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Origin of the pantropical and nutriceutical *Morinda citrifolia* L. (Rubiaceae): comments on its distribution range and circumscription

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ABSTRACT

Aim *Morinda citrifolia* L., commercially known as noni or the Indian mulberry plant, is morphologically variable and the only widely distributed member of the pantropical genus *Morinda sensu stricto* (Rubiaceae). This large distribution has been attributed partly to the ability of the seeds of the large-fruited *M. citrifolia* L. var. *citrifolia* L. to be transported by oceanic drifting. This form of *M. citrifolia* var. *citrifolia* has been predicted to be the progenitor colonizer of the island endemic *Morinda* species. Using a phylogenetic approach and large sampling of the widespread, large-fruited *M. citrifolia* var. *citrifolia*, we assessed the potential area of origin of *M. citrifolia* and tested the hypothesis that the large-fruited *M. citrifolia* var. *citrifolia* is an ancestral colonizer.

Location Tropics.

Methods We performed Bayesian analyses of 22 species of the tribe Morindeae (including 11 individuals of the three currently recognized varieties of *M. citrifolia*) based on combined nrETS, nrITS, *rps16* and *trnT-F* sequence data. Geographic origins of the studied taxa were mapped onto the Bayesian majority rule consensus tree.

Results Nine sequenced individuals of *M. citrifolia* from diverse geographic locations formed a highly supported clade, which was sister to the Australo-Micronesian clade that included *M. bracteata* var. *celebica* and *M. latibracteata*. These sister clades are part of the broader Asian, arborescent *Morinda* clade. We found no support for the current varietal classification of *M. citrifolia*.

Main conclusions Our analyses suggest a Micronesian origin of *M. citrifolia*. This implies that the large-fruited *M. citrifolia* var. *citrifolia* might well have been present in the Pacific before the arrival of the Micronesian and Polynesian ancestors from Southeast Asia. The wide distribution of this form of *M. citrifolia* var. *citrifolia* is attributed partly to the trans-oceanic dispersal of its buoyant seeds, self-pollination and its ability to produce flowers and fruits year-round. The hypothesis that the widespread, large-fruited *M. citrifolia* var. *citrifolia* is the progenitor colonizer of the island endemic *Morinda* species is inconsistent with its derived position within the Asian, arborescent *Morinda* clade and with the fact that the nine sampled individuals of *M. citrifolia* form a clade.

Keywords

Biogeography, DNA sequence data, Indian mulberry, *Morinda citrifolia*, *Morinda* s.s., Morindeae, noni, phylogeography, Rubiaceae, taxonomy.

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INTRODUCTION

Molecular markers have been used to assess phylogenetic affinities between cultivated crops and their wild relatives (e.g. *Gossypium* species, Wendel & Albert, 1992; *Solanum* species, Hosaka, 1995; *Triticum* species, Kellogg *et al.*, 1997; *Malus* species, Robinson *et al.*, 2001; *Artocarpus* species, Zerega *et al.*, 2004). In a recent molecular phylogenetic study by Razafimandimbison *et al.* (2009, Clade B in figure 2) *Morinda citrifolia* L., a major crop in many parts of the Pacific and tropical Asia but also growing naturally across much of the tropics, was nested in *Morinda* s.s. The three sampled individuals of *M. citrifolia* (one individual each of the large- and small-fruited forms of *M. citrifolia* L. var. *citrifolia* L. and *M. citrifolia* L. var. *potteri* O. Degen.) formed a well-supported clade in that analysis. The Australian *M. bracteata* var. *celebica*, currently merged in *M. citrifolia* var. *bracteata* (Roxb.) Kurz (Merrill, 1923), and the Micronesian *M. latibracteata* were resolved as sisters, making *M. citrifolia* paraphyletic.

Morinda citrifolia is one of the most well-known and studied species of Rubiaceae (coffee family). The large-fruited *M. citrifolia* var. *citrifolia* is of key interest to *Drosophila* researchers investigating the genetic resistance of one fruit fly species (*Drosophila sechellia*) capable of eating the ripe fruits, which are toxic to most insects (e.g. Jones, 2005; Kopp *et al.*, 2008). *Morinda citrifolia*, also an economically important species, commercially known as noni or Indian mulberry, is used in traditional medicine across much of the tropics and is marketed globally. Almost all noni fruits currently used for commercial products are from the large-fruited *M. citrifolia* var. *citrifolia* (Nelson & Elevitch, 2006; Scot C. Nelson, pers. comm.). The juice of noni, pressed from the fleshy multiple fruits, has become a popular nutraceutical in the past two decades, with claims made that the drink can treat, cure or prevent a range of diseases (Wang *et al.*, 2002, 2008; Chan-Blanco *et al.*, 2006; Deng *et al.*, 2007); however, clinical evidence supporting the medicinal efficacy of noni in humans

is currently lacking. In spite of its popularity, the origin and circumscription of *M. citrifolia* have been controversial and remain equivocal.

Morinda citrifolia as presently delimited has a pantropical distribution (Fig. 1), although its occurrence in continental Africa has been controversial, mainly because of Hiern's (1877, 1898) broad concept of *M. citrifolia*, which included the African *Morinda lucida*. The *Flora of Tropical East Africa* (Verdcourt, 1976) and the *Flora Zambesiaca* (Verdcourt, 1989) treated *M. citrifolia sensu* Hiern (1877) as *M. lucida*; however, this does not rule out the occurrence of the large-fruited *M. citrifolia* var. *citrifolia* in continental Africa. In fact, Diane Bridson (Royal Botanic Gardens, Kew) identified one specimen of *Morinda* (Kuchar 10031, KREMU) collected in 1978 from the upper edge of the sandy beach of Watamu peninsula (Kenya) as *M. citrifolia*. In addition, Klopper *et al.*'s (2006) checklist of the flowering plants of sub-Saharan Africa included *M. citrifolia*. Glen (2002) listed *M. citrifolia* among the cultivated plants in southern Africa. Based on the evidence presented above we consider the large-fruited *M. citrifolia* var. *citrifolia* to be present in continental Africa. This form of *M. citrifolia* var. *citrifolia* is pantropical and commonly grows along seashores (Fig. 1); however, it can also thrive on a wide range of habitats: low-elevation lava flows, rocky coasts, salty tide pools, open grasslands and lowlands, gulches and cliffs (e.g. Nelson & Elevitch, 2006). This wide distribution has been attributed to the efficient dispersal of its seeds, which can be transported by oceanic drifting. The seeds remain viable after floating in seawater for several months (Guppy, 1917). The pyrene (seed and the associated endocarp) of this form of *M. citrifolia* var. *citrifolia* is fairly large (c. 6 mm long, Nelson & Elevitch, 2006) and has a large air-filled cavity on its ventral surface, with the seed enclosed in its dorsal cavity. The seeds of the large-fruited *M. citrifolia* var. *citrifolia* are dispersed inland by fruit bats (e.g. Whittaker & Jones, 1994; Shilton, 1997). It is worth noting that this form of *M. citrifolia* var. *citrifolia* has also been introduced in historical times and is now reproduc-

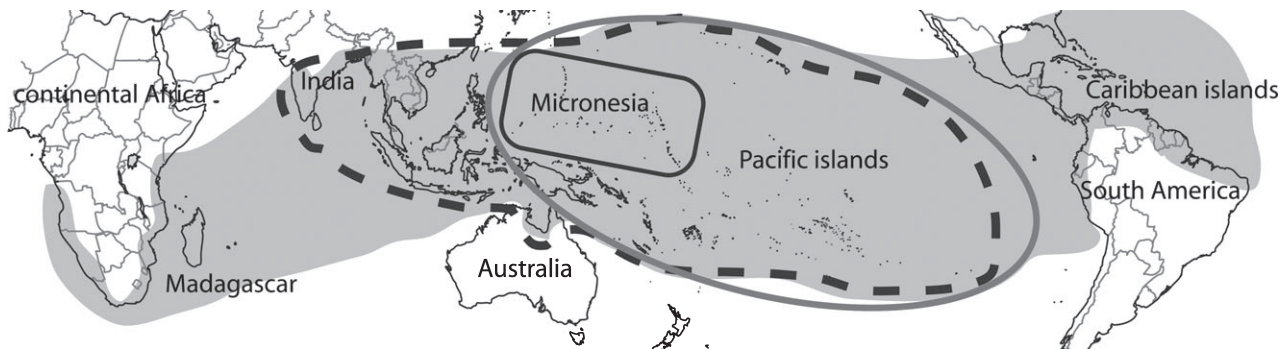


Figure 1 Geographical distribution of *Morinda citrifolia* L. The grey area denotes the current geographical distribution of *M. citrifolia* as presently delimited and the large-fruited *M. citrifolia* var. *citrifolia*; the black dashed line delimits that of *M. citrifolia* var. *bracteata* (including *M. bracteata* var. *celebica*); the grey line delimits that of *M. citrifolia* var. *potteri*; and the black line delimits that of the Micronesian small-fruited *M. citrifolia* var. *citrifolia*.

ing on its own in the wild (e.g. McClatchey, 2003; Nelson & Elevitch, 2006). The small-fruited *M. citrifolia* var. *citrifolia* (known as Micronesian lada) seems confined to Micronesia. Other varieties of *M. citrifolia* have more restricted distributions, with *M. citrifolia* var. *potteri* in the Pacific and *M. citrifolia* var. *bracteata* Indo-Australian (Fig. 1). There is no available information regarding the natural habitats and nature of dispersals of the small-fruited *M. citrifolia* var. *citrifolia*, *M. citrifolia* var. *bracteata* or *M. citrifolia* var. *potteri*.

There have been conflicting opinions regarding the area of origin of the large-fruited *M. citrifolia* var. *citrifolia* (e.g. Morton, 1992; McClatchey, 2003; Nelson & Elevitch, 2006). The prevailing view maintains that this plant originated in Southeast Asia and was dispersed by humans and/or sea currents to the Pacific islands and the rest of the tropics (e.g. Morton, 1992). The Southeast Asian ancestors of the Micronesian and Polynesian people are historically known to have migrated from Southeast Asia to the Pacific islands and brought with them fruits of the large-fruited *M. citrifolia* var. *citrifolia* among other essential items (e.g. Whistler, 1985, 1991; Abbott, 1992; Morton, 1992). It is, however, unknown whether or not this form of *M. citrifolia* var. *citrifolia* was already present in the Pacific before the arrival of the Micronesian and Polynesian ancestors, a question on which the historical record is silent. On the basis of the morphological-based phylogeny of the New Caledonian *Morinda* by Johansson (1994), McClatchey (2002, 2003), subsequently endorsed by Nelson & Elevitch (2006, p. 1), postulated that the large-fruited *M. citrifolia* var. *citrifolia* 'evolved in Australia, Borneo or intermediate Indonesia and New Guinea and was subsequently distributed by ocean current or birds, bats, or people to other parts of the Indian, and Pacific Oceans'. Furthermore, McClatchey (2003) predicted that the large-fruited *M. citrifolia* var. *citrifolia* is the progenitor colonizer that gave rise to the island endemic *Morinda* species, given its efficient seed dispersal mechanism. If this is correct, we expect the island endemic species of *Morinda* to be closely related to populations of the large-fruited *M. citrifolia* var. *citrifolia* occurring on their island(s).

Taxonomy of *Morinda citrifolia* varieties

Morinda citrifolia has long been regarded as morphologically heterogeneous (Hooker, 1880; Valetton, 1908; McClatchey, 2003; Nelson & Elevitch, 2006; Waki *et al.*, 2008); however, there have been no attempts to test its monophyly with a molecular-based phylogeny. Kurz (1877) was the first to establish infraspecific subdivisions for *M. citrifolia*. He included the continental Asian species *Morinda bracteata* in synonymy of *M. citrifolia* and recognized two varieties: *M. citrifolia* var. *citrifolia* and *M. citrifolia* var. *bracteata*. Hooker (1880) described *M. citrifolia* var. *elliptica* Hook. f., which was later recognized by Ridley (1918) as a separate species [*Morinda elliptica* (Hook. f.) Ridley]; this species has since been widely accepted by the Rubiaceae systematists (e.g. Puff *et al.*, 2007), consistent with Razafimandimbison *et al.*

(2009). The Asian species *Morinda tinctoria* Roxb. (= *M. coreia* Buch.-Ham.) was considered by many Indian botanists to be the wild progenitor of *M. citrifolia* (Hooker, 1880) and was formally placed by Kuntze (1891) in *M. citrifolia* as a separate variety, *M. citrifolia* var. *tinctoria* (Roxb.) O. Kuntze. This taxonomic decision was refuted by Razafimandimbison *et al.* (2009). Hiern (1898) merged the African *M. lucida* in *M. citrifolia* and recognized it at varietal level, *M. citrifolia* var. *lucida* (Benth.) Hiern, also refuted by Razafimandimbison *et al.* (2009). Furthermore, Domin (1929) described *M. citrifolia* var. *typica* Domin, a name currently considered by the International Plant Names Index (IPNI) as invalid because it was 'presumably meant to be the type variety' of *M. citrifolia*. *Morinda citrifolia* var. *potteri* was described by Degener (1949) based on a cultivated specimen originally from Fiji.

Today there are three varieties widely recognized in *M. citrifolia*: var. *bracteata*, var. *citrifolia* and var. *potteri* [= *M. citrifolia* L. f. *potteri* (O. Degen.) H. St. John; John, 1984]. *Morinda citrifolia* var. *bracteata* is easily distinguished from the other varieties by its conspicuous well-developed bracts subtending the inflorescence and its small fruits. Miquel (1857) described the distinct variety *M. bracteata* var. *celebica* Miq. based on a Sulawesi specimen, which was later synonymized by Merrill (1923) in *M. citrifolia* var. *bracteata*. Valetton (1908) considered *M. bracteata* var. *celebica* very distinct from *M. citrifolia* because of its enlarged calyx lobes but never recognized this entity at species level. *Morinda citrifolia* var. *potteri* differs from the other varieties of *M. citrifolia* in having variegated leaves and small fruits, foetid when ripe. It is worth noting, however, that a variegated form of the large-fruited *M. citrifolia* var. *citrifolia* is also found in many botanical gardens. The large-fruited *M. citrifolia* var. *citrifolia* appears to have evolved self-pollinating flowers from the ancestral condition of (hermaphroditic) outcrossing flowers (but see also Waki *et al.*, 2008); it flowers and produces fruits year-round. We have no information regarding the breeding systems of the small-fruited *M. citrifolia* var. *citrifolia*, var. *bracteata* or var. *potteri*. Fruits of the three recognized varieties of *M. citrifolia* are foetid-smelling when mature, although those of the large-fruited *M. citrifolia* var. *citrifolia* smell worse than others. The fruits of *M. citrifolia* are fleshy and generally much larger than those of the remaining species of *Morinda* s.s. (Razafimandimbison *et al.*, 2009); we consider these large and fleshy compound fruits (syncarps) the potential morphological synapomorphies of *M. citrifolia*. With respect to the delimitation of *M. citrifolia*, McClatchey (2003) presented the following three hypotheses. H1: the 'species comprises more than one variety'; H2: *M. citrifolia* includes 'more than one species'; and H3: *M. citrifolia* represents 'a single species with limited variation and cannot accurately be divided into separate varieties'.

The main objective of this study is to perform a phylogenetic analysis of *Morinda* s.s. based on combined nrETS, nrITS, *rps16* and *trnT-F* sequence data using a much larger sampling of the large-fruited *M. citrifolia* var. *citrifolia* to: (1) assess the potential area of origin of *M. citrifolia*; and (2) test the hypothesis that the large-fruited *M. citrifolia* var. *citrifolia* is

the progenitor colonizer of the island endemic *Morinda* species.

MATERIALS AND METHODS

Taxon sampling

Based on the results of Razafimandimbison *et al.* (2009) we restricted our analyses mainly to the members of *Morinda* s.s. *Morinda citrifolia* var. *bracteata* was represented by three wild specimens: one with conspicuous bracts subtending the inflorescences (= the type species of *M. bracteata*) from Thailand, and two with conspicuous enlarged calyx lobes (described as *M. bracteata* var. *celebica*) from Australia. *Morinda citrifolia* var. *potteri* was represented by one cultivated individual, and *M. citrifolia* var. *citrifolia* was represented by five wild specimens of the large-fruited form from Guyana (South America), Hawaii, New Caledonia, New Guinea and Puerto Rico (Caribbean region) and by two specimens of the small-fruited form [one grown from seeds collected in Palau (Micronesia) and currently cultivated at the National Tropical Botanical Garden, Hawaii (Pacific) and the other from Kosrae of the Federated State of Micronesia (Micronesia)]. Two species each of the allied genera *Appunia* Hook. f., *Coelospermum* Blume and *Gynochthodes* Blume were used as outgroup taxa to root the trees. We analysed a total of 32 accessions of Morindeae, including 16 species of *Morinda* s.s. and 11 individuals of *M. citrifolia* (including the two individuals of *M. bracteata* var. *celebica*) (Table 1).

DNA sequencing and phylogenetic analyses

DNA extraction, amplification and sequencing were conducted using the methods outlined in Razafimandimbison *et al.* (2009). We performed a Bayesian analysis based on a combined data set of nrETS, nrITS, *rps16* and *trnT-F*. Three unpublished internal transcribed spacer (ITS) sequences of *M. citrifolia* from GenBank (AY762832, AY762833 and AY762840) produced from wild individuals from Kosrae (Federal State of Micronesia), Australia and Puerto Rico, respectively, were included in the analysis. We re-identified the voucher specimens from which these sequences were produced as follows: a small-fruited *M. citrifolia* var. *citrifolia* (AY762832), a large-fruited *M. citrifolia* var. *citrifolia* (AY762833) and *M. bracteata* var. *celebica* (AY762840) (see Table 1). The same nucleotide substitution models as utilized in Razafimandimbison *et al.* (2009) were used for nrETS, nrITS and *trnT-F*, but the GTR + G model was used for the *rps16* data (Nylander, 2004). The settings for the Bayesian analysis were also the same as those used in Razafimandimbison *et al.* (2009). Country (with the exception of Hawaii and New Caledonia) origins of the terminal taxa were mapped onto the resulting Bayesian majority rule consensus tree; however, we used continental origins for the

sampled *Appunia guatemalensis* and *Morinda lucida*, as their country origins are not known.

RESULTS

Molecular phylogenetic analyses

The combined nrETS, nrITS, *rps16* and *trnT-F* matrix contained 4271 base pairs (bp), from which 411 (c. 10%) were parsimony-informative characters. The Bayesian majority rule consensus tree from the combined data shown in Fig. 2 is consistent with Razafimandimbison *et al.* (2009, Clade B in figure 1), except that *Morinda* s.s. (including the two African lianescent *M. longiflora* and *M. morindoides*) is poorly supported (posterior probability, PP = 0.55). All sampled arborescent *Morinda* species from the Caribbean islands, continental Africa and continental Asia, Micronesia and Australia, and the Samoan *Sarcopygme pacifica* Setch. Christoph. formed a well-supported lineage (PP = 1.00) (hereafter called the arborescent *Morinda* clade). Within the arborescent *Morinda* clade the sequenced continental Asian and Micronesian *Morinda* species and the sampled specimens of the three varieties of *M. citrifolia* together formed a well-supported group (PP = 1.00) (hereafter called the Asian *Morinda* clade, Fig. 2). Within the Asian *Morinda* clade, the continental Asian *Morinda* lineage comprising *M. angustifolia* and *M. scabrida* (PP = 1.00) was the first group to branch off, followed by another continental Asian *Morinda* lineage of *M. coreia* and *M. elliptica* (PP = 1.00). The latter group was resolved with weak support (PP = 0.69) as sister to a *Morinda* lineage including the sequenced Micronesian *Morinda* species and all sampled *M. citrifolia* (including *M. bracteata* var. *celebica*) from Australia, Guyana, Hawaii, Micronesia (Palau and Federal State of Micronesia), New Caledonia, New Guinea, Puerto Rico and Thailand. The Micronesian *M. pedunculata* was resolved with high support (PP = 1.00) as sister to a highly supported clade of all sequenced specimens of *M. citrifolia*, *M. bracteata* var. *celebica* and *M. latibracteata* (PP = 1.00). The two individuals of the Australian *M. bracteata* var. *celebica* formed a well-supported clade (PP = 1.00), sister to the Micronesian *M. latibracteata* (PP = 1.00); this Australo-Micronesian *Morinda* lineage was in turn sister to a highly supported lineage (PP = 1.00) including one specimen each of *M. citrifolia* var. *bracteata* (Thailand) and var. *potteri* (Hawaii) and five sampled accessions of the large-fruited form of *M. citrifolia* var. *citrifolia* (Guyana, Hawaii, New Caledonia, New Guinea and Puerto Rico) and two sequenced individuals of the small-fruited form of *M. citrifolia* var. *citrifolia* (Fig. 2). In sum, *M. citrifolia* as currently delimited is monophyletic only if the Australian *M. bracteata* var. *celebica*, currently classified in *M. citrifolia* var. *bracteata*, is excluded, consistent with Razafimandimbison *et al.* (2009). The results do not support the current varietal classification of *M. citrifolia*.

Table 1 List of taxa investigated in this study, voucher information, country origins and accession numbers.

Taxa	Voucher information/publications	Country/continental origins	nrETS	nrITS	rps16	trnT-F
<i>Appunia debilis</i> Sandwith	Razafimandimbison et al. (2009)	Guyana	F1907103**	F1907039**	GQ463257	F1906974**
<i>A. guatemalensis</i> Donn. Sm.	Razafimandimbison et al. (2008)	<i>Sine loc.</i> (Neotropics)	F1907104**	AM945191*	AM945306*	AM945332*
<i>Coelosperrnum balansanum</i> Baill.	Razafimandimbison et al. (2009)	New Caledonia	F1907107**	F1907041**	GQ463258	F1906978**
<i>C. paniculatum</i> F. Muell. var. <i>syncarpum</i> J. T. Johanss.	Razafimandimbison et al. (2009)	Australia	F1907111**	F1907045**	GQ463259	F1906982**
<i>Gynochthodes coriacea</i> Blume	Alejandro et al. (2005)	Indonesia	F1907112**	AM945192*	AM117311†	AJ847407†
<i>G. sessilis</i> Halford	Razafimandimbison et al. (2009)	Australia	F1907116**	F1907048**	GQ463260	F1906985**
<i>Morinda angustifolia</i> Roxb.	Razafimandimbison et al. (2009)	China	F1907116**	F1907050**	GQ463261	F1906987**
<i>M. citrifolia</i> var. <i>bracteata</i> (Roxb.) Kurz	Ryding 461 (UPS)	Thailand	GQ463239	GQ463245	GQ463263	GQ463251
<i>M. bracteata</i> Roxb. var. <i>celebica</i> Miq.	Razafimandimbison et al. (2009)	Australia	F1907119**	F1907054**	GQ463262	F1906991**
<i>M. bracteata</i> Roxb. var. <i>celebica</i> Miq. 1	P. S. Short 4842	Australia	F1907120**	AY762833††		
<i>M. buchii</i> Urb.	Razafimandimbison et al. (2009)	Haiti	F1907120**	F1907055**	GQ463264	F1906992**
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. (LF)	Taylor 11790 (MO)	Puerto Rico		AY762840††		
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. 1 (LF)	Drodz & Mozem 19981109 (UPS)	New Guinea	GQ463243	GQ463243	GQ461270	GQ463255
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. 2 (LF)	Mouly 227 (F)	New Caledonia	GQ463240	GQ463246	GQ463265	GQ463252
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. 3 (LF)	Lorence 9704 (PTBG)	Hawaii	GQ463242	GQ463248	GQ463269	GQ463254
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. 4 (LF)	Razafimandimbison et al. (2009)	Guyana	F1907125**	F1907060**	GQ463266	F1906997**
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. (SF)	NTBG 980177	Cultivated at National Tropical Botanical Garden (Hawaii, Pacific), from seed originally from Palau (Micronesia)	GQ463241	GQ463247	GQ463267	GQ463254
<i>M. citrifolia</i> L. var. <i>citrifolia</i> L. 1 (SF)	Lorence 7933 (PTBT)	Kosrae (Federated State of Micronesia)		AY762832††		
<i>M. citrifolia</i> L. var. <i>potteri</i> O. Degen.	Lorence 9706 (not 9704 as in Razafimandimbison et al., 2009) (PTBG)	Cultivated at National Tropical Botanical Garden (Hawaii, Pacific)	F1907127**	F1907062**	GQ463268	F1906999**
<i>M. coreia</i> Buch.-Ham.	Razafimandimbison et al. (2009)	Cultivated at National Tropical Botanical Garden No. 990204 (Hawaii, Pacific), from seed originally from India	F1907129**	F1907064**	GQ463271	F1907001**
<i>M. elliptica</i> (Hook. f.) Ridl.	Razafimandimbison et al. (2009)	Thailand	F1907131**	F1907066**	GQ463272	F1907003**
<i>M. geminata</i> DC.	Razafimandimbison et al. (2009)	Nigeria	F1907135**	F1907067**	GQ463273	F1907004
<i>M. latibracteata</i> Valetton	Razafimandimbison et al. (2009)	Palau (Micronesia)	F1907135**	F1907071**	GQ463274	F1907008**
<i>M. longiflora</i> G. Don	Razafimandimbison et al. (2009)	Ivory Coast	F1907115**	F1907049**	GQ463275	F1906986
<i>M. lucida</i> A. Gray	Razafimandimbison et al. (2009)	<i>Sine loc.</i> (Africa)	F1907137**	F1907073**	GQ463276	F1907010
<i>M. moensis</i> Alain	Razafimandimbison et al. (2009)	Cuba	F1907138**	AF002740\$	GQ463277	F1907011**
<i>M. morindoides</i> (Baker) Milne-Redh.	Razafimandimbison et al. (2009)	Ivory Coast	F1907140**	F1907075**	GQ463278	F1907013**

Table 1 Continued

Taxa	Voucher information/publications	Country/continental origins	nrETS	nrITS	rps16	trnT-F
<i>M. pedunculata</i> Valetou	Razafimandimbison <i>et al.</i> (2009)	Cultivated at National Tropical Botanical Garden No. 980153 (Hawaii, Pacific) from seed originally from Palau (Micronesia)	FJ907143**	FJ907077**	GQ463279	FJ907016**
<i>M. royoc</i> L.	Malcomber (2002); Razafimandimbison <i>et al.</i> (2009)	USA	FJ907146**	AF333845¶	GQ463280	FJ907020**
<i>M. scabrida</i> Craib	Larsen <i>et al.</i> 43537 (AAU)	Thailand	GQ463244	GQ463250	GQ463281	GQ463256
<i>M. titanophylla</i> E. M. A. Petit	Razafimandimbison <i>et al.</i> (2009)	D.R. of Congo	FJ907157**	F907092**	GQ463282	FJ907031**
<i>Sarcopygme pacifica</i> (Reinecke) Setch. & Christoph.	Tronchet <i>et al.</i> 222 (P)	Samoa		FJ907097**	GQ463283	FJ907036**

*Razafimandimbison *et al.* (2008); †Bremer & Eriksson (2009); ‡Alejandro *et al.* (2005); §Andersson & Rova (1999); ¶Malcomber (2002); **Razafimandimbison *et al.* (2009); ††Proujansky & Stern (GenBank unpublished).

DISCUSSION

Phylogenetic affinity and the potential area of origin of *Morinda citrifolia*

All sampled individuals of *M. citrifolia* are nested within the Asian *Morinda* clade (Fig. 2). In this clade the two-first lineages to branch off are composed entirely of species from continental Asia (China, India and Thailand). One interpretation of this pattern is that the clade had its origin in continental Asia (Fig. 2). *Morinda citrifolia* is most closely related to the Micronesian species *M. pedunculata* and *M. latibracteata* and Australasian *M. bracteata* var. *celebica* rather than to the sequenced continental Asian *Morinda* species (*M. angustifolia*, *M. scabrida*, *M. coreia* and *M. elliptica*). *Morinda pedunculata*, *M. latibracteata* and the small-fruited *M. citrifolia* var. *citrifolia* are all endemics to Micronesia, suggesting a Micronesian origin of *M. citrifolia* (Fig. 2). This finding does not support the Southeast Asian origin of *M. citrifolia* and implies that *M. citrifolia* is likely to have already been present in Micronesia at least before the arrival of the Micronesian ancestors from Southeast Asia over 3000 years ago. Given its efficient seed dispersal mechanism, the large-fruited *M. citrifolia* var. *citrifolia* might well have reached Polynesia and the rest of the Pacific before the arrival of the Southeast Asian ancestors of the Polynesian people. The historical record is silent on this, but molecular phylogenetic dating and biogeographical analyses of the tribe Morindeae might provide further evidence (study in progress).

The Australian origin of *M. citrifolia* put forward by McClatchey (2003), based on the sister-group relationship between *M. citrifolia* and the Australian *M. reticulata* in Johansson (1994), is not supported by Razafimandimbison *et al.* (2009), as these two species are consistently resolved into two distinct clades: the former in *Morinda sensu* Razafimandimbison *et al.* (2009, Clade B in Fig. 1) and the latter in *Coelospermum sensu* Razafimandimbison *et al.* (2009, Clade C in Fig. 1). McClatchey's (2003) hypothesis is based on the morphological phylogeny of Johansson (1994), which is erroneously rooted with three lianescent *Morinda* species, *M. glomerata*, *M. grayi* and *M. myrtifolia*, which all belong to a lianescent, derived *Gynochthodes* (Razafimandimbison *et al.*, 2009; Clade D in Fig. 1).

Is the widespread, large-fruited *Morinda citrifolia* var. *citrifolia* the progenitor colonizer of island endemic *Morinda* species?

The hypothesis that the widespread, large-fruited *M. citrifolia* var. *citrifolia* could have been the progenitor colonizer that gave rise to numerous island endemic *Morinda* species (McClatchey, 2003) is not supported by our analyses. *Morinda citrifolia* is derived within the Asian *Morinda* clade (Fig. 2) and is more closely related to the Southeast Asian, arborescent *Morinda* species than to the sampled lianescent *Morinda* species from Australia, the Pacific and Madagascar (now

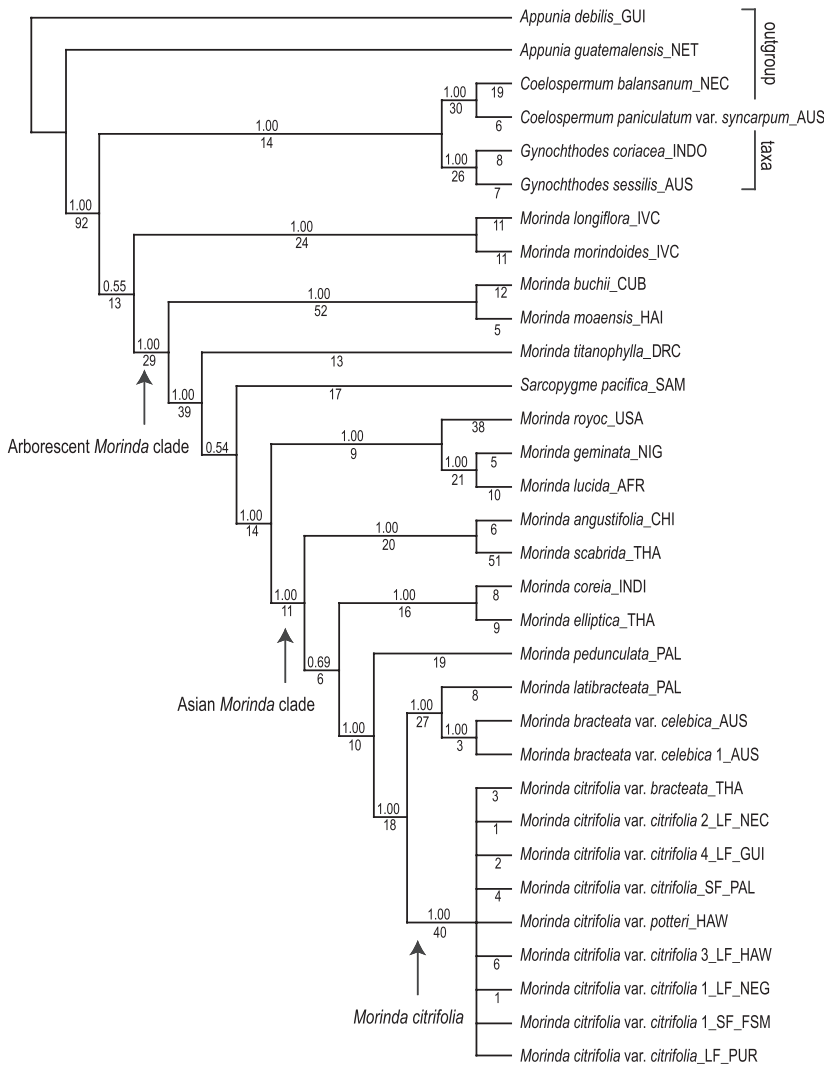


Figure 2 Bayesian majority rule consensus tree of *Morinda sensu* Razafimandimbison *et al.* (2009) based on combined nrETS, nrITS, *rps16* and *trnT-F* data from 22 Morindeae taxa. *Morinda citrifolia* var. *citrifolia* is a single taxon represented by 11 specimens. Values above the nodes are posterior probabilities and numbers below the nodes are numbers of evolutionary steps. LF and SF denote the large- and small-fruited forms of *M. citrifolia* var. *citrifolia*, respectively; the bracket delimits the outgroup taxa. AFR, Africa; AUS, Australia; CUB, Cuba; CHI, China; FST, Federated State of Micronesia; GUY, Guyana; HAI, Haiti; HAW, Hawaii; INDI, India; INDO, Indonesia; IVC, Ivory Coast; NEC, New Caledonia; NEG, New Guinea; NET, Neotropics; NIG, Nigeria; PAL, Palau; PUR, Puerto Rico; DRC, Republic Democratic of Congo; SAM, Samoa; and USA, United State of America.

members of *Gynochthodes sensu* Razafimandimbison *et al.*, (2009), where the large-fruited *M. citrifolia* var. *citrifolia* is commonly found. In addition, the hypothesis is inconsistent with the fact that the nine sampled individuals of *M. citrifolia* from diverse geographic locations form a monophyletic group (Fig. 2). Howarth *et al.* (2003) have reported similar evolutionary patterns in the genus *Scaevola* (Goodeniaceae), which has two widespread strand species: one occurring throughout the Indian and Pacific regions and the other in tropical America and Africa.

Comments on the geographical distribution of the large-fruited *Morinda citrifolia* var. *citrifolia*

Morinda citrifolia is the only species of *Morinda* s.s. with a pantropical distribution. The remaining *Morinda* species are all regional endemics. The wide distribution of the large-fruited *M. citrifolia* var. *citrifolia* has been attributed partly to natural dispersal by oceanic floating (seeds remain viable after floating in seawater for several months; Guppy, 1917; Morton, 1992). The acquisition of the buoyant seeds alone is not, however,

enough to explain its wide distribution, as the same type of seeds is found in other Morindeae species (e.g. *Morinda royoc* and *Appunia guatemalensis*, Hayden & Dwyer, 1969) that have much narrower geographic distributions than *M. citrifolia*. In fact, the large-fruited *M. citrifolia* var. *citrifolia* has the ability to self-pollinate and produce flowers and fruits year-round, allowing it to adapt easily to and colonize a range of new habitats. This form of *M. citrifolia* var. *citrifolia* also has been introduced in historical times and is now reproducing in the wild, making it difficult (if not impossible) to know whether or not its natural distribution is much narrower than its current distribution. It has yet to be verified whether or not the fruits of *M. citrifolia* var. *bracteata*, *M. citrifolia* var. *potteri* and the small-fruited *M. citrifolia* var. *citrifolia* also have buoyant seeds.

Comments on the circumscription of *Morinda citrifolia*

Our analyses further confirm that *Morinda coreia*, *M. elliptica* and *M. lucida* are not closely related to the presently delimited *M. citrifolia*, consistent with Razafimandimbison *et al.* (2009). The present study indicates that the sequenced individual of

M. citrifolia var. *bracteata* (with conspicuous bracts subtending the inflorescence) from Thailand and the two sampled individuals of *M. bracteata* var. *celebica* from Australia are not closely related to each other. The sister-group relationship between the Australian *M. bracteata* var. *celebica* and the Micronesian *M. latibracteata* is confirmed (27 molecular synapomorphies, PP = 1.00) (Fig. 2). Both taxa have enlarged calyx lobes, although they are relatively smaller in *M. bracteata* var. *celebica*. The texture and size of leaves also differ between these two taxa. Merrill (1923) synonymized *M. bracteata* var. *celebica* in *M. citrifolia* var. *bracteata sensu* Kurz (1877); however, our analysis suggests that it should be excluded from *M. citrifolia* and recognized at species level in order to make *M. citrifolia* monophyletic. On the other hand, it would be advisable to sequence accessions of *M. bracteata* var. *celebica* from Celebes (now Sulawesi, Indonesia), where the type specimen was originally collected (Miquel, 1857), before making any taxonomic adjustment.

Waki *et al.*'s (2008) cluster analysis based on 58 morphological traits of *M. citrifolia* showed that *M. citrifolia* var. *citrifolia*, *M. citrifolia* var. *bracteata* and *M. citrifolia* var. *potteri* formed a group. The samples of *M. citrifolia* var. *bracteata* investigated in that study match the description of the Australian *M. bracteata* var. *celebica* used in this study. The present analyses demonstrate that *M. citrifolia* is monophyletic only if the Australian *M. bracteata* var. *celebica* is excluded. Our analysis reveals for the first time discrepancies between the levels of molecular and morphological variation in *M. citrifolia*, with the former being relatively low and the latter being high. In addition, we find no molecular support for the current varietal classification of *M. citrifolia*. The DNA sequence data from nrETS, nrITS, *rps16* and *trnT-F* markers appear to support recognition of a single species with limited molecular sequence variation that cannot accurately be described as more than one variety, consistent with hypothesis H3 of McClatchey (2003). On the other hand, it is possible that with more extensive sampling this widespread species could present greater molecular variation. Similarly, data from low-copy nuclear or microsatellite markers may reveal more structure than the sequence data from the four selected markers. Accordingly, we maintain for now the current varietal classification of *M. citrifolia* pending further analyses.

CONCLUSIONS

The present study focuses on the origin of the well-known noni or Indian mulberry plant (*Morinda citrifolia*) using a phylogenetic approach. Our results confirm the monophyly of *M. citrifolia* exclusive of the Australian *M. bracteata* var. *celebica* and suggest a Micronesian origin of *M. citrifolia*, which implies that the large-fruited *M. citrifolia* var. *citrifolia* is likely to have been present in the Pacific before the arrival of the Micronesian and Polynesian ancestors from Southeast Asia. The wide distribution of the large-fruited *M. citrifolia* var. *citrifolia* is attributed partly to the acquisition of its self-pollination and buoyant fruits and to its ability to produce

flowers and fruits year-round. We demonstrate that this form of *M. citrifolia* var. *citrifolia* could not have been the progenitor colonizer of the island endemic *Morinda* species. Finally, our findings concerning the phylogenetic relationships of *M. citrifolia* will allow noni researchers to focus their investigations into medicinal and biochemical properties on closely related species.

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BIOSKETCH

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