



Molecular phylogenetics and generic assessment in the tribe Morindeae (Rubiaceae–Rubioidae): How to circumscribe *Morinda* L. to be monophyletic?

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ABSTRACT

Most of the species of the family Rubiaceae with flowers arranged in head inflorescences are currently classified in three distantly related tribes, Naucleae (subfamily Cinchonoideae) and Morindeae and Schradereae (subfamily Rubioideae). Within Morindeae the type genus *Morinda* is traditionally and currently circumscribed based on its head inflorescences and syncarpous fruits (syncarps). These characters are also present in some members of its allied genera, raising doubts about the monophyly of *Morinda*. We perform Bayesian phylogenetic analyses using combined nrETS/nrITS/trnT-F data for 67 Morindeae taxa and five outgroups from the closely related tribes Mitchelleae and Gaertnereae to rigorously test the monophyly of *Morinda* as currently delimited and assess the phylogenetic value of head inflorescences and syncarps in *Morinda* and Morindeae and to evaluate generic relationships and limits in Morindeae. Our analyses demonstrate that head inflorescences and syncarps in *Morinda* and Morindeae are evolutionarily labile. *Morinda* is highly paraphyletic, unless the genera *Coelospermum*, *Gynochthodes*, *Pogonolobus*, and *Sarcopygme* are also included. Morindeae comprises four well-supported and morphologically distinct major lineages: *Appunia* clade, *Morinda* clade (including *Sarcopygme* and the lectotype *M. royoc*), *Coelospermum* clade (containing *Pogonolobus* and *Morinda reticulata*), and *Gynochthodes*–*Morinda* clade. Four possible alternatives for revising generic boundaries are presented to establish monophyletic units. We favor the recognition of the four major lineages of Morindeae as separate genera, because this classification reflects the occurrence of a considerable morphological diversity in the tribe and the phylogenetic and taxonomic distinctness of its newly delimited genera.

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1. Introduction

A recent molecular phylogenetic study of Razafimandimbison et al. (2008) based on five plastid gene and nrITS regions led to the establishment of new tribal limits for the species-rich Psychotriaceae alliance of the subfamily Rubioideae (Rubiaceae or coffee family). These authors circumscribed the tribe Morindeae in a narrow sense to include only six genera (*Appunia* Hook.f., *Coelospermum* Blume, *Gynochthodes* Blume, *Morinda* L., *Pogonolobus* F. Muell., and *Siphonandrium* K. Schum.). The members of Morindeae can be diagnosed by the following features: massive T-shaped placentae inserted in the middle of the septum with two anatropous ovules per carpel and pyrenes with a single lateral germination slit (Igersheim and Robbrecht, 1993). Some genera traditionally associated with Morindeae are currently classified in the following tribes: Colletocemateae Rydin & B. Bremer (*Colletocema* E.M.A

Petit), Lasiantheae B. Bremer & Manen (*Lasianthus* Jack), Mitchelleae Razafim. & B. Bremer (*Damnacanthus* C.F. Gaertn. and *Mitchella* L.), and Prismaticerideae Ruan (*Prismaticeris* Thw. and its allied genera). The Samoan genus *Sarcopygme* Setch. & Christoph., classified by Darwin (1979) in Morindeae, was excluded from Morindeae sensu Razafimandimbison et al. (2008) (hereafter called Morindeae) mainly because of its numerous (up to 100) and synchronous flowers with uniovulate locules.

Morindeae is a pantropical group of ca. 160 species assigned to six genera whose generic limits are controversial and remain unsettled. Of these genera, the most species-rich genus is *Morinda*, one of the 24 rubiaceae genera that Linnaeus described in his volume Species Plantarum (Linnaeus, 1753). Linnaeus (1753) included three species (*M. citrifolia* L., *M. royoc* L., and *M. umbellata* L.) in his genus *Morinda*, which can be characterized by a combination of its head inflorescences and syncarpous fruits (=syncarps or multiple fruits with ovaries fused). Head inflorescences (also known as capitula, Johansson, 1994) in *Morinda* sensu Linnaeus (1753) consist of two to many flowers clustered together on a common receptacle. These heads are either solitary (i.e., one cluster of

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flowers on a peduncle = single head) or umbel-like; the later are unbranched and comprised of two to many heads. Within each capitulum the number of flowers varies from 2 to 50 but is fairly constant within species. Steyermark (1972) merged the neotropical Morindeae genus *Appunia* bearing head inflorescences/inflorescences composed of free flowers/fruits in *Morinda*. This taxonomic decision broadened the morphological concept of *Morinda* and resulted in disagreements among Rubiaceae specialists over its circumscription. For example, Hayden and Dwyer (1969), Johansson (1987), Burger and Taylor (1993), and Lorence (1999) retained *Appunia* at generic level, while Andersson (1992), Boom and Delprete (2002), and Taylor and Steyermark (2004) included *Appunia* in *Morinda*. The occurrence of head inflorescences and syncarpous fruits in some members of its allied genera of *Morinda* (*Coelospermum* and *Gynochthodes*) raises doubts regarding the monophyly of the genus. *Morinda* currently comprises ca. 130 species of lianescent, arborescent, and suffrutescent plants whose phylogenetic affinities with the other Morindeae genera have never been assessed.

The main objective of this study is to reconstruct a robust phylogeny of the tribe Morindeae using combined plastid (*trnT-F*) and nuclear (nrETS and nrITS) DNA sequence data. The resulting phylogeny will be used to: (1) rigorously test the monophyly of *Morinda* as presently delimited; (2) evaluate the phylogenetic value of head inflorescences and syncarps traditionally and currently used for circumscribing genera in *Morinda* and Morindeae; (3) and assess the current generic relationships and limits in Morindeae.

2. Materials and methods

2.1. Taxon sampling

We investigated a total of 67 taxa of Morindeae, including *Morinda* (ca. 41 species), the neotropical genus *Appunia* (six species), the Australasian genera *Coelospermum* (six species) and *Gynochthodes* (four species) and the monotypic New Guinean and northern Australian genus *Pogonolobus* (one individual). We were unable to identify six New Caledonian *Morinda* specimens (*Morinda* sp. 3–7 and 9) using the last taxonomic treatment of *Morinda* for this region (Johansson, 1994); some of them may represent undescribed new *Morinda* species. Two Australian *Morinda* (*Morinda* sp. 1 and 2) and one Malagasy *Morinda* (*Morinda* sp. 8) specimens are undescribed new species. *Morinda citrifolia* was represented by one individual each of its three varieties, var. *bracteata*, var. *citrifolia*, and var. *potteri*. The Samoan genus *Sarcopygme*, represented by one individual of its type species, *S. pacifica* (Reinecke) Setch. & Christoph., was also included in the analyses because Darwin (1979) placed it in Morindeae. No sequenceable material of the New Guinean monotypic genus *Siphonandrium* was available. Five outgroup taxa were selected on the basis of the molecular phylogenetic study of Razafimandimbison et al. (2008). From the sister tribe Mitchelleae Razafim. & B. Bremer one species of *Damnacanthus* and one species of *Mitchella* were used and from the next closest tribe Gaetnereae Bremek. ex S.P. Darwin two species of the paleotropical genus *Gaertnera* Lam. and one species of the neotropical *Pagamea* Aubl. were utilized. We investigated a total of 72 taxa for this study (see Table 1).

2.2. DNA extraction, amplification, and sequencing

Sequence data from the (nuclear ribosomal) nrETS, nrITS, and (chloroplast) *trnT-F* regions, used alone or in combination with other chloroplast markers (e.g., Razafimandimbison et al., 2005, 2008), have recently been proven useful for inferring phylogenetic relationships within Rubiaceae. Total DNA was extracted from leaves dried in silica gel (Chase and Hills, 1991) and/or herbarium

material for all investigated taxa, except *Sarcopygme pacifica* and isolated following the mini-prep procedure of Saghai-Marooof et al. (1984), as modified by Doyle and Doyle (1987). For *S. pacifica* total DNA was extracted from a dry young inflorescence (Tronquet 749, P1). Isolated DNA was amplified and sequenced according to the protocols outlined in the following articles: Razafimandimbison et al. (2005) for nrETS, Razafimandimbison and Bremer (2002) for *trnT-F*, and Razafimandimbison and Bremer (2001) and Razafimandimbison et al. (2004) for nrITS. The primers from these previous studies were used for the nrETS, nrITS, and *trnT-F* regions. In all PCRs, one reaction was run using water instead of DNA as a negative control to check for contamination. All sequencing reactions were performed using the Big Dye[®] Terminator v3.1 Cycle Sequencing kit and Big Dye[®] Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) and sequences were analyzed with the 3100 Genetic Analyzer (Applied Biosystems).

2.3. Phylogenetic analyses

Sequence fragments were assembled using the Staden package (Staden, 1996). All new sequences have been submitted to GenBank (FJ906973–FJ907161, Table 1). For each DNA sequence region (or marker) all new sequences and published ones taken from GenBank were aligned together using the computer program CLUSTAL-X (Thompson et al., 1997) to produce an initial alignment and manually adjusted using software SeAl v.2.0 (Rambaut, 1996). Insertion/deletion events were inferred by eye and gaps were treated as missing data in the alignments. The aligned matrices were analyzed with Bayesian inference without coded indels.

Separate and combined Bayesian analyses of sequence data were performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For each of the three DNA sets, the best performing nucleotide substitution model was selected using the computer programs MrModeltest 2.0 (Nylander, 2001) and MrAIC ver. 1.4.3 (Nylander, 2004). The best performing evolutionary models were estimated under three different model selection criteria: Akaike information criterion (AIC) (Akaike, 1974), AICc (a second order AIC, necessary for small samples) and the Bayesian information criterion (BIC) (Schwartz, 1978). All combined Bayesian analyses were conducted with four independent Markov chain runs for 5×10^6 Metropolis-coupled MCMC generations, with tree sampling every 1×10^3 generations. Trees sampled from the first 2×10^6 generations were discarded as burn-in (as detected by plotting the log likelihood scores against generation number). We partitioned the combined data sets into two partitions: partition # 1 with GTR + G applied to the nrITS and *trnT-F* data; and partition # 2 with HKY + G applied to the ETS data. In all analyses, partitions were unlinked so that each partition was allowed to have its own sets of parameters. Flat prior probabilities were specified according to suggestions produced by the software MrAIC (Nylander, 2004). All separate and combined Bayesian analyses were repeated two times using different random starting trees to evaluate the convergence of the likelihood values and posterior probabilities. All saved trees (after excluding burn-ins) from the two independent runs were pooled for a consensus tree. Groups characterized by posterior probabilities over 95% were regarded as strongly supported.

3. Results

3.1. Phylogenetic analyses

A total of 202 sequences were used, of which 189 (ca. 94%) are published here. The combined nrETS/nrITS/*trnT-F* matrix contained 3654 base pairs (bp), of which 662 bp (ca. 18%) were parsimony

Table 1

List of taxa investigated in this study, voucher information, country origins, and accession numbers.

Taxa	Voucher information	Country origins	nrETS	nrITS	trnT-F
<i>Appunia brachycalyx</i> (Bremek.) Steyerem.	Granville 5443 (BR)	French Guyana (France)		FJ907038	FJ906974
<i>Appunia calycina</i> (Benth.) Sandwith	McDowell 2413 (US)	Guyana			FJ906975
<i>Appunia debilis</i> Sandwith	McDowell 5728 (ETSU)	Guyana	FJ907103	FJ907039	FJ906976
<i>Appunia guatemalensis</i> Donn.Sm.	Razafimandimbison et al. (2008)		FJ907104	AM945191 ^a	AM945332 ^a
<i>Appunia odontocalyx</i> Sandwith	Smith et al. 1350 (US)	Peru	FJ907105	FJ907040	FJ906977
<i>Appunia tenuiflora</i> (Benth.) B.D. Jacks	Hoffmann 966 (US)	Guyana	FJ907106		
<i>Coelospermum balansanum</i> Baill.	Mouly 318 (P)	New Caledonia (France)	FJ907107	FJ907041	FJ906978
<i>Coelospermum crassifolium</i> J.T. Johanss.	Johansson 85 (P)	New Caledonia (France)	FJ907108	FJ907042	FJ906979
<i>Coelospermum dasylobum</i> Halford & A.J. Ford	Q7385 (BRI)	Australia		FJ907043	FJ906980
<i>Coelospermum monticola</i> Baill. ex Guillaumin	Razafimandimbison et al. (2008)		FJ907110	AM945194 ^a	AM945334 ^a
<i>Coelospermum paniculatum</i> F. Muell. var. <i>syncarpum</i> J.T. Johanss.	Q8854 (BRI)	Australia	FJ907111	FJ907045	FJ906982
<i>Damnacanthus indicus</i> C.F. Gaertn.	Razafimandimbison et al. (2008)		FJ907101	AY514061 ^b	AM945335 ^a
<i>Gaertnera phyllosepala</i> Baker	Razafimandimbison et al. (2008)		FJ907100	AM945200 ^a	AM945340 ^a
<i>Gaertnera phyllostachya</i> Baker	Razafimandimbison et al. (2008)		FJ907099	AM945201 ^a	AM945341 ^a
<i>Gynochthodes coriacea</i> Blume	Alejandro et al. (2005)		FJ907112	AM945192 ^a	AJ847407 ^c
<i>Gynochthodes epiphytica</i> (Rech.) A.C. Sm. & S.P. Darwin	Smith 9377 (S)	Fiji	FJ907113	FJ907046	FJ906983
<i>Gynochthodes oresbia</i> Halford	RJ1411 (BRI)	Australia	FJ907114	FJ907047	FJ906984
<i>Gynochthodes sessilis</i> Halford	PIF28127 (BRI)	Australia		FJ907048	FJ906985
<i>Mitchella repens</i> L.	Ellison 781 (S)	USA	FJ907102	FJ907037	FJ906973
<i>Morinda ammitia</i> Halford & A.J. Ford	Bremer and Bremer 3909 (UPS)	Australia		FJ907051	FJ906988
<i>Morinda angustifolia</i> Roxb.	No voucher	Cult. Xishuangbann Trop. Bot. Gard. (China)	FJ907116	FJ907050	FJ906987
<i>Morinda bracteata</i> Kurz. var. <i>celebica</i> Miq.	AF4789 (BRI)	Australia	FJ907119	FJ907054	FJ906991
<i>Morinda buchii</i> Urb.	Ekman 2452 (S)	Haiti	FJ907120	FJ907055	FJ906992
<i>Morinda bucidifolia</i> A. Gray	Smith 4645 (S)	Fiji	FJ907121	FJ907056	FJ906993
<i>Morinda candollei</i> (Montrouz.) Beauvis. 1	McPherson and Munzinger 701 (UPS)	New Caledonia (France)	FJ907122	FJ907057	FJ906994
<i>Morinda candollei</i> (Montrouz.) Beauvis. 2	Johansson 15 (S)	New Caledonia (France)	FJ907123	FJ907058	FJ906995
<i>Morinda candollei</i> (Montrouz.) Beauvis. 3	Mouly 190 (P)	New Caledonia (France)	FJ907151	FJ907086	FJ907025
<i>Morinda candollei</i> (Montrouz.) Beauvis. 4	Mouly 137 (P)	New Caledonia (France)	FJ907148	FJ907083	FJ907022
<i>Morinda candollei</i> (Montrouz.) Beauvis. 5	Mouly 140 (P)	New Caledonia (France)	FJ907156	FJ907091	FJ907030
<i>Morinda canthoides</i> (F. Muell.) Halford & R.J.F. Hend.	Q8878 (BRI)	Australia	FJ907124	FJ907059	FJ906996
<i>Morinda citrifolia</i> L. var. <i>citrifolia</i> L. (LF)	McDowell 5742 (ETSU)	Guyana	FJ907125	FJ907060	FJ906997
<i>Morinda citrifolia</i> L. var. <i>citrifolia</i> L. (SF)	Lorence 9705 (PTBG)	Palau	FJ907126	FJ907061	FJ906998
<i>Morinda citrifolia</i> L. var. <i>potteri</i> O. Degen.	Lorence 9704 (PTBG)	Cult. at Natl. Trop. Bot. Gard. (Hawaii, USA)	FJ907127	FJ907062	FJ906999
<i>Morinda collina</i> Schltr.	Johansson 124 (S)	New Caledonia (France)	FJ907128	FJ907063	FJ907000
<i>Morinda coreia</i> Buch.-Ham.	Lorence 9460 (PTBG)	India	FJ907129	FJ907064	FJ907001
<i>Morinda deplanchei</i> (Hook. f.) Baill. ex K. Schum.	Johansson 57 (S)	New Caledonia (France)	FJ907130	FJ907065	FJ907002
<i>Morinda elliptica</i> (Hook.) Ridl.	Larsen et al. 41223 (AAU)	Thailand	FJ907131	FJ907066	FJ907003
<i>Morinda geminata</i> DC.	Gledhill 848 (P)	Nigeria		FJ907067	FJ907004
<i>Morinda glaucescens</i> Schltr.	Johansson 81 (S)	New Caledonia (France)	FJ907132	FJ907068	FJ907005
<i>Morinda grayi</i> Seem.	Smith 1521 (S)	Fiji	FJ907133	FJ907069	FJ907006
<i>Morinda jasminoides</i> A. Cunn.	Q8836 (BRI)	Australia	FJ907134	FJ907070	FJ907007
<i>Morinda latibracteata</i> Valetton	Lorence 8777 (PTBG)	Palau	FJ907135	FJ907071	FJ907008
<i>Morinda longiflora</i> 1 G. Don	Andru 5003 (P)	Ivory Coast	FJ907115	FJ907049	FJ906986
<i>Morinda longiflora</i> 2 G. Don	63PT00539 (P)	Ivory Coast	FJ907136	FJ907072	FJ907009
<i>Morinda lucida</i> A. Gray	BR-19733106	Cult. Belgium Botanical Garden	FJ907137	FJ907073	FJ907010
<i>Morinda moaensis</i> Alain	Rova et al. 2213 (GB)	Cuba	FJ907138		FJ907011
<i>Morinda mollis</i> A. Gray	Degener 15262 (S)	New Caledonia (France)	FJ907139	FJ907074	FJ907012
<i>Morinda morindoides</i> (Baker) Milne-Redh.	Leeuwenberg 2249 (P)	Ivory Coast	FJ907140	FJ907075	FJ907013
<i>Morinda myrtifolia</i> A. Gray	Johansson 98 (S)	New Caledonia (France)	FJ907141		FJ907014
<i>Morinda neocaledonica</i> (S. Moore) Guillaumin	Johansson 54 (S)	New Caledonia (France)	FJ907142	FJ907076	FJ907015
<i>Morinda pedunculata</i> Valetton	Lorence 9461 (PTBG)	Palau	FJ907143	FJ907077	FJ907016
<i>Morinda podistra</i> Halford & A.J. Ford	AF4753 (BRI)	Australia	FJ907144	FJ907078	FJ907017
<i>Morinda reticulata</i> Benth.	KRM4638 (BRI)	Australia	FJ907109	FJ907044	FJ906981
<i>Morinda retusa</i> Poir.	De Block et al. 636 (BR)	Madagascar		FJ907079	FJ907018
<i>Morinda royoc</i> L. 1	Leyman 126 (BR)	Unknown	FJ907145	FJ907080	FJ907019
<i>Morinda royoc</i> L. 2	Lorence 8419 (PTBG)	Florida (USA)	FJ907146		FJ907020
<i>Morinda</i> sp. 1	Q8853 (BRI)	Australia	FJ907118	FJ907053	FJ906989
<i>Morinda</i> sp. 2	AF3963 (BRI)	Australia	FJ907117	FJ907052	FJ906990
<i>Morinda</i> sp. 3	McPherson and Munzinger 18243 (UPS)	New Caledonia (France)	FJ907154	FJ907089	FJ907028
<i>Morinda</i> sp. 4	Mouly 16 (P)	New Caledonia (France)	FJ907152	FJ907087	FJ907026
<i>Morinda</i> sp. 5	Mouly 310 (P)	New Caledonia (France)	FJ907153	FJ907088	FJ907027
<i>Morinda</i> sp. 6	McPherson and Munzinger 18075 (P)	New Caledonia (France)	FJ907147	FJ907082	FJ907021
<i>Morinda</i> sp. 7	Mouly 399 (P)	New Caledonia (France)	FJ907150	FJ907085	FJ907024
<i>Morinda</i> sp. 8	Kårehed et al. 218 (UPS)	Madagascar	FJ907147	FJ907084	FJ907023
<i>Morinda</i> sp. 9	Mouly 302 (P)	New Caledonia (France)	FJ907155	FJ907090	FJ907029
<i>Morinda titanophylla</i> E.M.A. Petit	Troupin 10732 (BR)	R.D. of Congo	FJ907157	FJ907092	FJ907031
<i>Morinda umbellata</i> L. 1	Wambeck and Wanntorp 2622 (S)	Sri Lanka	FJ907158	FJ907093	FJ907032
<i>Morinda umbellata</i> L. 2	Q8839 (BRI)	Australia	FJ907160	FJ907094	FJ907034

(continued on next page)

Table 1 (continued)

Taxa	Voucher information	Country origins	nrETS	nrITS	trnT-F
<i>Morinda umbellata</i> L. 3	Takeuchi and Ama 15319 (BR)	New Guinea (France)	FJ907159	FJ907094	FJ907033
<i>Pogonolobus guianensis</i> Aubl.	Razafimandimbison et al. (2008)		FJ907098	AF333846 ^d	AM945342 ^a
<i>Pogonolobus reticulatus</i> F. Muell.	Q8840 (BRI)	Australia	FJ907161	FJ907096	FJ907035
<i>Sarcopygme pacifica</i> (Reinecke) Setch. & Christoph.	Tronchet et al. 222 (P)	Samoa (USA)		FJ907097	FJ907036

Andersson and Rova (1999).

^a Razafimandimbison et al. (2008).

^b Bremer and Manen (2000).

^c Alejandro et al. (2005).

^d Malcomber (2002).

informative characters (PIC). Of the 662 bp PIC 224 (ca. 34%) were from the nrETS data, 239 (ca. 36%) from the nrITS data, and 207 (ca. 30%) from the trnT-F data. The separate Bayesian analyses of the nrETS, nrITS, and trnT-F data produced Bayesian majority rule consensus trees with similar topologies (not shown). Visual inspection of the trees showed no well-supported conflict between them; accordingly, we merged the sequence data of the three markers for combined analyses.

The Bayesian majority rule consensus tree (from 6000 trees) from the combined nrETS/nrITS/trnT-F data was almost fully resolved (Fig. 1). The six sampled *Appunia* species [including the type species *A. tenuiflora* (Benth.) B.D. Jacks] formed a well-supported clade (A in Fig. 1; posterior probability or PP = 1.00), which was resolved with high support (PP = 1.00) as sister to a large clade containing the rest of the sampled Morindeae taxa. *Morinda* as presently delimited was resolved as paraphyletic, because *Sarcopygme*, *Coelospermum*, *Pogonolobus*, and *Gynochthodes* were all embedded within the large *Morinda* clade (Fig. 1). The next lineages to branch off after the *Appunia* clade (A in Fig. 1) were a largely arborescent *Morinda* clade (B in Fig. 1; including the only two African lianescent *Morinda* species, *Sarcopygme pacifica*, *M. citrifolia*, and the lectotype *M. royoc*; PP = 0.97) and the *Coelospermum* clade (C in Fig. 1; including the Australian and New Guinean *Pogonolobus reticulatus* F. Muell. and the Australian *Morinda reticulata* Benth.; PP = 1.00), respectively. This *Coelospermum* clade was in turn resolved as sister to a large lianescent *Gynochthodes*–*Morinda* clade (D in Fig. 1; PP = 1.00), within which all sampled species of *Gynochthodes* (including the type species *G. coriacea* Blume) formed a well-supported subclade (PP = 1.00) sister to a small *Morinda* subclade (PP = 1.00). This *Gynochthodes* subclade (including three *Morinda* species, PP = 1.00) was resolved as sister to a large lianescent *Morinda* subclade (PP = 1.00). Within the largely arborescent *Morinda* clade (B in Fig. 1; PP = 0.97) the type species of the Samoan *Sarcopygme*, *S. pacifica* and the African *M. titanophylla* formed a weakly supported group (PP = 0.81) and the two varieties of *M. citrifolia* (var. *citrifolia* L. and var. *potteri* O. Degen.) formed a well-supported group (PP = 1.00); *M. bracteata* Kurz. var. *celebica* Miq., now merged in *M. citrifolia* L. var. *bracteata* Kurz. (Merrill, 1923), and the Micronesian *M. latibracteata* Val. formed a well-supported group, which in turn was sister to the *M. citrifolia* var. *citrifolia*–var. *potteri* clade.

4. Discussion

4.1. Phylogenetic relationships in Morindeae

4.1.1. Monophyly of *Appunia* Hook.f.

Appunia, originally described by Hooker (1873), is a neotropical genus with ca. 16 species of shrubs or small trees (Steyermark, 1967; Govaerts et al., 2006). The generic status of *Appunia* has been controversial for the last 40 years (e.g., Steyermark, 1972; Johansson, 1987). Many authors (e.g., Steyermark, 1967; Johansson, 1987; Burger and Taylor, 1993; Lorence, 1999; Borhidi and Diego-Pérez,

2002) recognized *Appunia* as separate genus. In subsequent publications Steyermark (1972), Andersson (1992), Boom and Delprete (2002), Taylor and Steyermark (2004), and more recently Govaerts et al. (2006) merged the genus in *Morinda*. In Razafimandimbison et al. (2008), *Appunia*, represented only by *A. guatemalensis* Donn.Sm., was resolved as sister to a clade containing *Morinda citrifolia*, *Coelospermum monticola* Baill. ex Guillaumin, *Gynochthodes coriacea*, and *Gynochthodes* sp. This position is further corroborated by this study, which investigates six of 16 *Appunia* species (type species *A. tenuiflora* included), *Coelospermum*, *Gynochthodes*, and *Morinda*. These analyses support the monophyly of *Appunia*, which is distinct from the other Morindeae genera by having a combination of head inflorescences composed of free flowers, club-shaped stigmas, and simple, non-syncarpous fruits.

4.1.2. Paraphyly of *Morinda* L.

Morinda sensu Linnaeus (1753) is non-monophyletic because all three accessions of *M. umbellata* are more closely related to *Coelospermum* sensu Johansson (1988), *Gynochthodes* and *Pogonolobus* than to either *M. citrifolia* or *M. royoc* (Fig. 1). *Morinda* as presently delimited by a combination of head inflorescences, bifid stigmas, and syncarpis is highly paraphyletic, unless *Coelospermum*, *Gynochthodes*, *Pogonolobus*, and *Sarcopygme* are also included (Fig. 1). This is the first study to demonstrate the paraphyly of the presently circumscribed *Morinda* and lability of head inflorescences and syncarpous fruits in *Morinda* and Morindeae (Fig. 1).

4.1.3. *Sarcopygme* Setch. & Christoph.

The Samoan genus *Sarcopygme*, consisting of five species of small trees, was originally established by Setchell and Christophersen (1935) based on *Sarcocephalus pacificus* Reinecke (Reinecke, 1898), which belonged to the tribe Naucleaeae (Cinchonoideae). The authors (Setchell and Christophersen, 1935) argued that their new genus showed “a superficial resemblance to *Sarcocephalus* in the fruiting heads but is different from the latter in its single ovules in each locule of the ovary rather than numerous ovules per cell in *Sarcocephalus*”. Setchell and Christophersen (1935) postulated that *Sarcopygme* is most closely related to *Morinda* but is distinct from the latter by its caducous stipules, large involucre bracts, simultaneous opening of all flowers in the head (i.e., synchronous flowering heads), distinct calyces and club-shaped stigmas. *Sarcopygme* is additionally distinct in its monocaul trunks and relatively large leaves that are clustered at the apex. Darwin (1979) classified *Sarcopygme* in Morindeae because of its solitary and erect ovules, raphide crystals, valvate aestivation of corolla lobes, and multiple fruits. On the other hand, Johansson (1987) qualified it as a genus of uncertain taxonomic position. A narrow circumscription of Morindeae proposed by Igersheim and Robbrecht (1993), also endorsed by Razafimandimbison et al. (2008), excluded *Sarcopygme* from Morindeae because of its uniovulate locules and unbranched stigmas. The present analyses, however, show that *Sarcopygme*, represented here by the type species *S. pacifica*, belongs to Morindeae,

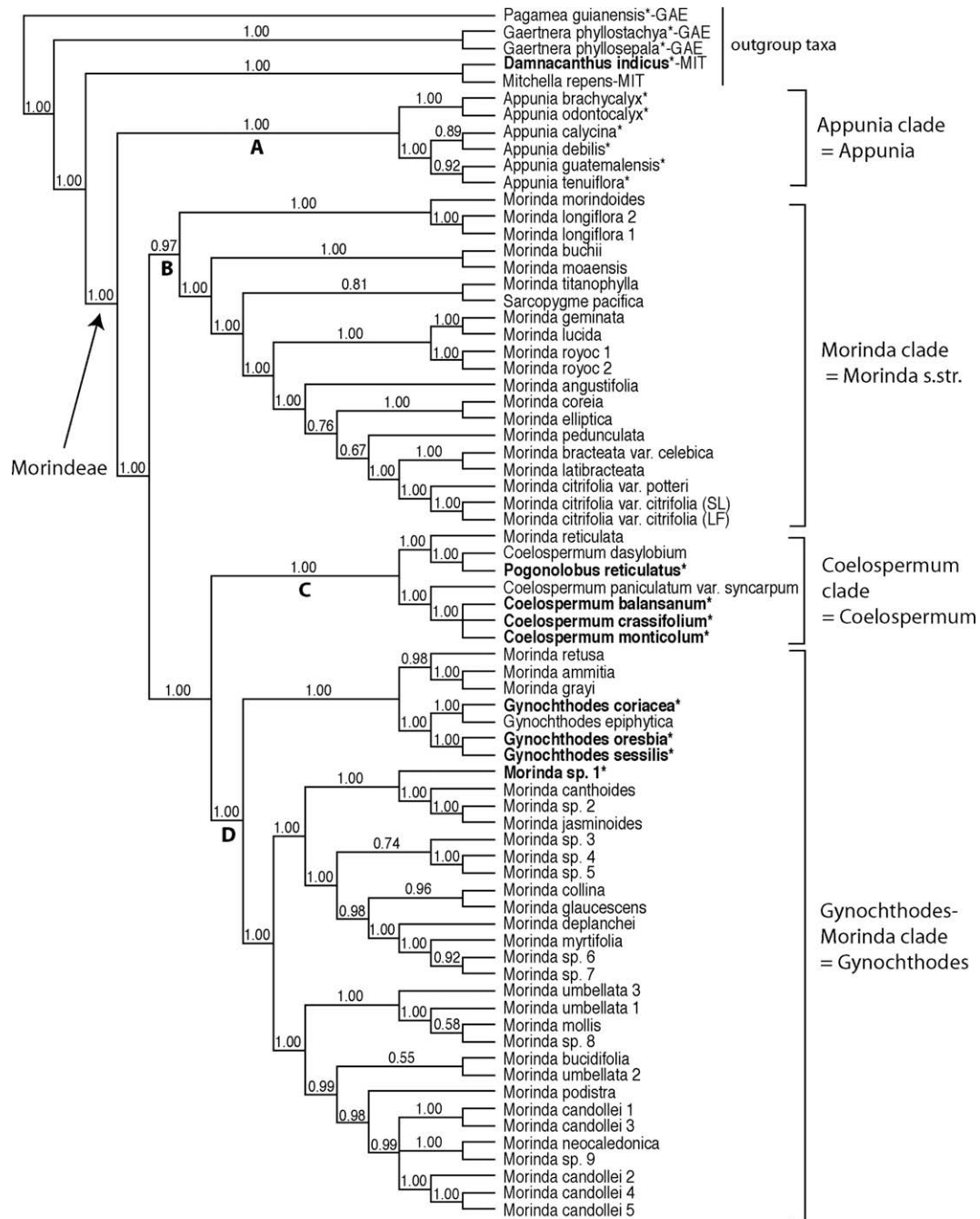


Fig. 1. Bayesian majority rule consensus tree from the combined nrETS/nrITS/trnT-F data of 67 Morindeae taxa and five outgroup taxa from the tribes Gaertnereae and Mitchelleae. Values above nodes are Bayesian posterior probabilities. The vertical bar delimits the outgroup taxa; GAE = Gaertnereae and MIT = Mitchelleae; LF and SF stand for the large- and small-fruited *Morinda citrifolia* var. *citrifolia*, respectively; brackets delimit the major lineages of Morindeae corresponding to our newly defined genera. Taxa in boldface are with non-headed inflorescences and taxa with asterisks (*) are without syncarpous fruits (syncarps).

consistent with Setchell and Christophersen's (1935) decision; the tree-like *S. pacifica* is nested in the largely arborescent *Morinda* clade (B in Fig. 1; PP = 1.00).

4.1.4. *Pogonolobus* F. Muell. and paraphyly of *Coelospermum* Blume

The Australian and New Guinean *Pogonolobus* was originally described by Mueller (1858) as a monotypic genus based on its arborescent habit and flowers with pubescent corolla lobes, and exerted anthers. The genus was later merged by Benth (1867) in *Coelospermum* [*C. reticulatum* (F. Muell.) Benth.] based on its exerted anthers, but Johansson (1987) re-established *Pogonolobus* based on palynological characters. *Coelospermum* is a small genus of seven species of mainly lianescent plants, which are characterized by the

following combination of characters according to Johansson (1988): terete branches, sheathing stipules, paniculate or corymbiform and puberulous inflorescences, white corollas with both long and short hairs inside the tube, mostly simple fruits with ovules inserted at the middle of the septum, seeds shortly winged at one end and pollen grains with large lumina. Like *Morinda*, the circumscription of *Coelospermum* is highly controversial. The Australian *Morinda reticulatus* was transferred by Baillon (1879) to *Coelospermum* as *Coelospermum decipiens* Baill.; however, this decision was not followed by many Rubiaceae specialists (e.g., Johansson, 1987). The present analyses demonstrate that *Coelospermum* sensu Johansson (1988) is paraphyletic, unless the Australian *M. reticulata* and *P. reticulatus* are also included (C in Fig. 1). This finding is consistent

with the decisions of *Bentham* (1867) and *Baillon* (1879) to merge these two species into *Coelospermum* but inconsistent with *Johansson* (1987). The broadly delimited *Coelospermum* (including *M. reticulatus* and *Pogonolobus*) can be characterized by its mainly lianescent habit and terminal inflorescences composed flowers with anthers well-exserted beyond the corolla lobes. The *Coelospermum* clade is deeply nested between the largely arborescent *Morinda* (B in Fig. 1) and the *Gynochthodes*–*Morinda* (=D in Fig. 1) clades, and these together are sister to the *Appunia* clade (A in Fig. 1). Within the *Coelospermum* clade the *M. reticulata*–*Pogonolobus*–*C. dasylobum* subclade is defined by interpetiolar stipules and hairy corolla lobes.

4.1.5. Monophyly of *Gynochthodes* Blume

Gynochthodes is a genus of ca. 18 lianescent species with wind-ing stems, which are distributed from continental southeast Asia south- and eastwards through Malesia to Micronesia, Fiji, and northern Australia. According to *Johansson* (1987), the genus can be distinguished from *Morinda* and *Coelospermum* by its stipules and bracts, which have marginal hairs, its axillary, racemose or cymose inflorescences with white and shortly pedunculate flowers in whorls, and flowers with recurved calyx tubes, corollas with long hairs within the tubes and on the adaxial side of the lobes. Our analyses strongly support the monophyly of *Gynochthodes* (type species *G. coriacea* included), which is resolved as sister to a small *Morinda* subclade of three *Morinda* species (*M. retusa* Poir., *M. ammitia* Halford & A.J. Ford, and *M. grayi* Seem.). This *Gynochthodes*–*Morinda* subclade is also deeply nested among the largely *Morinda* clade sister to the *Appunia* clade (Fig. 1).

4.1.6. *Siphonandrium* K. Schum.

Siphonandrium is a dioecious monotypic genus from New Guinea with scandent habit, umbel- to head-like inflorescences, and flowers with anthers fused into a tube (*Igersheim* and *Robbrecht*, 1993). This latter character is unique within Morindeae and is very rare in Rubiaceae [but present in e.g., the genera *Argostemma* Wall. (*Argostemmataeae* Bremek. ex Verdc.) and *Strumpfia* Jacq. (*Urophylleae* Bremek. ex Verdc.)]. No sequenceable material of *S. intricatum* K. Schum. is available for this study and therefore its position within the tribe has yet to be investigated. Based on its scandent habit and dioecious flowers the genus is possibly closely related to the *Gynochthodes*–*Morinda* clade (=D in Fig. 1).

4.2. Generic circumscriptions in Morindeae

The present study clearly indicates that *Morinda* as presently delimited is highly paraphyletic, unless *Coelospermum*, *Gynochthodes*, *Pogonolobus*, and *Sarcopygme* are also included. In other words, head inflorescences and syncarpous fruits are evolutionarily labile in *Morinda* and Morindeae. Therefore, new generic limits of Morindeae are needed. Below we present four possible alternatives for revising generic boundaries to establish monophyletic groups.

4.2.1. Alternative # 1

One is to recognize a broad circumscription of *Morinda* (*Appunia*, *Coelospermum*, *Gynochthodes*, *Pogonolobus*, *Sarcopygme*, and *Siphonandrium* included) without infrageneric subdivision. *Morinda* sensu lato can thus be diagnosed by its massive and T-shaped placenta inserted in the middle of the septum and two anatropous ovules per carpel (excepting *Sarcopygme*), although these are not obvious characters; the genus is additionally characterized by the frequent occurrence of head inflorescences/infructescences.

4.2.2. Alternative # 2

A second scenario is to adopt a broad circumscription of *Morinda* and recognize the four well-supported major lineages

(see A–D in Fig. 1) at subgeneric level. Either of these two alternatives would render Morindeae monogeneric and require a total of 29 new combinations and two new names in *Gynochthodes* and *Pogonolobus*. All described species of *Appunia* have already been transferred by *Steyermark* (1972) to *Morinda*. The second alternative would require new descriptions of four new subgenera.

4.2.3. Alternative # 3

The third alternative is to maintain *Appunia* (A in Fig. 1) as a distinct genus but merge *Coelospermum*, *Gynochthodes*, *Pogonolobus*, *Sarcopygme*, and *Siphonandrium* in *Morinda*. According to *Steyermark* (1967), *Appunia* is mainly distinct from *Morinda* (*Coelospermum*, *Gynochthodes*, *Pogonolobus*, and *Sarcopygme* not included) by its head inflorescences composed of free flowers/fruits and club-shaped stigmas, rather than head inflorescences formed by flowers with ovaries fully connate and two-branched styles in *Morinda*. Following this circumscription, however, *Morinda* is shown to be paraphyletic in this study. Plus, recognizing *Appunia* at generic level would render *Morinda* a morphologically heterogeneous genus with no obvious morphological synapomorphy. In other words, it would make *Morinda* s.l. (*Coelospermum*, *Gynochthodes*, *Pogonolobus* and *Sarcopygme* included) rather difficult to delimit, as headed and non-headed inflorescences (*Gynochthodes* and some *Coelospermum* species) and club-shaped (*Sarcopygme*) and two-branched stigmatic lobes [all *Morinda* sensu *Linnaeus* (1753)] are all present. The two stigmatic lobes are further subdivided into three lobes in some lianescent *Morinda* species (e.g., *M. collina* Schltr., *Johansson*, 1994: 32).

4.2.4. Alternative # 4

The fourth alternative is to recognize the four major lineages (A–D in Fig. 1) as separate genera: *Appunia*; *Morinda* s.str. (including *Sarcopygme*, the only two lianescent African species, *M. longiflora* and *M. morindoides*, all neotropical tree-like or suffrutescent *Morinda* species, and all arborescent and suffrutescent Asian and African *Morinda* species, all with large flowers); *Coelospermum* (including *M. reticulata* and *Pogonolobus*); and *Gynochthodes* (including all lianescent *Morinda* species from Australia, the Pacific, tropical and subtropical Asia, and Madagascar). For the *Gynochthodes*–*Morinda* clade (=D in Fig. 1) four validly published generic names, *Gynochthodes*, *Sphaerophora* Blume (*Blume*, 1850), *Pogonanthus* Montrouz. (*Montrouzier*, 1860), and *Imantina* Hook.f. (*Hooker*, 1873), are available, with the former genus having priority over the latter two names. This group can be defined by its lianescent habit, small flowers with partly exerted anthers and this scenario would require up to 80 new combinations. Within the *Gynochthodes*–*Morinda* clade (D in Fig. 1) recognizing the sister groups, subclade *Gynochthodes* (including *M. ammitia*, *M. grayi*, and *M. retusa*) and subclade *Morinda*, as separate genera does not seem an attractive solution, because there is no obvious character for distinguishing them.

We favor the fourth of these four alternative realignments, the recognition of the four major lineages (A–D in Fig. 1) as separate genera, because this classification reflects the occurrence of a considerable morphological diversity in Morindeae and the phylogenetic and taxonomic distinctness of its newly delimited genera. For now we maintain the New Guinean genus *Siphonandrium* as a distinct genus. Table 2 summarizes all five accepted genera of Morindeae and finally, all necessary new combinations will be published elsewhere.

4.3. Keys to the accepted genera of Morindeae

Below we present keys that can be used to identify all accepted genera of Morindeae on the basis of morphological traits.

Table 2

List of genera accepted here and their synonyms, geographic distributions, and number of species.

Genera accepted in Morindeae	Synonyms	Geographic distributions	Number of species
<i>Appunia</i> Hook.f.	Bellynkxia Müll. Arg.	Neotropics	ca. 16
<i>Coelospermum</i> Blume	Holostyla Endl.; Merismostigma S. Moore; Olostyla DC.; <i>Pogonolobus</i> F. Muell.	Australasia	ca. 9
<i>Gynochthodes</i> Blume	Imantina Hook.f.; Pogonanthus Montrouz.; Sphaerophora Blume; Tetralopha Hook.f.	Australasia and Madagascar	80–100
<i>Morinda</i> L.	Appunetia Good; <i>Sarcopygme</i> Setch. & Christoph	Pantropical	30–35
<i>Siphonandrium</i> K. Schum. ^a		New Guinea	1

^a Not included in this study.

- 1a. Arborescent, rarely suffrutescent (the African *M. angolensis* Good and the Haitian *M. buchii* Urb.) and lianescent [the African *M. longiflora* G. Don and *M. morindoides* (Baker) Milne-Redh.], large (corolla tubes/corolla lobes > 1) and hermaphroditic flowers.....2.
- 2a. Flowers free, congested, stigmatic lobes club-shaped, fruits simple, free*Appunia*.
- 2b. Flowers fused basally or partly or completely, stigmatic lobes bifid, fruits syncarpous (syncarps).....*Morinda* s.str.
- 1b. Lianescent, rarely arborescent (the New Guinean and Australian *Pogonolobus reticulatus* and Hawaiian *Morinda trimera* Hillebr.), small (corolla tubes/corolla lobes < 1, except *Gynochthodes sublancoolata* Miq. and *M. trimera*) and polygamous or dioecious flowers.....3.
- 3a. Anthers fused into a tube.....*Siphonandrium*.
- 3b. Anthers not fused into a tube.....4.
- 4a. Inflorescences mostly paniculate, sometimes corymbs, anthers well exerted beyond the corolla tubes.....*Coelospermum*.
- 4b. Inflorescences never paniculate, anthers partly exerted.....*Gynochthodes*.

It is important to note that seven of the eight described African species of *Morinda* s.str. have much larger flowers [corolla tubes ranging from 12 to 40 mm (rarely 80 mm) long and corolla lobes varying between 3 and 14 mm (rarely 22 mm) long] than the species of *Appunia* and the remaining species of *Morinda* s.str. The flowers of *Coelospermum* as defined here are much larger [corolla tubes ranging from 3 to 7 mm (rarely 11 mm) long and corolla lobes varying from 4.5 to 16 mm long] than that of the newly delimited *Gynochthodes* [corolla tubes ranging from 0.7 to 5.5 mm long and corolla lobes varying from 1.5 to 11 mm long].

5. Conclusions

The present study demonstrates for the first time that *Morinda* as presently delimited is highly paraphyletic, unless *Coelospermum*, *Gynochthodes*, and *Sarcopygme* are also included. Both head inflorescences and multiple fruits are evolutionarily labile in *Morinda* and Morindeae. The tribe Morindeae can be subdivided into four well-supported major lineages, which can be characterized by a combination of growth habit, inflorescence type and position, infructescence type, flower size, and breeding systems: *Appunia* clade, *Morinda* clade (including *M. royoc*, the type species of the genus, and *Sarcopygme*), *Coelospermum* clade (including *Pogonolobus* and *Morinda reticulata*), and *Gynochthodes*–*Morinda* clade. Four possible alternatives for revising generic boundaries are presented to establish monophyletic units. We favor the recognition of the four well-supported and morphologically distinct major lineages of Morindeae (A–D in Fig. 1) as separate genera, because this classification reflects the occurrence of a considerable morphological diversity in the tribe and the phylogenetic and taxonomic distinctness of its newly delimited genera.

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