Genera included: *Corynanthe*, *Pausinystalia*, *Pseudocinchona*.

Subtribe Naucleinae DC., Prodromus Systematis Naturalis 4: 343 (1830) (as Cinchoneae subtribe Naucleeae)
—Subtribe Sarcocephalinae DC., Prodromus Systematis Naturalis 4: 367 (1830) (as Gardeniaceae subtribe Sarcocephalinae)
—Anthocephalinae Ridsdale, Blumea 24: 320 (1978a)
—Neolamarckiinae Robbrecht, Opera Botanica Belgica 1: 179 (1988)
Shrubs or trees. Stipules entire or shallowly bifid. Young inflorescences not surrounded by calyptra-like bracts. Inflorescences terminal. Flowers homostylous. Corolla lobe imbricate in bud. Stigmas fusiform (spindle-shaped), with receptive areas restricted to the base; ovary bicarpellate, with numerous ovules in each locule, mainly pendulous. Infructescences multiple fruits or formed by free fruits, indehiscent. Seeds unwinged.
Genera included: *Burttdavya*, *Nauclea*, *Neolamarckia*, *Ochreinauclea*, *Sarcocephalus*

Subtribe Breoniinae Razafimandimbison and B. Bremer, subtrib. nov.
Type genus: *Breonia*


Luculia grandifolia
Outgroup
Exostema lineatum-CHI
Cinchona pubescens-CIN
Cephalanthus natalensis
Cephalanthus salicifolius
Cephalanthus glabratus
Cephalanthus occidentalis
Clade A
Sinoadina racemosa
Adina rubella
Haldina cordifolia
Pertusadina eurhyncha
Adinauclea fagifolia
Metadina trichotoma
Pertusadina malaccensis
Adina pilulifera
Pertusadina hainanensis
Clade B
Myrmeconauclea strigosa
Neonauclea brasii
Neonauclea clemensii
Neonauclea forsteri
Neonauclea longipedunculata
Ochreinauclea maingayi
Clade C
Ludekia borneensis
Breonadia salicina
Neobreonia decaryana
Breonia macrocarpa
Breonia perrieri
Gyrostipula comoriensis
Gyrostipula foveolata
Janotia macrostipula
Breonia chinensis
Breonia havilandiana
Clade D
Hallea rubrostipulata
Hallea stipulosa
Mitragyna inermis
Mitragyna diversifolia
Mitragyna roduntifolia
Clade E
Corynanthe paniculata
Pausinystalia macroceras
Pausinystalia johimbe
Pau. lane-poolei subsp. ituriense
Pseudocinchona mayumbensis
Pseudocinchona pachyceras
Clade F
Uncaria sp.
Uncaria africana
Clade G
Uncaria guianensis
Uncaria rhynchophylla
Clade H
Sarcocephalus latifolius
Sarcocephalus pobeguinii
Burttdavya nyassica
Neolamarckia cadamba
Nauclea diderrichii
Nauclea xanthoxylon
Clade I
Nauclea orientalis
Nauclea subdita
Clade J
NAUCLEEAE SENSU LATO
100
62
56
74
83
87
72
96
100
60
71
94
99
68
94
67
82
71
89

Useful recent treatments: Homolle (1938), Leroy (1975a), Ridsdale (1975), Razafimandimbison (2002)

Subtribes distinctus bracteis calyptriformibus inflorescentiam juvenem cingentibus et discis ad infructescentiae maturitatem e floris nectariis accrescentibus.

Trees or shrubs. Stipules entire. Young inflorescences completely surrounded by calyptra-like bracts. Inflorescences axillary. Flowers homostylous. Corolla lobe imbricate in bud. Stigmas clavate to globose; ovary bicarpellate, with 1 to 15 ovules in each locule, pendulous. Infructescences multiple fruits, indehiscent, with accrescent nectary disks derived from the post-growth of floral nectaries during infructescence development. Seeds unwinged or winged.

Genera included: *Breonadia*, *Breonia*, *Gyrostipula*, *Janotia*.

Subtribe Adininae Ridsdale, Blumea 24: 319 (1978a).

Useful recent study: Ridsdale (1978a)

Trees or shrubs. Stipules entire or shallowly bifid. Young inflorescences surrounded or not (in *Ludekia*) by calyptra-like bracts. Inflorescences terminal or axillary. Flowers homostylous. Corolla lobes valvate but subimbricate at the apex or imbricate. Stigmas clavate to globose, ovary bicarpellate, with ovary 1 to 14 ovules in each locule. Infructescences formed by free, capsular fruits. Seeds winged.

Genera included: *Adina*, *Adinauclea*, *Haldina*, *Ludekia*, *Metadina*, *Myrmeconauclea*, *Neonauclea*, *Pertusadina*, *Sinoadina*.

DISCUSSION

Molecular evolution—Table 4 shows that the *trnT-F* data provide more phylogenetically informative characters (131) than the *rbcL* data (53) in Naucleeae, suggesting that the *trnT-F* regions might be useful for inferring phylogenetic relationships at higher taxonomic levels (tribes) in Rubiaceae. However, the ITS data yield many more parsimony-informative characters (210) than the *trnT-F* data, corroborating the usefulness of ITS for addressing phylogenetic relationships at tribal level or below in Rubiaceae (e.g., McDowell and Bremer, 1998; Person, 2000; Lantz, Andreasen, and Bremer, 2002).

Several studies (e.g., Gielly and Taberlet, 1994; McDade and Moody, 1999) have shown that the spacer portion of the *trnT-F* region is evolving more rapidly than the intron. This is also corroborated by our results, with both *trnL-F* and *trnT-L* spacers providing the same number of variable sites (71) and more than that of the *trnL* intron (31). Our studies also showed that the *trnT-L* spacer had more phylogenetically informative characters (all variable sites) than the *trnL-F* spacer (only 31 of 71 variable sites), suggesting that the combination of these three adjacent regions evolving at different rates is useful for inferring phylogenetic relationships in Rubiaceae.

Subtribal limits in Naucleeae s.l.—Both strict consensus trees (Figs. 2 and 3) from the combined analyses identify the same lineages and also receive much higher support than any of the strict consensus trees from the separate analyses. We chose the strict consensus tree from the combined molecular-morphological analysis (Fig. 3) over the strict consensus tree from the combined molecular analysis (Fig. 2) for making conclusions on intratribal classifications, the generic limits, and relationships among the subtribes of Naucleeae s.l. because it is the best supported hypothesis, maximizing congruence among all of the characters sampled (e.g., Nixon and Carpenter, 1997).

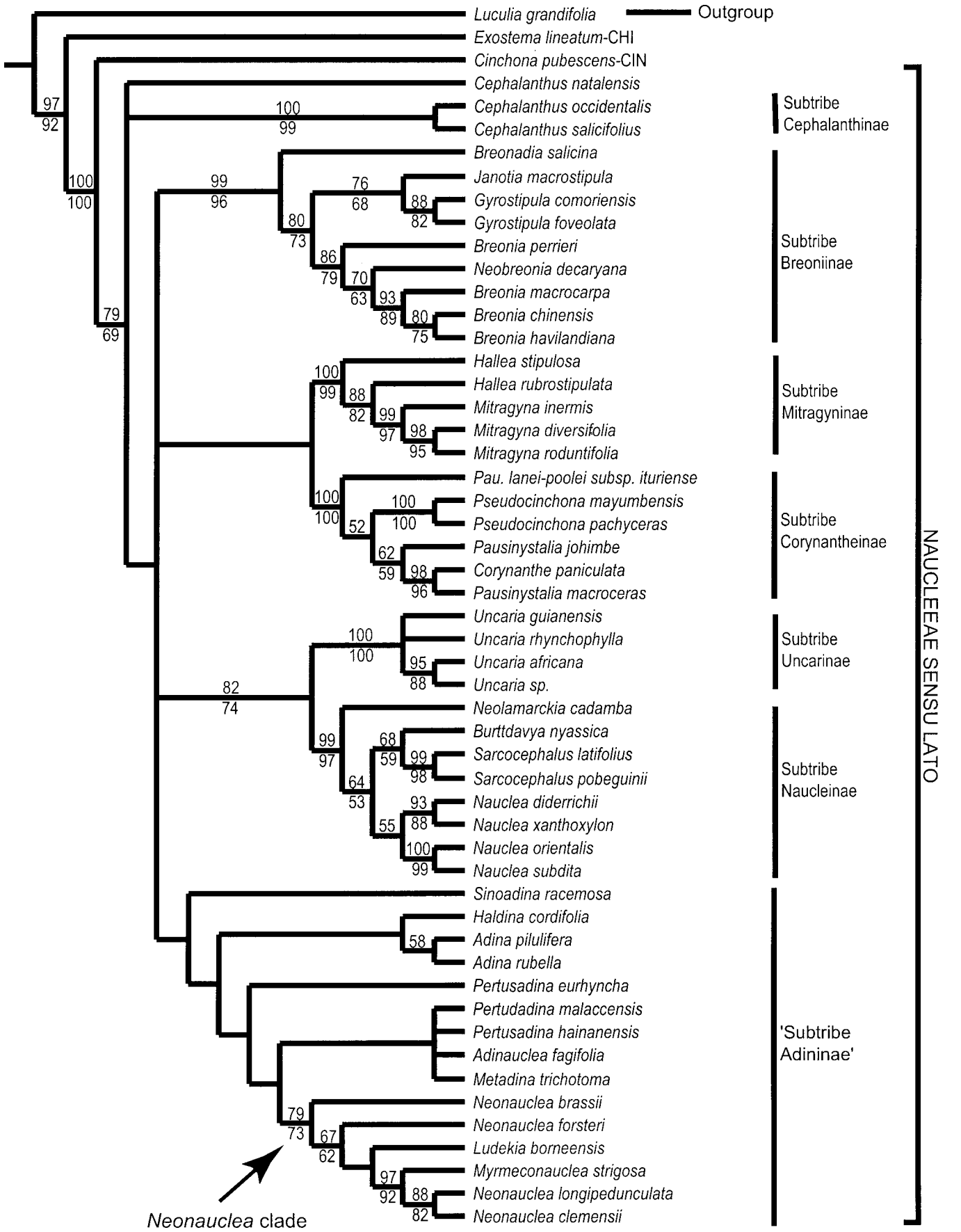
Haviland (1897) and Ridsdale's (1978a) subtribal limits are partly supported by our results. The combined molecular-morphological tree (Fig. 3) highly supports (JK = 100, BS = 100) the monophyly of the subtribes Anthocephalidae sensu Haviland (Naucleinae sensu Razafimandimbison and Bremer), Mitragynae sensu Haviland (Mitragyninae), and Uncariae sensu Haviland (Uncarinae). We perceive no support for the subtribe Cephalanthidae sensu Haviland because its members came out in five separate and distinct clades (Cephalanthinae, Breoniinae, Adininae). The subtribe Naucleinae sensu Ridsdale (same as Naucleinae sensu Razafimandimbison and Bremer excluding *Neolamarckia*) constitutes a moderately monophyletic group (JK = 84, BS = 76). Naucleinae sensu Ridsdale and Neolamarckiinae sensu Robbrecht (containing the single genus *Neolamarckia*) are resolved together as a monophyletic group (JK = 100, BS = 99), supporting the inclusion of *Neolamarckia* in Naucleinae sensu Ridsdale. Recognition of Neolamackiinae (Haviland, 1897; Ridsdale, 1978a) as a separate subtribe is mainly based on one autapomorphic character state: ovaries with upper parts divided into two or four cavities due to false septa. The subtribe Adininae sensu Ridsdale, containing Breoniinae, and Adininae sensu Razafimandimbison and Bremer, is not resolved as a monophyletic group.

The present study shows that a new intratribal classification of Naucleeae s.l. is needed. The combined molecular-morphological tree (Fig. 3) depicts that the tribe can be subdivided into seven subtribes: Cephalanthinae, Mitragyninae, Uncarinae, Corynantheinae, Naucleinae, Breoniinae (all strongly supported), and ''Adininae'' (poorly supported). Both Naucleinae (as Anthocephalidae sensu Haviland) and Neolamarckiinae (as Anthocephalinae sensu Ridsdale) have been used in earlier subtribal classifications (Haviland, 1897; Ridsdale, 1978a). Naucleinae (Candolle, 1830) has priority over Neolamarckiinae (Robbrecht, 1988). ''Adininae'' receives poor support (JK and BS < 50) in both combined trees (Figs. 2 and 3). However, we have chosen a conservative approach to provisionally maintain Adininae as a separate subtribe to accommodate *Adina*, *Adinauclea*, *Haldina*, *Metadina*, *Ludekia*, *Myrmeconauclea*, *Neonauclea*, *Pertusadina*, and *Sinoadina*.

If compared with the previous subtribal classifications of Naucleeae (Haviland, 1897; Ridsdale, 1978a) it appears that Haviland's scheme is best supported by our data. Of the seven subtribes recognized here, two (Breoniinae and Corynantheinae) are newly described, two (Adininae and Cephalanthinae) have received very different circumscriptions (Table 2), and two (Mitragyninae and Uncarinae) have identical circumscriptions as Haviland's (1897). The circumscription of Anthocephalidae sensu Haviland differs from our Naucleinae only be-

←

Fig. 1. Jackknife consensus tree of Naucleeae obtained from *trnT-F* data of 55 taxa. Vertical bars correspond to the limits of lineages. Brace indicates the members of Naucleeae s.l. Tribal positions are indicated by a three-letter suffix: CIN = Cinchoneae and CHI = Chioccoceae.


Luculia grandifolia
Outgroup
Exostema lineatum-CHI
Cinchona pubescens-CIN
Cephalanthus natalensis
Cephalanthus occidentalis
Cephalanthus salicifolius
Subtribe Cephalanthinae
Breonadia salicina
Janotia macrostipula
Gyrostipula comoriensis
Gyrostipula foveolata
Breonia perrieri
Neobreonia decaryana
Breonia macrocarpa
Breonia chinensis
Breonia havilandiana
Subtribe Breoniinae
Hallea stipulosa
Hallea rubrostipulata
Mitragyna inermis
Mitragyna diversifolia
Mitragyna roduntifolia
Subtribe Mitragyninae
Pau. lanei-poolei subsp. ituriense
Pseudocinchona mayumbensis
Pseudocinchona pachyceras
Pausinystalia johimbe
Corynanthe paniculata
Pausinystalia macroceras
Subtribe Corynantheinae
Uncaria guianensis
Uncaria rhynchophylla
Uncaria africana
Uncaria sp.
Subtribe Uncarinae
Neolamarckia cadamba
Burttdavya nyassica
Sarcocephalus latifolius
Sarcocephalus pobeguinii
Nauclea diderrichii
Nauclea xanthoxylon
Nauclea orientalis
Nauclea subdita
Subtribe Naucleinae
Sinoadina racemosa
Haldina cordifolia
Adina pilulifera
Adina rubella
Pertusadina eurhyncha
Pertudadina malaccensis
Pertusadina hainanensis
Adinauclea fagifolia
Metadina trichotoma
'Subtribe Adininae'
Neonauclea brassii
Neonauclea forsteri
Ludekia borneensis
Myrmeconauclea strigosa
Neonauclea longipedunculata
Neonauclea clemensii
Neonauclea clade
NAUCLEEAE SENSU LATO

cause of the inclusion of *Burttdavya*, which was described after his worldwide revision for Naucleeae (Haviland, 1897).

Relationships among subtribes—Our results support the basal position of Cephalanthinae in Naucleeae s.l. Cephalanthinae is resolved with high support (JK = 100, BS = 100) as sister to the rest of Naucleeae placed in a large clade. However, even the combination of four data sets (three molecular and one morphological) did not provide enough informative characters to resolve the relationships among the six subtribes within this large clade. The poor resolution can be explained by the inadequate number of synapomorphic characteristics shared among these lineages, which are mostly united by homoplastic characters. In other words, this pattern may also reflect rapid early diversification of these subtribes, such that few or no mutations apparently became fixed in their common ancestors. Although Ridsdale's subtribal concepts (1978a) are only partly supported by our results, his conclusions about the relationships between his subtribes are corroborated by our studies: he says ''the subtribes here recognized are relatively homogeneous but these subtribes have only a rather low level of relationship with each other'' (Ridsdale, 1978a, p. 309). Most of the synapomorphic characteristics support the nodes that define both the subtribes and their internal nodes; this suggests that the diversification of and within the subtribes at both molecular and morphological levels must have occurred rather slowly after their early rapid radiation, such that there has been enough time for both morphological and molecular changes to accumulate along branches.

Relationships within subtribes and generic limits—Our intention is to establish new generic limits that are as consistent as possible with the previous classifications of Naucleeae and to minimize nomenclatural changes. In this study, we used two criteria for evaluating the status of the genera (with two or more species) currently placed in Naucleeae s.1. as well as generic recognition (Backlund and Bremer, 1998): (1) genera must be monophyletic and diagnosed by inclusive morphological synapomorphies, allowing recognition of their members; and (2) they must be easy to identify and separate from each other. Assessment and recognition of the monotypic genera are done based on a combination of the following three criteria: (a) if they are not nested within well-defined genera; (b) if they have at least two autapomorphic characters, allowing them to be recognized very easily; (c) and if their relationships with other genera are not strongly supported.

Cephalanthinae—Unlike its taxonomic position, the recognition *Cephalanthus* at generic level has been widely accepted. We maintain *Cephalanthus* sensu Ridsdale (1976), represented by three species here, as a separate genus because it is strongly supported as a monophyletic group, although we have been unable to find any unique morphological synapomorphy that unites *C. natalensis* and the American and Asian species.

Mitragyninae—Leroy (1975a) segregated three of the four African species (*Mitragyna ciliata*, *M. rubrostipulata*, and *M. stipulosa*) from *Mitragyna* s.1. and placed them in the new genus *Hallea* based on the following characters: monopodial growth, relatively large leaves, axillary inflorescences on lateral twigs, the abaxial side of corolla lobes densely pubescent and prolonged into short appendages, and stigmas with the receptive areas covering the entire surfaces of the stigmatic lobes. *Mitragyna* s.s. are characterized by having small leaves, terminal inflorescences, sympodial growth, the outside of corolla lobes glabrous and without appendages, and stigmas with the receptive areas restricted towards the distal and proximal ends of the stigmatic lobes. These architectural and floral structure differences correlate with wood and leaf anatomy (see Leroy, 1975a) and some differences in the spectrum of alkaloids. Also, they are supported by chorology: *Hallea* are exclusively restricted to rheophytic habitats such as swampy forest in East and Central Africa, whereas *Mitragyna* are mostly Asian, with only one African representative (*M. inermis*), which is restricted to the Sudanian regions. *Hallea* has been recognized as a separate genus by several authors (e.g., Huysman, Robbrecht, and Smets, 1994). However, Ridsdale (1978a) retained *Hallea* in *Mitragyna*.

Our strict consensus combined molecular-morphological tree (Fig. 3) strongly shows with high support (JK = 100, BS = 100) that *Hallea* and *Mitragyna* s.s. are more closely related to each other than they are to the rest of Naucleeae s.1.; this is consistent with morphological evidence. *Mitragyna* s.1. (including *Hallea*), an Afro-Asian genus with nine species (four African and five Asian), is diagnosed by mitriform stigmatic lobes and three-zonocolporate pollen grains with endoapertures that are always H-shaped (Huysmans, Robbrecht, and Smets, 1994). Also, the combined molecular-morphological tree supports the monophyly of *Mitragyna* s.s., represented by three species here, but neither reject nor support the monophyly of *Hallea*. However, the paraphyly of *Hallea* is highly supported by the combined molecular tree (Fig. 2); therefore, we merge *Hallea* with *Mitragyna*, as suggested by Ridsdale (1978b).

Uncarinae—The generic status of *Uncaria*, represented in our analysis by four species, has never been disputed due to its unique climbing habit and paired fang hooks that make this genus very distinct from the other members of Naucleeae s.1. Our study strongly supports the monophyly of *Uncaria*, supporting its generic status.

Corynantheinae—The African genus *Corynanthe* was originally described by Welwitsch (1869) based on a single species (*C. paniculata*) from Angola. Later, more species (including the African ''yohimbe tree'' [*C. johimbe*]) were recognized. Because of the presence of johimbine (or yohimbe), a substance with medicinal properties, *Corynanthe* attracted early systematic attention. The anatomical and pharmacological studies by Dupouy and Beille (1905), which confirmed J.-B. Pierre's conclusions (unpublished data, Laboratoire Phanérogamie, Paris) based on morphological observations, revealed some evidence to support the distinctions between *Corynanthe paniculata* (with an infundibular corolla tube, exserted style

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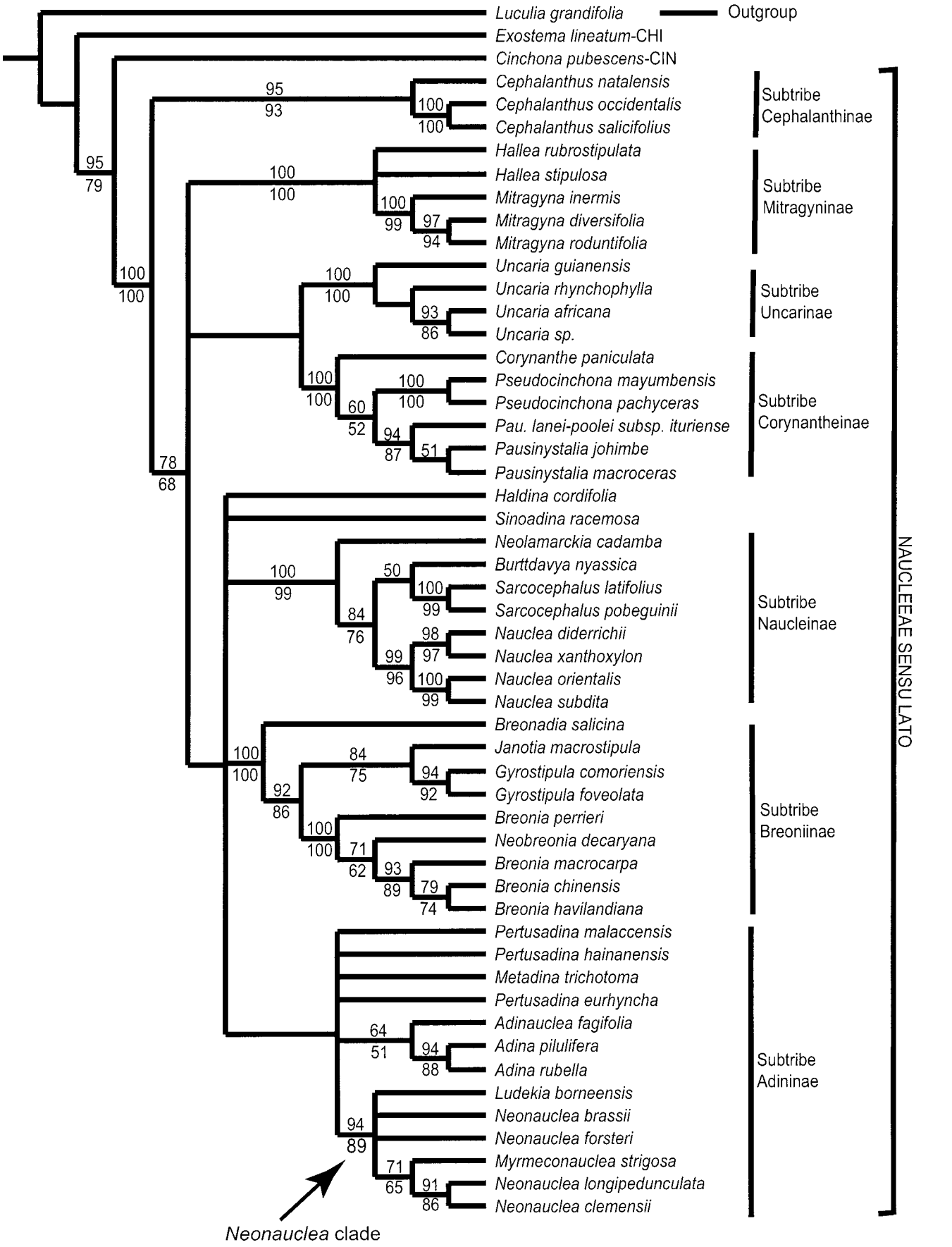
Fig. 2. Strict consensus tree of 15 equally most parsimonious trees of Naucleeae s.l. from 53 taxa based on combined molecular data. Numbers above and below nodes are jackknife and bootstrap support values of that particular node. Vertical bars correspond to the limits of the lineages. Brace indicates the members of Naucleeae s.l. Tribal positions are indicated by a three-letter suffix: CHI = Chioccoceae and CIN = Cinchoneae.


Luculia grandifolia
Outgroup
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Cephalanthus occidentalis
Cephalanthus salicifolius
Subtribe Cephalanthinae
Hallea rubrostipulata
Hallea stipulosa
Mitragyna inermis
Mitragyna diversifolia
Mitragyna roduntifolia
Subtribe Mitragyninae
Uncaria guianensis
Uncaria rhynchophylla
Uncaria africana
Uncaria sp.
Subtribe Uncarinae
Corynanthe paniculata
Pseudocinchona mayumbensis
Pseudocinchona pachyceras
Pau. lanei-poolei subsp. ituriense
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Pausinystalia macroceras
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Neolamarckia cadamba
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Sarcocephalus latifolius
Sarcocephalus pobeguinii
Nauclea diderrichii
Nauclea xanthoxylon
Nauclea orientalis
Nauclea subdita
Subtribe Naucleinae
Breonadia salicina
Janotia macrostipula
Gyrostipula comoriensis
Gyrostipula foveolata
Breonia perrieri
Neobreonia decaryana
Breonia macrocarpa
Breonia chinensis
Breonia havilandiana
Subtribe Breoniinae
Pertusadina malaccensis
Pertusadina hainanensis
Metadina trichotoma
Pertusadina eurhyncha
Adinauclea fagifolia
Adina pilulifera
Adina rubella
Ludekia borneensis
Neonauclea brassii
Neonauclea forsteri
Myrmeconauclea strigosa
Neonauclea longipedunculata
Neonauclea clemensii
Subtribe Adininae
NAUCLEEAE SENSU LATO
Neonauclea clade

and anthers, spherical and undivided stigmas, and mainly loculicidal capsules) and *C. johimbe* (with corolla tubes differentiated in a basal narrowly and shortly cylindrical part bearing an apical bladder, included style and anthers, bilobed stigmas, and mainly septicidal capsules). Consequently, they placed *C. johimbe* in the separate genus, *Pausinystalia*. These morphological distinctions between *Corynanthe* and *Pausinystalia* defined by Dupouy and Beille (1905) have been followed by several authors (e.g., Brandt, 1922; De Wildeman, 1922; Good, 1926; Hallé, 1966; Stoffelen, Robbrecht, and Smets, 1996). Chevalier (1909) questioned the homogeneity of *Corynanthe* sensu Dupouy and Beille (containing *C. pachyceras* and *C. paniculata*) and adopted a new circumcription by restricting it to the type species with five-merous flowers, exserted style and anthers, and loculicidal capsules and placing the species with four-merous flowers, exserted style and anthers, and largely septicidal capsules (*C. pachyceras*) in his new genus *Pseudocinchona* (*P. africana*). Chevalier (1926) subsequently made the new combination *Pseudocinchona pachyceras* (K. Schum.) A. Chev. because the epithet "*pachyceras*" (Schumann, 1901) has priority over that of "*africana*" (Chevalier, 1909). *Pseudocinchona* is additionally characterized by the occurrence of two alkaloids, corynanthine and corynantheine, which are absent in *Pausinystalia* and *Corynanthe* s.s. (*C. paniculata*). Raymond-Hamet's (1941) study confirmed that johimbine is indeed absent in *Pseudocinchona* but present in *Pausinystalia johimbe* and *Corynanthe paniculata*. Also, he found out that johimbine is absent in *Pausinystalia mayumbensis* (Good, 1926) and therefore transferred this latter to *Pseudocinchona*. Hallé (1966) treated the two species of *pseudocinchona* (*P. mayumbensis* and *P. pachyceras*) in *Corynanthe* and instead recognized them at subgeneric level. However, Stoffelen, Robbrecht, and Smets (1996) also included *Pseudocinchona* in *Corynanthe*, but without recognizing them at the infrageneric level.

The combined molecular-morphological tree (Fig. 3) strongly supports *Pausinystalia* sensu Stoffelen, Robbrecht, and Smets (1996) (represented by three species here) as a separate monophyletic group. However, *Corynanthe* sensu Stoffelen, Robbrecht, and Smets (1996), represented here by all three species, is resolved with high support as paraphyletic, with its type species *C. paniculata* placed as sister to a clade containing *Pausinystalia* sensu Stoffelen, Robbrecht, and Smets, *Corynanthe mayumbensis*, and *C. pachyceras*. The combined molecular tree (Fig. 2) places *C. paniculata* with high support nested between *Pausinystalia johimbe* and *P. macroceras*, making *Pausinystalia* sensu Stoffelen, Robbrecht, and Smets (1996) paraphyletic; this result is also consistent with phytochemical data (*C. paniculata* and *Pausinystalia* have johimbine, a substance not found in *Pseudocinchona* [Chevalier, 1909; Raymond-Hamet, 1941]). In both combined trees, *Pseudocinchona* (represented by *P. mayumbensis* and *P. pachyceras*) is shown with strong support to be monophyletic. Based on all evidence presented above, we argue that the generic status of *Corynanthe* sensu Stoffelen, Robbrecht, and Smets (1996) is untenable. Three alternative solutions are possible. One is to restrict *Corynanthe* to *C. paniculata*, resurrect *Pseudocinchona* sensu Chevalier (1909, 1926), and maintain the current circumscription of *Pausinystalia* (Stoffelen, Robbrecht, and Smets, 1996). The second is to sink *Pausinystalia* in *Corynanthe* (the older name) and retain all the described taxa within the *Corynanthe* lineage without infrageneric subdivision; this would maximize the nomenclature stability, but would make *Corynanthe* rather heterogenous morphologically. Third is to include *Pausinystalia* in *Corynanthe* sensu Stoffelen, Robbrecht, and Smets (1996) and recognize *Corynanthe paniculata*, *Pseudocinchona*, and *Pausinystalia* at the subgeneric level. We favor the first alternative because this does not require any nomenclatural change (maximizing stability) and also reflects the distinctness of these three genera.

TABLE 4. Comparisons of the different data sets.

Data sets	Number of investigated taxa	Number of characters[a]	Number of informative characters
ITS[b]	53	641	210 (32.76%)
rbcL[b]	53	1415	53 (3.7%)
trnT-F	55	1988	131 (6.6%)
Combined molecular	53	4044	381 (9.5%)
Combined molecular-morphology	53	4093	426 (10.5%)

[a] Length of aligned sequences (including phylogenetically scored, informative indels).

[b] Trees not presented.

Naucleinae sensu Razafimandimbison and Bremer—Nauclea sensu Haviland (1897) is characterized by a combination of two characters: spindle-shaped stigmatic lobes with the receptive areas restricted only to the base and multiple fruits. Ridsdale's narrow generic concept of *Nauclea* includes only the species with Y-shaped placentae attached to the upper third of the septum and multiple fruits (Ridsdale, 1975, 1978a). Consequently, he reinstated the African genus *Sarcocephalus* and recognized the new genus *Ochreinauclea* to accommodate *Nauclea*, both *maingayi* and *N. missionis*, which Ridsdale thought to have pseudomultiple fruits.

Nauclea sensu Ridsdale (1975, 1978a), represented by four species in our analysis, is resolved with high support (JK = 99, BS = 96) as monophyletic. The Y-shaped placenta appears to be the single morphological synapomorphy of *Nauclea* sensu Ridsdale. Therefore, we will maintain the present circumscription of *Nauclea* (Ridsdale, 1978a).

The two species of *Sarcocephalus* sensu Ridsdale (1975) analyzed here form a highly supported monophyletic group (JK = 100, BS = 99). Both *Nauclea* sensu Ridsdale and *Sarcocephalus* have multiple fruits, but this latter is distinct from the former by its small deltoid stipules with obtuse to slightly notched apices, calyx lobes prolonged by small appendages, discoidal placentae rather than large stipules and corolla lobes without appendages, and Y-shaped placentae as in *Nauclea* sensu Ridsdale (1975). We therefore maintain the generic status of *Sarcocephalus*.

The generic status of the East African monotypic genus *Burttdavya* is maintained based on its linear-oblong placentae

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Fig. 3. Strict consensus tree of 38 equally most parsimonious trees of Naucleeae s.l. from 53 taxa based on combined molecular-morphological data. Numbers above and below nodes are jackknife and bootstrap support values of that particular node. Vertical bars correspond to the limits of the lineages. Brace indicates the members of Naucleeae s.l. Tribal positions are indicated by a three-letter suffix: CHI = Chioccoceae and CIN = Cinchoneae.

and free indehiscent fruits without exocarps (Ridsdale, 1975); *Burttdavya* is placed with weak support (JK = 50, JK < 50%) as sister to *Sarcocephalus*.

The Asian genus *Ochreinauclea* was not included in our combined analyses because *O. maingayi* appeared in two different clades in the nuclear and chloroplast-based phylogenies (see Razafimandimbison and Bremer, 2002). However, it was included in the ITS, *rbcL*, and *trnT-F* analyses. In the ITS tree, *O. maingayi* was placed within Naucleinae sensu Razafimandimbison and Bremer with high support as closely related to *Neolamarckia cadamba*. This relationship is corroborated by the occurrence of both of the two morphological synapomorphies (spindle-shaped stigmas and receptive areas restricted to the base of the stigmatic lobes) for Naucleinae in *Ochreinauclea*. However, the *rbcL* (Razafimandimbison and Bremer, 2002) and *trnT-F* (Fig. 1) trees both placed *Ochreinauclea* in the *Neonauclea* clade. We have interpreted *Ochreinauclea maingayi* as a nothospecies, i.e., a hybrid between *Neolamarckia* and one taxon from the *Neonauclea* clade (Razafimandimbison and Bremer, 2002). *Ochreinauclea* is characterized by multiple fruits, a feature also present in *Nauclea* and *Sarcocephalus*, and winged seeds, a feature commonly found in the *Neonauclea* clade, but absent in Naucleinae. *Neolamarckia* and *Ochreinauclea* both have conical terminal vegetative buds. The combination of indehiscent, multiple fruits and winged seeds together are unique for *Ochreinauclea* and rare in Rubiaceae. The Article H.5 in the International Code of Botanical Nomenclature (Saint Louis Code) 2000 (Greuter et al. 2000: p. 107) states that ''the appropriate rank of a nothotaxon is that of the postulated or known parent taxa.'' Therefore, like *Neolamarckia*, the generic status of *Ochreinauclea* can be maintained. Its unique features make *Ochreinauclea* easy to distinguish from the other Naucleeae genera.

Neolamarckia is an Asian genus, which was originally described by Richard (1830) as *Anthocephalus* based on a nonexistent specimen of Sonnerat (see discussions in Razafimandimbison [2002] and references therein). *Neolamarckia* is diagnosed by having branched placentae attached to the upper third of the septa and ovaries that in their upper parts are split into two or four locules, but their lower parts are divided into two locules by the false septa in the upper parts. The combined molecular-morphological tree (Fig. 3) placed *Neolamarckia* with high support (JK = 100, BS = 99) as sister to a clade containing *Burttdavya*, *Nauclea* sensu Ridsdale, and *Sarcocephalus*; this suggests that *Neolamarckia* is a distinct lineage and thus its generic status could be maintained.

Breoniinae—Breoniinae contains all the investigated members of the Malagasy Naucleeae (*Breonia*, *Gyrostipula*, and *Janotia*) and the Afro-Malagasy genus *Breonadia*.

Breonadia can easily be recognized by its verticillate leaves and intrapetiolar stipules. Our results placed *Breonadia* as basal and sister to a highly supported clade (JK = 92, BS = 86) containing *Breonia*, *Gyrostipula*, and *Janotia*. This shows that *Breonadia* is not closely related to *Adina* sensu Haviland, as previously suggested by Haviland (1897), but instead its position is consistent with Ridsdale's decision to separate *Breonadia* from *Adina* s.1. Its basal position and unique features suggest that its generic status could be maintained.

Breonia was originally described by Richard (1830) as an endemic genus from Madagascar. Ridsdale (1975), in his revision of the African and Malagasy Naucleeae, adopted a narrow circumscription of *Breonia*, excluding *Breonia decaryana* and *B. keliravina*, both of which have flattened terminal vegetative buds, calyx tubes of adjacent flowers partly fused, and carpels containing a single ovule per locule. These two species were recognized as a separate genus *Neobreonia* (Ridsdale, 1975). Recently, a revision of *Breonia* has been completed (Razafimandimbison, 2002). It contains 20 species and *Neobreonia decaryana* has been reincluded; this decision was based on the results of our phylogenetic analyses of the ITS and *rbcL* and morphological data sets (Razafimandimbison and Bremer, 2002) and is further supported by the present study.

Capuron (1972) originally described two new species of *Neonauclea* from Madagascar, *N. foveolata* and *N. macrostipula*, mainly because of their dehiscent capsular fruits and bicornate seeds. A. Homolle (unpublished data, Laboratoire Phanérogamie, Paris) considered these two species of *Neonauclea* to be better treated in *Adina*. Leroy (1975b) strongly argued that the characters observed in these two species (axillary inflorescence, absence of interfloral bracteoles and calyx appendages) did not fit either *Adina* (presence of interfloral bracteoles) or *Neonauclea* (inflorescence terminal and calyx lobes prolonged by long appendages). As a result, he classified *N. foveolata* and *N. macrostipula* in the separate genera *Gyrostipula*, with two species (*G. comoriensis* and *G. foveolata*), and *Janotia*, with one species (*J. macrostipula*).

In the strict consensus tree of the combined molecular-morphological data (Fig. 3), *Gyrostipula* appears monophyletic with high support (JK = 94, BS = 92). This is corroborated by a number of autapomorphies: convolute, red, long terminal vegetative buds, calyx tubes densely pubescent, and red placentae persistently attached to the septa after the fruits dehisce and release the mature seeds. *Gyrostipula* and *Janotia* are resolved as more closely related to each other than they are to the other members of Breoniinae, a relationship also corroborated by one morphological synapomorphy, ovules attached side by side to the base of the placentae. *Janotia* can easily be distinguished from *Gyrostipula* by its terminal vegetative buds with semi-persistent, complanate large foliaceous stipules and long filiform calyx lobes. We here retain both genera based on the principle of ease of identification (Backlund and Bremer, 1998).

Adininae sensu Razafimandimbison and Bremer—Adina sensu Haviland, consisting of nine species (*Adina cordifolia*, *A. microcephala*, *A. multifolia*, *A. oligocephala*, *A. pilulifera*, *A. polycephala*, *A. racemosa*, *A. rubella*, and *A. rubescens*), is characterized by filiform to clavate interfloral bracteoles, valvate (though sometimes apically subimbricate) corolla lobes, and free, capsular fruits with the calyx remnants falling off together with the central axis. Ridsdale (1978a) considered Haviland's *Adina* as a heterogeneous assemblage of taxa; he adopted a much narrower generic concept for *Adina* by restricting it to the three Asian species *A. pilulifera*, *A. rubella*, and *A. dissimilis*, which are diagnosed by having loosely defined terminal vegetative buds surrounded by spreading stipules. Ridsdale subsequently recognized all of the other species in separate genera: *Adina fagifolia* as *Adinauclea*, *A. microcephala* as *Breonadia*; *A. cordifolia* as *Haldina*; *A. oligocephala* as *Metadina*; *A. polycephala* as *Khasiaclunea*; *A. multifolia* and *A. rubescens* as *Pertusadina multifolia* and *P. eurhyncha*, respectively, and *A. racemosa* as *Sinoadina*. He stated that only few small characters separated these minor genera. *Adinauclea* has spathulate to spathulate-clavate interfloral bracts and valvate but subimbricate at the apex corolla lobes.

TABLE 5. List of genera accepted here and their synonyms.

Accepted genera	Synonyms
Adina sensu Ridsdale (1978a)	
Adinauclea sensu Ridsdale (1978a)	
Breonadia sensu Ridsdale (1975)	
Breonia sensu Razafim. (2002)	*Cephalidium* A. Rich.; *Franchetia* Baill.; *Elattospermum* Sol.; *Neobreonia* Ridsdale
Burttdavya sensu Ridsdale (1975)	
Cephalanthus sensu Ridsdale (1976)	*Acrodryon* Spreng.; *Axolus* Tafin.; *Eresimus* Rafin.
Corynanthe sensu Welw. (1869) and Chevalier (1909)	
Gyrostipula sensu J.-F. Leroy (1975a)	
Haldina sensu Ridsdale (1978a)	
Janotia sensu J.-F. Leroy (1975a)	
Ludekia sensu Ridsdale (1978a)	
Metadina sensu Ridsdale (1978a)	
Mitragyna sensu Ridsdale (1978b)	*Hallea* J.-F. Leroy
Myrmeconauclea sensu Ridsdale (1978a)	
Nauclea sensu Ridsdale (1975)	
Neolamarckia sensu Bosser (1984)	*Anthocephalus* A. Rich.
Neonauclea sensu Ridsdale (1978a)	
Ochreinauclea sensu Ridsdale (1978a)	
Pausinystalia sensu Stoffelen, Robbrecht, and Smets (1996)	
Pertusadina sensu Ridsdale (1978a)	
Pseudocinchona sensu Chevalier (1909)	
Sarcocephalus sensu Ridsdale (1975)	
Sinoadina Ridsdale (1978a)	
Uncaria sensu Ridsdale (1978b)	*Ourouparia* Aubl.

Ridsdale (1978a) argued that these features suggest that *Adinauclea* is more closely related to *Adina* than it is to *Neonauclea*. However, *Adinauclea* also has flattened terminal vegetative buds, a feature absent in *Adina* and Ridsdale's segregate genera but commonly found in *Neonauclea* and its satellite genera. *Metadina* can be easily diagnosed by having numerous (20–30) heads arranged in a compound thyrse. *Pertusadina* is characterized by a combination of conical terminal vegetative buds, axillary inflorescences, and capsular fruits. Finally, *Sinoadina* is characterized by terminal inflorescences with 7–11 heads arranged in a simple thyrse.

Our results are largely inconclusive for assessing the relationships and generic limits among *Adinauclea*, *Metadina*, and *Pertusadina*. For now, we maintain their generic status. *Adina* sensu Ridsdale (1978a) is resolved with strong support (JK = 94, BS = 88) as a monophyletic group, supporting its generic status. *Haldina* and *Sinoadina* are left unresolved as separate lineages within a collapsed clade containing Naucleinae, Breoniinae, and Adininae; we also continue to maintain their generic status. Adding one more data set from a low-copy nuclear gene, such as phytochrome B, which has been shown to be useful for resolving relationships among and generic limits of many groups (e.g., Simmons et al., 2000 and Mathews, Tsai, and Kellogg, 2000), would perhaps be helpful in resolving relationships among *Adina* sensu Haviland (1897) and rigorously evaluate their generic status.

Neonauclea sensu Merrill (1915) was a large Asian genus including about 70 species and characterized by terminal inflorescences and free capsular fruits. Merrill (1920) recognized *Neonauclea strigosa* as the separate genus *Myrmeconauclea* because of its unique features (pseudomultiple fruits and seeds with very long ventral wings). Ridsdale accepted *Myrmeconauclea*, but still viewed *Neonauclea* sensu Merrill as an overly heterogeneous assemblage of taxa; he adopted a new circumscription for *Neonauclea* by restricting it to the species with corolla lobes prolonged by obtrigonal to spathulated, deciduous appendages. He recognized the following genera as separate genera: *Neonauclea bernardoi* as *Ludekia*, *N. oligocephala* as *Khasiaclunea*, and *N. zeylanica* as *Diyaminauclea*. *Ludekia* is distinct from the rest of Naucleeae genera by its globose stigmatic lobes with 7–9 prominently longitudinal ridges.

Ludekia borneensis, *Myrmeconauclea strigosa*, *Neonauclea brassii*, *N. clemensii*, *N. forsteri*, and *N. longipedunculata* constitute a strongly monophyletic group (JK = 94, BS = 89), corroborating their close relationships. However, the relationships between *Ludekia* and *Neonauclea* sensu Ridsdale are unresolved. *Myrmeconauclea* is nested within *Neonauclea* sensu Ridsdale, represented here by four species, indicating that *Neonauclea* sensu Ridsdale (1989) is paraphyletic and needs to be recircumscribed. Two alternative solutions are possible. One would be to merge *Ludekia* and *Myrmeconauclea* in *Neonauclea*, making this latter as monophyletic; this would maximize taxonomic stability and minimize nomenclatural changes, but would also make *Neonauclea* rather heterogeneous morphologically. A second would be to retain the two well-defined genera (*Ludekia* and *Myrmeconauclea*) as separate genera; this would require then considerable splitting of the paraphyletic *Neonauclea* s.s. into several small genera, which might also cause a lot of nomenclatural changes. The first alternative is a more logical approach, but we have avoided recognizing morphologically heterogeneous genera. Also, we have investigated only four (of 65) species *Neonauclea* sensu Ridsdale (1989). A molecular phylogenetic study using fast-evolving markers and including several species of *Neonauclea* sensu Ridsdale is needed to specifically address the limits of the genera within *Neonauclea* clade. For now, we continue to maintain the generic status of *Lukedia*, *Myrmeconauclea*, and *Neonauclea*.

In conclusion, phylogenetic studies of Naucleeae s.l. based on the four data sets (three molecular, ITS, *rbcL*, and *trnT-F*, and one morphological) further confirm the monophyly of

Naucleeae s.l., also including *Cephalanthus*, *Corynanthe*, *Mitragyna*, s.l. (including *Hallea*), *Pausinystalia*, and *Uncaria*. Anthocephalidae, Mitragynae, and Uncariae all sensu Haviland are highly supported as monophyletic; however, Cephalanthidae sensu Haviland is shown to be paraphyletic. Naucleinae sensu Ridsdale (1975) is moderately supported as monophyletic. Naucleinae sensu Ridsdale and Neolamackiinae sensu Robbrecht (1994) form a strongly supported monophyletic group. Our results neither support nor reject the monophyly of Adininae sensu Ridsdale (containing Adininae sensu Razafimandimbison and Bremer and Breoniinae). We disregard Haviland and Ridsdale's subtribal limits and recognize a new subtribal classification that contains seven morphologically distinct subtribes: Cephalanthinae, Mitragyninae, Uncarinae, Corynantheinae, Naucleinae, Breoniinae, and Adininae. Cephalanthinae is resolved as basal and sister to the rest of Naucleeae; however, the relationships among the other major lineages remain largely unresolved. Finally, we retain the generic status of the following 22 genera: *Adina*, *Adinauclea*, *Breonadia*, *Breonia*, *Burttdavya*, *Cephalanthus*, *Gyrostipula*, *Haldina*, *Janotia*, *Ludekia*, *Metadina*, *Mitragyna*, *Myrmeconauclea*, *Nauclea*, *Neolamarckia*, *Neonauclea*, *Ochreinauclea*, *Pausinystalia*, *Pertusadina*, *Sarcocephalus*, *Sinoadina*, and *Uncaria*. *Corynanthe* is restricted to its type species, *C. paniculata*. *Hallea* is reincluded in *Mitragyna*. The African genus *Pseudocinchona* is reestablished. We were unable to assess the relationships among *Adinauclea*, *Adina*, *Metadina*, and *Pertusadina* due to lack of resolution. Finally, all accepted genera and their synonymies are given in Table 5.

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