Phylogeny of *Euclinia* and allied genera of Gardenieae (Rubiaceae), and description of *Melanoxerus*, an endemic genus of Madagascar

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DOI http://dx.doi.org/10.12705/634.2

Abstract We performed molecular phylogenetic analyses of the *Randia* clade of the tribe Gardenieae using both plastid and nuclear DNA data. In the phylogenetic hypotheses presented, the African genera *Calochone*, *Euclinia*, *Macrosphyra*, *Oligocodon*, *Pleiocoryne*, and *Preussiodora* are resolved as a monophyletic group. Support is also found for a clade of the Neotropical genera *Casasia*, *Randia*, *Rosenbergiodendron*, *Sphinctanthus*, and *Tocoyena*. This Neotropical clade is resolved as sister group to the African clade in analyses of combined plastid and nuclear data. The genus *Euclinia* appears polyphyletic. The species described from Madagascar represent an independent lineage, the position of which is moreover found to be incongruent between datasets. Plastid and ribosomal DNA data support a sister-group relationship to the mainland African clade, whereas the low-copy nuclear gene *Xdh* supports a closer relationship to the Neotropical genera. The phylogenetic reconstructions also indicate that *Casasia* and *Randia* are not monophyletic as presently circumscribed. A taxonomic proposal is made for the recognition of the Malagasy taxon at generic level as *Melanoxerus*.

Keywords *Euclinia*; Gardenieae; Ixoroideae; Madagascar; molecular phylogenetics; *Randia*; Rubiaceae; systematics; *Xdh*

■ INTRODUCTION

The genus *Euclinia* Salisb. of the coffee family (Rubiaceae) as currently circumscribed comprises three species with a widely disjunct distribution in tropical Africa. *Euclinia longiflora* Salisb. occurs in West and Central Africa, *E. squamifera* (R.D.Good) Keay in western Central Africa, and *E. suavissima* (Homolle ex Cavaco) J.-F.Leroy in Madagascar. Salisbury (1808) first described *Euclinia longiflora* from a plant raised from seeds collected in Sierra Leone. Although he initially (Salisbury, 1808: pl. 93) – "I cannot hesitate" – considered it a species of *Randia* L., this placement was amended in the errata and "Index sexualis" of the same book, wherein he instead recognized the taxon at generic level, with a name in reference to its "beautifully reclining branches". The name *Randia longiflora* had already been published by Lamarck (1789), whose resurrection of *Randia* is also mentioned in Salisbury's description. Unfortunately, the diagnostic characteristics of the new genus were not much discussed by Salisbury, who apparently planned to detail that elsewhere ("*in alio loco fusius exponam*"; Salisbury, 1808: Index Sexualis). Subsequently, in the first classification of the tribe Gardenieae by Candolle (1830), *Euclinia longiflora* was subsumed in a broadly circumscribed *Randia*, and was for a long time known as *Randia macrantha* (Schult.) DC. This classification was also adopted in the system by Hooker (1873: 88, 89), although with comments on *Randia* as being poorly distinguished from *Gardenia* J.Ellis (i.e., by fruits appearing bilocular [*Randia*] or unilocular [*Gardenia*]), and that both genera were "polymorphic".

In a major revision of the African Gardenieae, Keay (1958) recognized 21 genera all of which at some time had been considered part of *Randia* or *Gardenia*. Among the resurrected names were *Euclinia*, to which he also transferred a second species, *E. squamifera*, from *Gardenia*. One of the characters considered especially important by Keay (1958) was pollen grain aggregation. Pollen grains dispersed in tetrads, a feature which is otherwise rare in Rubiaceae, is characteristic of *Euclinia* as well as of *Gardenia* s.str. and *Randia* s.str. (*Randia* is now considered a strictly Neotropical genus as the result of several subsequent revisions, e.g., Yamazaki, 1970; Tirvengadum, 1978, 1983; Wong, 1984; Fosberg, 1987; Puttock, 1999). Robbrecht & Puff (1986) suggested that Gardenieae characterized by pollen tetrads might constitute a natural group. However, molecular phylogenetic studies of the tribe by Andreasen & Bremer (2000), and Persson (2000), showed that pollen tetrads probably have evolved independently at least three times within Gardenieae. In the latter study, *Euclinia* was found resolved in a clade (informally named the *Randia* clade) together with the African genera *Calochone* Keay, *Macrosphyra* Hook.f., *Oligocodon* Keay, and *Preussiodora* Keay, and the Neotropical genera *Casasia* A.Rich., *Randia*, *Rosenbergiodendron* Fagerl., *Sphinctanthus* Benth., and *Tocoyena* Aubl. Of these, all but the latter three genera have pollen dispersed in tetrads (Keay, 1958; Persson, 1993; Gustafsson, 1998). Relationships within the *Randia* clade were largely unresolved in the study

Received: 31 Aug 2013 | returned for first revision: 2 Dec 2013 | last revision received: 25 Feb 2014 | accepted: 7 Mar 2014 | not published online ahead of inclusion in print and online issues

by Persson (2000), although the genera with pollen dispersed as monads (i.e., *Rosenbergiodendron*, *Sphinctanthus* and *Tocoyena*) formed a supported group, informally named the *Rosenbergiodendron* clade.

Gustafsson & Persson (2002) further investigated the phylogeny of the *Randia* clade and *Randia* in particular, using nuclear ribosomal DNA. Although relationships within the group were not well-supported, and the data did not support (or contradict) the monophyly of either *Randia* or *Casasia*, they did find support for the *Rosenbergiodendron* clade, as well as for a clade comprising all included African genera (*Calochone*, *Macrosphyra*, *Oligocodon*, and *Preussiodora*; *Euclinia* was not included in the study). In a recent study by Mouly & al. (2014), *Euclinia* was included and nested within a well-supported clade of the African genera. *Euclinia* was sister group to a *Calochone*-*Macrosphyra* clade. Sister group to this clade was a clade represented by *Oligocodon* and *Pleiocoryne* Rauschert, two of the genera which Keay (1958) had separated from *Gardenia*, whereas *Preussiodora*, segregated from *Randia* by Keay (1958), was sister group to the rest of the African genera in the analyses of Mouly & al. (2014). Although generic relationships within the African clade were well supported in the latter study, the phylogenetic relationships to, and among the Neotropical genera, were poorly supported, as in previous molecular phylogenetic studies of the *Randia* clade (i.e., Persson, 2000; Gustafsson & Persson, 2002).

The third species of *Euclinia*, *E. suavissima*, an endemic of Madagascar, was also originally described as *Gardenia* (Cavaco, 1967). It was subsequently transferred to *Euclinia* by Leroy (1974), who unfortunately did not give any comment on his rationale. This species has since been little-studied. Preliminary phylogenetic analyses done as part of an ongoing project on the biogeography of the Rubiaceae of Madagascar have indicated that this taxon is part of the *Randia* clade, although not closely related to *Euclinia*. In the present study we perform molecular phylogenetic analyses of the *Randia* clade using both nuclear and plastid DNA, with the specific aims of resolving generic relationships in the group, and testing the monophyly of *Euclinia* as currently circumscribed.

■ MATERIALS AND METHODS

Taxon sampling. — Besides all three species of *Euclinia*, the taxon sampling included representatives of all genera of the *Randia* clade of Persson (2000) and Mouly & al. (2014), i.e., *Calochone*, *Macrosphyra*, *Oligocodon*, *Pleiocoryne*, and *Preussiodora* from Africa, and *Casasia*, *Randia*, *Rosenbergiodendron*, *Sphinctanthus*, and *Tocoyena* from the Neotropics. The sampling from the former group included all known species except for two species of *Macrosphyra*. Malagasy *Euclinia* was sampled from northern (Daraina; "*Euclinia* sp. 1"), western (Beanka; "*Euclinia* sp. 2"), and southern (Zombitse; *E. suavissima*) Madagascar. Several other Gardenieae (including most African genera) were also sampled; i.e., *Aidia* Lour., *Aoranthe* Somers, *Atractocarpus* Schltr. & K.Krause, *Aulacocalyx* Hook.f., *Catunaregam* Wolf, *Coddia* Verdc., *Gardenia* J.Ellis, *Genipa* L., *Hyperacanthus* E.Mey. ex Bridson, *Morelia* A.Rich. ex DC., *Massularia* (K.Schum.) Hoyle, *Rothmannia* Thunb., and *Schumanniophyton* Harms. The outgroup also included *Alberta magna* E.Mey. and *Burchellia bubalina* (L.f.) Sims. An overview of the taxon sampling is given in Appendix 1.

DNA extraction, amplification, and sequencing. — DNA was extracted following the protocol of Doyle & Dickson (1987), and purified using the QIAquick PCR purification kit following the instructions of the manufacturer (Qiagen, Hilden, Germany). Part of the external transcribed spacer (ETS) of the nuclear ribosomal DNA (rDNA), as well as the plastid DNA (cpDNA) regions *rpl32* (*ndhF*-*rpl32*-*trnL*), *matK* (*trnK*-*matK*-*trnK*), *trnT-F* (*trnT*^{UGU}-*trnL*^{UAA}-*trnL*^{UAA}-*trnF*^{GAA}), and the *rps16* intron were amplified using the primers in Table 1. For the plastid regions a PCR program was used comprising an initial denaturation step at 95°C for 2 min, followed by 37 cycles of denaturation (95°C, 30 s), annealing (50°C, 1 min), and extension (72°C, 2 min), and a final extension step of at 72°C for 7 min. The PCR program outlined in Razafimandimbison & al. (2005) was used for ETS. The low-copy nuclear gene (nDNA) coding for xanthine dehydrogenase (*Xdh*), found to be phylogenetically informative in studies of Orchidaceae (Górniak & al., 2010) and across the angiosperms (Morton, 2011), was also investigated in this study. It was amplified in two parts using the primers in Table 1, and a Touchdown PCR protocol comprising an initial DNA denaturation step at 95°C for 2 min, followed by 37 cycles of denaturation (95°C, 1 min), annealing (61°C–55°C, 1 min; temperature lowered 1°C per cycle until 55°C was reached), and extension (72°C, 1 min 30 s), and a final extension step at 72°C for 5 min. PCR products were cleaned using Multiscreen Filter plates (Millipore, Billerica, Massachusetts, U.S.A.). PCR products were directly sequenced using the amplification primers and the BigDye terminator cycle sequencing kit, and subsequently analysed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, U.S.A.) or ABI PRISM 3730XL, as part of the EZ-seq v.2.0 sequencing service provided by Macrogen Europe (Amsterdam, The Netherlands). Sequence reads were assembled using the Staden package v.1.5.3 (Staden, 1996). The *Xdh* sequences typically showed very few ambiguous base-pair readings. These were coded according to IUPAC degenerative codes. Sequences new to this study (157) were deposited in GenBank (Appendix 1). Additional sequences (39) were obtained from GenBank (for references, see Appendix 1).

Data analyses. — Sequences were sorted by size and aligned using MUSCLE v.3.8.31 (default settings; Edgar, 2004), except for the *Xdh* sequences for which only one indel was apparent (a 3-bp deletion in *Rothmannia*). Alternative sequence versions of suspected sequence inversions were subsequently separated from each other in the alignments (but not excluded from the analyses; i.e., basepairs 602–615 of AM117289, 606–619 of KJ136943, 1068–1106 of KJ136927, and 1553–1641 of KJ136920). Phylogentic reconstruction was done by Bayesian Markov chain Monte Carlo (MCMC) inference (Yang & Rannala, 1997), using the program MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck,

2003). The four plastid DNA regions were combined and analysed together, with each region as a separate partition with unlinked model parameter estimates (except topology). The rDNA data was analysed separately and in combination with the cpDNA data, whereas the *Xdh* region was only analysed separately due to well-supported incongruences with the other datasets. Nucleotide substitution models indicated as best-fit to the data under the corrected Akaike information criterion (AICc), as implemented in MrAIC v.1.4.4 (Nylander, 2004; a script dependent on the program PHYML v.3.0; Guindon & Gascuel, 2003), were used for each dataset (ETS, GTR+Γ; *matK*, GTR+Γ; *rpl32*, GTR+Γ; *rps16*, GTR+I+Γ, *trnT-F*, GTR+I+Γ; *Xdh*, GTR+Γ). The analyses comprised two runs of four MCMC chains each, and were monitored for 10^7 generations, with every 1000th generation sampled. The chain heating parameter was 0.15. The initial 25% of the sampled trees were considered burn-in and excluded from the consensus. Effective sample sizes (ESS) of the model parameters were checked using Tracer v.1.5 (Rambaut & Drummond, 2007), in order to ensure an ESS >200, as recommended by Drummond & al. (2007) for adequate representation of the posterior probability.

The data was also analysed using the maximum parsimony optimality criterion as implemented in PAUP* v.4.0b10 (Swofford, 2002), and were performed using heuristic searches with the tree bisection-reconnection (TBR) branch swapping algorithm, Multrees on, 1000 random sequence addition replicates and a maximum of 10 trees saved per replicate. Clade support was estimated using 1000 bootstrap replicates, with three random addition replicates per replicate. A bootstrap proportion equal to or greater than 70%, and posterior probabilities equal to or greater than 0.95 were considered well supported (cf. Alfaro & al., 2003).

Table 1. Primers used for amplification and/or sequencing, with references for previously published sequences.

Region	Primer	Primer sequence from 5′ end	Reference
ETS	18S-E	GCAGGATCAACCAGGTAGCA	Baldwin & Markos (1998)
	ETS-Erit-F	CTTGTATGGGTTGGTTGGA	Negrón-Ortiz & Watson (2002)
ndhF-rpl32-trnL	ndhF16F	GAAAGGKATGATCCACCCGTATTG	newly designed
	rpl32-F	CAGTTCCAAAAAAACGTACTTC	Shaw & al. (2007)
	rpl32-R	CCAATATCCCTTYYTTTTCCAA	Shaw & al. (2007)
	rpl32-2R	ATTGGGTCGATTTTTGAGTG	newly designed
	trnL(uag)	CTGCTTCCTAAGAGCAGCGT	Shaw & al. (2007)
rps16 intron	rpsF	GTGGTAGAAAGCAACGTGCGACTT	Oxelman & al. (1997)
	rpsR2	TCGGGATCGAACATCAATTGCAAC	Oxelman & al. (1997)
trnK-matK-trnK	trnK-3914F	TGGGTTGCTAACTCAATGG	Johnson & Soltis (1994)
	matK1F	ACTGTATCGCACTATGTATCA	Sang & al. (1997)
	matK807R	ACTCCTGAAAGATAAGTGGA	newly designed
	matK4bR	GCRTCTTTTACCCAATAGCGAAG	newly designed
	matK1198F	CTGTGTTAGATATACNAATACCCC	Andersson & Antonelli (2005)
	matK6R	TTCTAGMATTTGACTCCGTACC	Bremer & al. (2002)
	matK1760F	TRGGCTATCTTTCAAGYGTGCG	newly designed
	trnK-2R	AACTAGTCGGATGGAGTAG	Johnson & Soltis (1994)
trnT-F	trnT-F_a1F	ACAAATGCGATGCTCTAACC	Bremer & al. (2002)
	trnT-F_iR	CCAACTCCATTTGTTAGAAC	Bremer & al. (2002)
	820F	GAATCGAYCSTTCAAGTATTC	Rydin & al. (2008)
	940R	GATTYTATCATTTCYGTVTMYGC	Rydin & al. (2008)
	1250F	ATGGCGAAATTGGTAGACGC	Rydin & al. (2008)
	1880F	TCAAAYGATTCACTCCATAGTC	Rydin & al. (2008)
	2670R	GATTTTCAGTCCTCTGCTCTACC	Rydin & al. (2008)
	c	CGAAATCGGTAGACGCTACG	Taberlet & al. (1991)
	d	GGGGATAGAGGGACTTGAAC	Taberlet & al. (1991)
Xdh	Xdh481F	CATTGCTACGGTCNTCTCAWG	newly designed
	Xdh1170F	GCYGGNACCARATAAGGAATG	newly designed
	Xdh1247R	TCCTGCAGCCATCCAAAGA	newly designed
	Xdh1641R	CATCTTCYTTCARCACAATGTC	newly designed

Scanning electron microscopy (SEM). — A piece of an anther of *Euclinia suavissima* (*Phillipson 3055*; P) was sampled and treated with 30%, 50%, 70%, and 90% ethanol, and then acetone in order to remove substances covering the pollen exine (cf. Hesse & Waha, 1989). The sample was then mounted on an aluminium stub and photographed (uncoated) using a JSM-7401 field emission scanning electron microscope (JEOL, Tokyo, Japan).

■ RESULTS

The aligned matrix of combined plastid sequences comprised 7810 basepairs (bp), 358 of which were phylogenetically informative (*matK*: *n* = 36, 116/2565 informative basepairs; *rps16*: *n* = 35, 40/890 informative basepairs; *rpl32*: *n* = 32, 116/2349 informative basepairs; *trnT-F*: *n* = 35, 86/1959 informative basepairs). The aligned ETS sequences (*n* = 24) included 156 informative characters of a total of 459 bp, and the *Xdh* matrix (*n* = 34) comprised 1151 bp, 154 of which were phylogenetically informative.

The phylogenetic hypothesis inferred from the Bayesian MCMC analyses of the combined plastid DNA is shown in Fig. 1 as a 50% majority-rule consensus tree, with posterior probablities (PP) indicated above each clade. Also indicated are the clade bootstrap (BS) support values from the parsimony analysis (below clades), as well as the corresponding support values from the analyses of the plastid DNA data together with ETS. Results from the analyses of ETS and the *Xdh* gene are similarily shown in Figs. 2 and 3, respectively. The phylogenetic reconstructions of the cpDNA data were overall well resolved, albeit several of the clades received low support. The ETS trees were not as well resolved, although the clades that did receive high posterior probability were congruent with those retrieved from the cpDNA data. In contrast, the analyses of *Xdh* data revealed several well-supported incongruences relative to cpDNA and ETS data.

The *Randia* clade was resolved with strong support in all analysed datasets (cpDNA, PP, 1.00; BS, 100%; ETS, PP, 1.00; BS, 73%; *Xdh*, PP, 1.00; BS, 99%). Resolved as sister group to the *Randia* clade in the analyses of cpDNA was a clade comprising the genera *Aidia*, *Aoranthe*, *Atractocarpus*, *Catunaregam*, *Hyperacanthus*, *Morelia*, and *Massularia* (PP, 1.00; BS, 54%). This clade was also resolved and well-supported in the analyses of *Xdh* (PP, 1.00; BS, 99%). Within the *Randia* clade, *Calochone*, *Euclinia*, *Macrosphyra*, *Oligocodon*, *Pleiocoryne*, and *Preussiodora* from mainland Africa formed a well-supported clade in analyses of cpDNA (PP, 1.00; BS, 85%), ETS (PP, 1.00; BS, 87%), and *Xdh* (PP, 1.00; BS, 93%). The sampled Malagasy *Euclinia* also formed a well-supported clade (PP, 1.00; BS, 100%, for all three datasets), which was resolved as sister to the mainland African clade in analyses of cpDNA data (PP, 1.00; BS, 97%) and ETS (PP, 1.00; BS, 96%). This (African and Malagasy) clade was in turn sister group to a clade of Neotropical genera (*Casasia*, *Randia*, *Rosenbergiodendron*, *Sphinctanthus*, *Tocoyena*). In contrast, the analyses of *Xdh* supported the Malagasy clade as sister group to the Neotropical clade (PP, 0.99; BS, 69%). Support for the Neotropical clade was low in analyses of cpDNA data (PP, 0.69), but it was well supported by ETS (PP, 0.96; BS, 67%) and *Xdh* (PP, 0.99; BS, 82%) data.

Relationships within the African clade were well resolved in all analysed datasets. *Euclinia* appeared polyphyletic; *E. suavissima* was sister group to the clade of the remaining African genera (cpDNA and ETS data), whereas *E. longiflora* and *E. squamifera* were nested within the latter clade and well supported as sister clade to a *Calochone-Macrosphyra* clade (PP, 1.00; BS, 88%). Resolved as sister to the *Euclinia-Calochone-Macrosphyra* clade was in turn a clade comprising *Oligocodon* and *Pleiocoryne*. Support for the latter clade was found both from cpDNA (PP, 1.00; BS, 78%), and *Xdh* data (PP, 0.95; BS, 72%), however, in the *Xdh* tree the *Oligocodon-Pleiocoryne* clade was instead inferred as sister group to *Macrosphyra* (PP, 0.98; BS, 80%). *Preussiodora* was sister group to the rest of the mainland African genera in all three datasets. A likely synapomorphy of the mainland African genera was the loss of a glutamic acid in the maturase K (*matK*) sequence (aa 466, 467, or 468). This mutation was not shared by *Euclinia suavissima*.

Relationships among the Neotropical genera were overall not well supported. However, *Rosenbergiodendron* and *Sphinctanthus* formed a clade in all analysed datasets (cpDNA, PP, 1.00; BS, 96%; ETS, PP, 1.00; BS, 100%; *Xdh*, PP, 1.00; BS, 97%). *Tocoyena* was well supported as sister group to the latter clade by cpDNA data only (PP, 1.00; BS, 100%). *Casasia* and *Randia* were not resolved as monophyletic. The sampled species of *Casasia* and *Randia aculeata* L. formed a well-supported clade in all analysed datasets (cpDNA, PP, 0.99; BS, 77%; ETS, PP, 1.00; BS, 91%; *Xdh*, PP, 0.98; BS, 56%). *Casasia* appeared paraphyletic in analyses of cpDNA, because *C. calophylla* A.Rich. formed a clade with *Randia aculeata* (PP, 1.00; BS, 75%), and this clade was in turn sister to *Casasia clusiifolia* (Jacq.) Urb. This topology was also inferred from the *Xdh* data. In contrast, the parsimony analysis of ETS supported *Casasia* as monophyletic (BS, 91%), however, the posterior probability of this node was low (PP, 0.71). In the analyses of the combined cpDNA and ETS, *Casasia* was well supported as paraphyletic (PP, 0.97), wheras the parsimony analysis showed low support for a monophyletic *Casasia* (57%). The parsimony analyses of ETS also differed from the Bayesian analyses in inferring *Tocoyena* as sister group to the *Casasia-Randia* clade, although bootstrap support for this relationship was low (BS, 59%). *Randia armata* (Sw.) DC. and *R. carlosiana* K.Krause formed a well-supported clade (cpDNA, PP, 1.00; BS, 99%, *Xdh*, PP, 1.00; BS, 100%). However, none of the datasets resolved this clade together with *R. aculeata* or the *Casasia-Randia* clade.

■ DISCUSSION

In this study we have analysed combined cpDNA (*matK*, *rps16*, *rpl32*, *trnT-F*), rDNA (ETS), and nDNA (*Xdh*) data in order to resolve generic relationships in the *Randia* clade and in particular to test the monophyly of *Euclinia*. In the phylogenetic

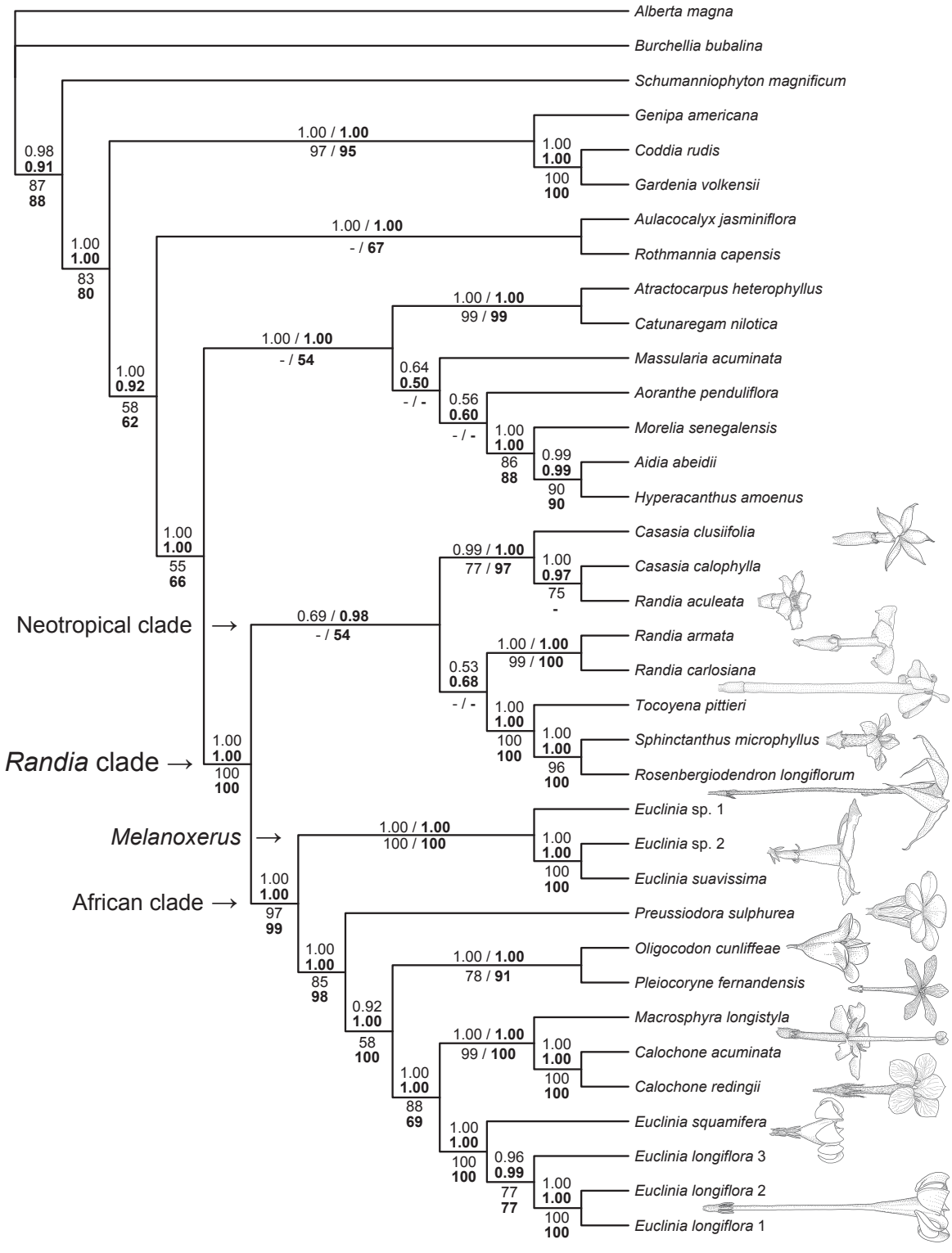


Fig. 1. Majority-rule consensus tree from the Bayesian MCMC analyses of the combined cpDNA (*rps16*, *rpl32*, *maK*, *trnT-F*), and rDNA (ETS) data. Posterior probabilities are indicated above, and bootstrap support values from the parsimony analysis below branches (a dash indicates a posterior probability of <0.50, or a bootstrap support of <50%). Values in bold are from the combined analyses of cpDNA and rDNA data. Drawings (not to scale) drawn/redrawn from: *Casasia clusiifolia* – Rogers (1987); *Randia aculeata* – Fig. 4D (*Stevens 30468*; MO); *Randia armata* and *Tocoyena pittieri* – images by the Environmental Sciences Program, Smithsonian Tropical Research Institute; *Sphinctanthus microphyllus* – *Hassler 7297* (P, image); *Rosenbergiodendron longiflorum* – *Hallé 543* (P, image); *Melanoxerus suavissius* (*Euclinia suavissima*) – *Allorge 2428* (P, image); *Oligocodon cunliffeae* – Keay (1958); *Macrosphyra longistyla* – Fig. 4G; *Calochone redingii*, *Euclinia longiflora*, *E. squamifera*, *Pleiocoryne fernandensis*, and *Preussiodora sulphurea* – Hallé (1970).

hypotheses presented, the *Randia* clade is sister group to a clade of Paleotropical genera, i.e., *Aidia*, *Aoranthe*, *Atractocarpus*, *Catunaregam*, *Hyperacanthus*, *Morelia*, and *Massularia*. Except for the last genus, these genera correspond to either the *Aidia* clade or the *Porterandia* clade of the more broadly sampled study by Mouly & al. (2014). Whereas relationships among *Massularia*, the *Aidia*-, *Porterandia*-, and *Randia* clades were poorly resolved in that study, the representatives of the three former clades included in the present study are strongly supported as monophyletic, both by plastid and *Xdh* sequence data (as is the sister-group relationship to the *Randia* clade). However, relationships within the *Aidia*-*Aoranthe*-*Atractocarpus*-*Catunaregam*- *Hyperacanthus*-*Morelia*-*Massularia* clade are still largely unresolved (Figs. 1, 3).

Systematic relationships of the *Randia* clade. — In this study we found the African genera of the *Randia* clade (i.e., *Calochone*, *Euclinia*, *Macrosphyra*, *Oligocodon*, *Pleiocoryne*, *Preussiodora*) resolved as a clade. This is congruent with the molecular phylogenetic studies by Gustafsson & Persson (2002) and Mouly & al. (2014). The nuclear data also support a clade comprising the Neotropical genera *Casasia*, *Randia*, *Rosenbergiodendron*, *Sphinctanthus*, and *Tocoyena*. The African and Neotropical clades are sister groups in the phylogenetic analyses of the combined plastid and nuclear data (Fig. 1). However, analyses of the *Xdh* gene support a shared ancestry between the Neotropical clade and *Euclinia suavissima* (Fig. 3). All three datasets analysed in this study (cpDNA, rDNA, nDNA) indicate that *Euclinia* is not monophyletic as presently circumscribed. *Euclinia longiflora* and *E. squamifera* form a clade that is nested within the African clade, whereas depending on the dataset, the Malagasy *E. suavissima* either was sister group to the rest of the African taxa (i.e., in analyses of cpDNA and ETS), or to the Neotropical clade (i.e., in analyses of *Xdh*). Although these results support the exclusion of *E. suavissima* from *Gardenia* by Leroy (1974), its inclusion in *Euclinia* is not supported. Consequently, we henceforth discuss this taxon as a separate genus, *Melanoxerus* (see Taxonomic synopsis).

The cause of the incongruence in the inferred phylogenetic position of *Melanoxerus* is not evident, and could have resulted from paralogy or incomplete lineage sorting of the *Xdh* gene, or an ancient hybridization event. The last explanation, however, would be remarkable given the widely disjunct extant distributions of the three groups. The cpDNA and *Xdh* data also support incongruent phylogenetic relationships among the mainland

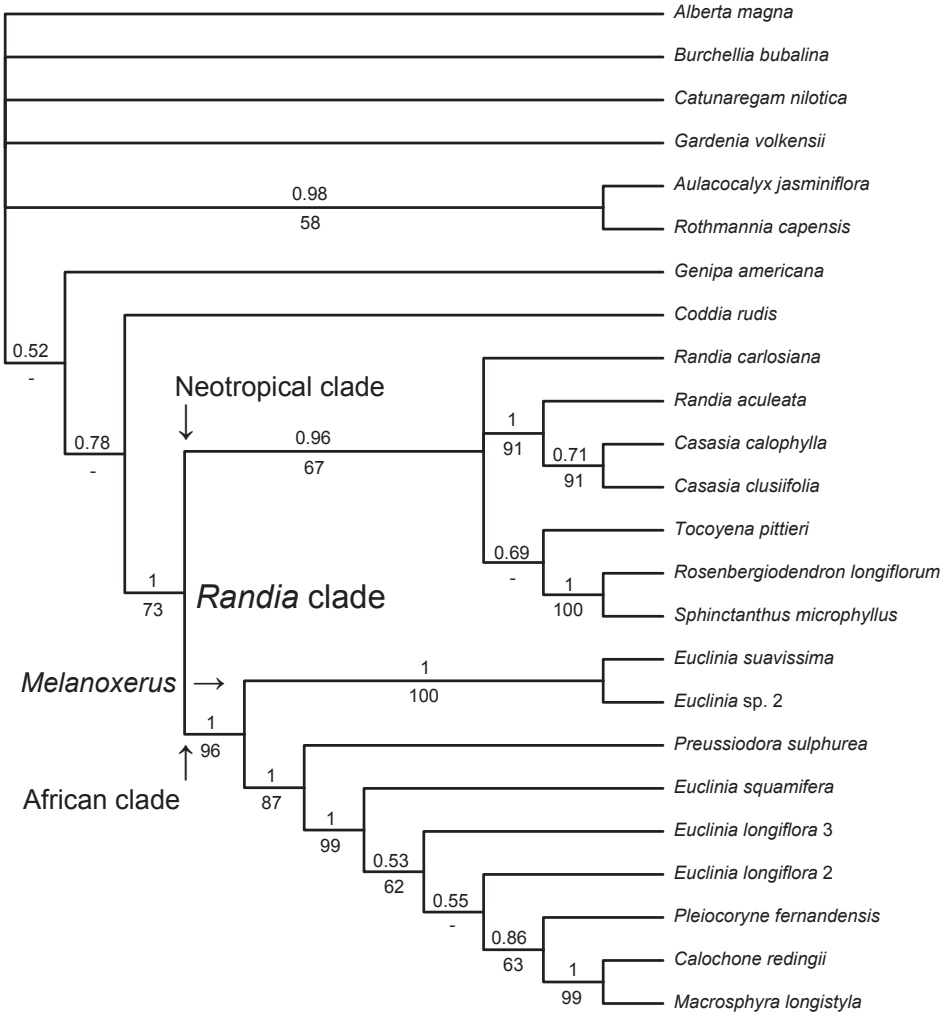


Fig. 2. Majority-rule consensus tree from the Bayesian MCMC analyses of ETS, with clade support values (posterior probabilities) indicated above, and bootstrap support values from the parsimony analysis below branches.

African genera. *Oligocodon* and *Pleiocoryne* are supported as sister groups by both datasets, but whereas this clade is resolved as sister group to the *Euclinia-Calochone-Macrosphyra* clade in the cpDNA tree, in the *Xdh* tree clade it is instead nested within this clade as sister group to *Macrosphyra* (which in the cpDNA and ETS trees appears closer to *Calochone*). Further research is needed in order to better understand relationships among these genera. Notably, both species of *Calochone* are represented in this study, and the genus is supported as monophyletic both by plastid DNA, and by *Xdh* (sequencing of the *C. acuminata* ETS was unsuccessful).

The Neotropical clade is strongly supported as monophyletic by ETS and *Xdh* data, but only weakly supported by the combined plastid data. Relationships within the clade are poorly resolved. This was also the result of the phylogenetic analyses of rDNA data (ITS, 5S-NTS) by Gustafsson & Persson (2002). The

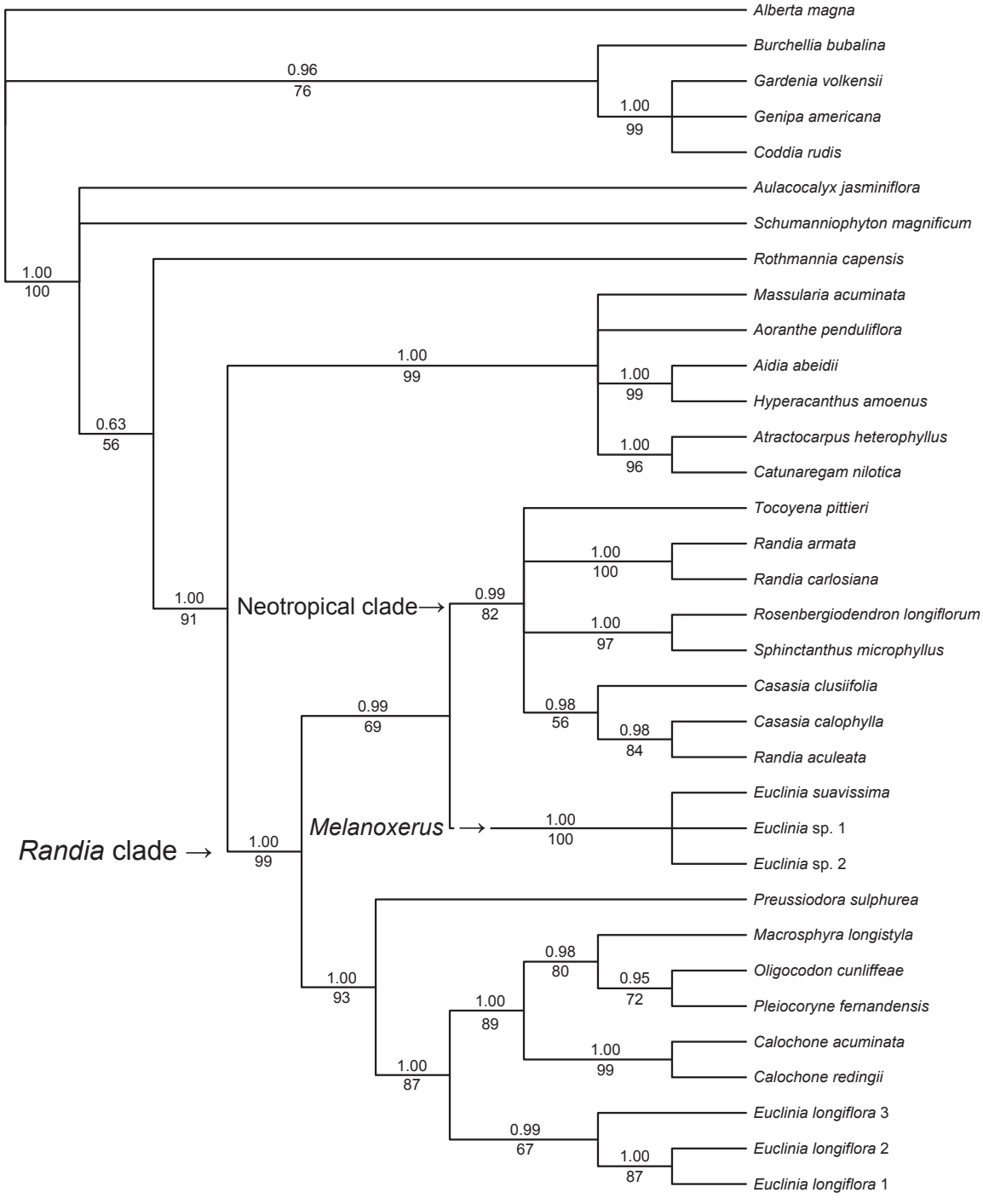
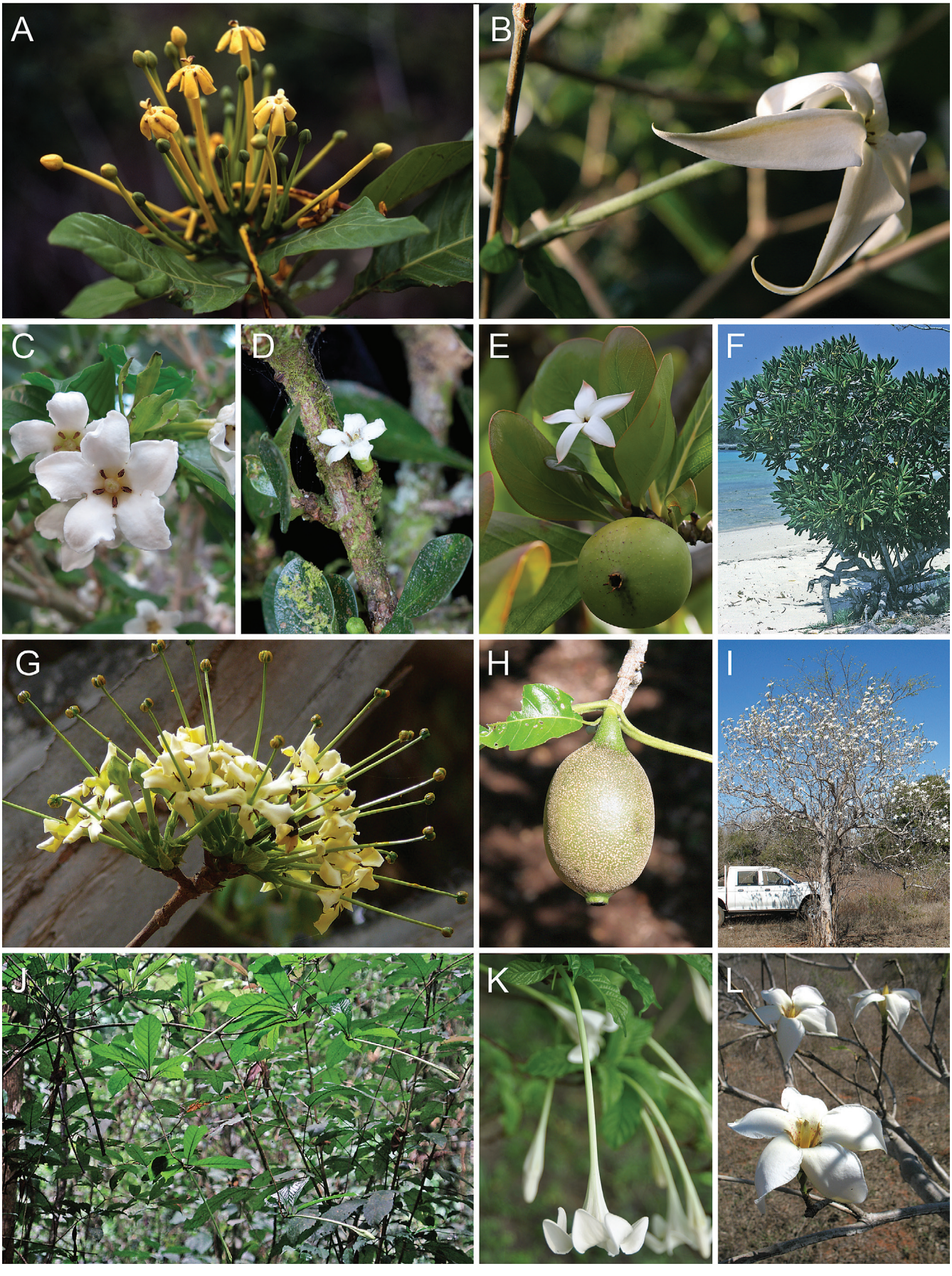


Fig. 3. Majority-rule consensus tree from the Bayesian MCMC analyses of *Xdh*, with clade support values (posterior probabilities) indicated above, and bootstrap support values from the parsimony analysis below branches.


A
B
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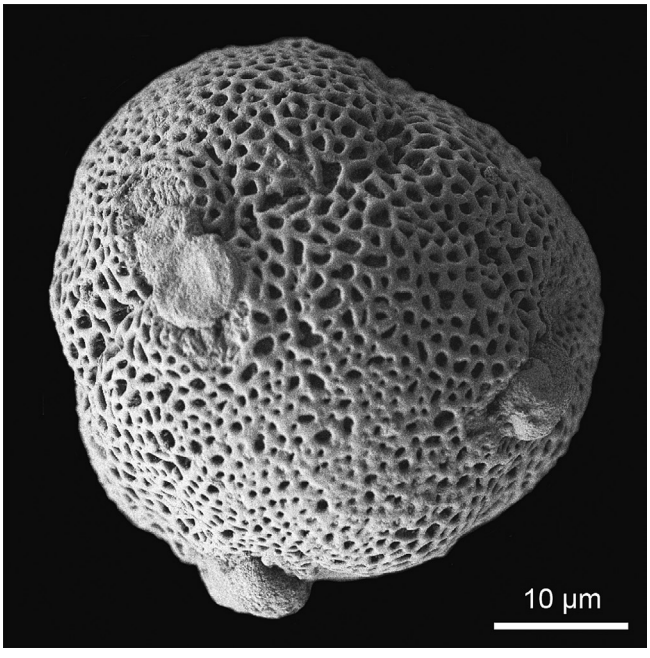

Fig. 5. Pollen tetrad of *Melanoxerus suavissius* (*Euclinia suavissima*) with apertures (pores) of individual grains aligned in pairs – *Phillipson 3055* (P), ×1600 magnification.

scarcity of molecular synapomorphies and consequently the difficulties in resolving relationships among the Neotropical taxa of the *Randia* clade could indicate that this lineage has undergone rapid radiation (cf. Wendel & Doyle, 1998). In relation to this, the assymmetry in species diversity within the *Randia* clade, where the number of Neotropical species is many times higher than those found in Africa (144 vs. 10; not including *Melanoxerus*; Govaerts & al., 2013), may be of interest. Although relationships among the Neotropical taxa are poorly resolved, our results indicate that *Casasia* and *Randia* are not monophyletic as presently circumscribed. The former is paraphyletic; in analyses of cpDNA and nDNA *Casasia calophylla* is more closely related to *Randia aculeata*, than to *C. clusiifolia*. Monophyly of *Casasia* was, however, supported in the parsimony analysis of ETS. *Randia* is consequently polyphyletic. This result indicates that the name *Casasia* may be better treated as a synonym of *Randia*. In fact, Grisebach (1866), stating that he could not find any characters for distinguishing these two genera, reduced *Casasia* to a synonym. However, Hooker (1873) did not agree and effectively reinstated the name, citing the distinctiveness of the venation and shape of the leaves (i.e., narrowly lanceolate leaves with striate venation; present in *C. calophylla*, *C. haitiensis* Urb. & Ekman, and *C. nigrescens* (Griseb.) C.Wright ex Urb., but not in the other species). The validity of maintaing the genus has subsequently been questioned by Lorence & Dwyer (1987).

Even if *Casasia* were to be considered a synonym of *Randia*, *Randia* is still not supported as monophyletic; a result congruent with that of Gustafsson & Persson (2002). In their analyses, which included a broad sampling of *Randia*, an informally named Central American clade (including *Randia aculeata*), and a South American clade (including *Randia armata* and *R. carlosiana*), formed a polytomy together with *Casasia*, and the *Rosenbergiodendron* clade. In our Bayesian analyses of cpDNA and nDNA, the *R. armata–R. carlosiana* clade is more closely related to the *Rosenbergiodendron* clade than to *Randia aculeata*, however, this relationship is weakly supported (and only resolved in the Bayesian analyses). The monophyly of *Randia* should be further investigated.

Notes on morphological variation within the *Randia* clade. — The taxa of the *Randia* clade are morphologically diverse. They form small shrubs, trees, or are more or less scandent. Notably, the African genera, i.e., *Calochone*, *Euclinia*, *Macrosphyra*, *Oligocodon*, *Pleiocoryne*, and *Preussiodora*, all have a more or less scandent habit (Hutchinson & Dalziel, 1931; Keay, 1958; Hallé, 1970; Bridson & Verdcourt, 1988). This character could consequently represent a potential synapomorphy for this clade. *Melanoxerus*, in contrast, forms trees, and the Neotropical genera of the *Randia* clade mostly form shrubs or small trees (although a few species of *Randia* are also scandent). Inflorescences are usually terminal in the *Randia* clade, and may consist of many (*Calochone*, *Macrosphyra*, *Pleiocoryne*, *Tocoyena*), few (*Oligocodon*, *Sphinctanthus*), or solitary flowers (*Euclinia*, *Melanoxerus*, *Rosenbergiodendron*). In dioecious *Randia* (and *Casasia*) the female flowers are usually solitary to few, and the male flowers few to many (Gustafsson & Persson, 2002; and references therein). There is much variation in flower morphology (Figs. 1, 4). Notably, extraordinarily long-tubular (sphingophilous) corollas appear to have evolved independently within several genera, e.g., *Euclinia longiflora* (Fig. 4K; to 24 cm; Hallé, 1970), *Tocoyena longiflora* Aubl. (to 26 cm; Silberbauer-Gottsberger & al., 1992), and *Rosenbergiodendron longiflorum* (Ruiz & Pav.) Fagerl. (Fig. 4B; to 30 cm; Gustafsson, 1998). The flowers are bisexual, except in *Randia* (most species; and *Casasia*), in which the flowers are unisexual and dioecious (Burger & Taylor, 1993; Lorence, 2012). Most genera of the *Randia* clade (including *Melanoxerus*; Fig. 5), have pollen dispersed in tetrads, except *Rosenbergiodendron*, *Sphinctanthus*, and *Tocoyena*, all of which disperse pollen in monads (Keay, 1958; Persson, 1993; Gustafson, 1998). Pollen tetrads may consequently represent a synapomophy for the *Randia* clade, in particular given the phylogenetic position of the *Rosenbergiodendron* clade inferred in this study (i.e., nested within the Neotropical clade). The fruits of the *Randia* clade are bicarpellate, with two parietal placentas, containing few to many, horizontally inserted, lenticular seeds, immersed in a fleshy pulp (fruits often appear unilocular as the expanding placentas as well as the septa may become uniformily fleshy;

◀ **Fig. 4.** Selected species of the *Randia* clade. **A,** *Tocoyena pittieri*; **B,** *Rosenbergiodendron longiflorum*; **C,** *Randia carlosiana*; **D,** *Randia aculeata*; **E–F,** *Casasia clusiifolia*; **G,** *Macrosphyra longistyla*; **H–I, L,** *Melanoxerus suavissimus* (*Euclinia suavissima*); **J–K,** *Euclinia longiflora*. — Image credits/copyrights: A, Johan Rova; B–C, H, Kent Kainulainen; D, Olga M. Montiel; E, Paul Rebmann, http://wildflphoto.com; F, J, Christian Puff; G, Andreas Tervort; I, L, Peter B. Phillipson; K, Edd Russell (D, I, and L, from Tropicos, botanical information system at the Missouri Botanical Garden, http://www.tropicos.org, used with permission; G, from Brunken & al., 2008–, used with permission).

Keay, 1958; Hallé, 1967; Robbrecht & Puff, 1986). The fruits, which often turn yellow or orange (or white) at maturity, are in many species conspicously lenticellate, and can range in size from less than one centimeter diameter in some species of *Randia* (Burger & Taylor, 1993; Lorence, 2012) to up to ten centimeters in diameter in *Tocoyena pittieri* (Standl.) Standl. (Dwyer, 1968). In many species of the *Randia* clade flowers, leaves, or fruit pulp turn blackish when drying.

As mentioned, a distinguishing character between *Euclinia* and *Melanoxerus* can be found in the habit. Whereas *Euclinia* are erect (treelets), scrambling, or more or less climbing shrubs with spindly branches (Fig. 4J; Hutchinson & Dalziel, 1931, as *Randia macrantha*; Hallé, 1970; Bridson & Verdcourt, 1988), *Melanoxerus* forms trees of up to 20 m height (Fig. 4I; Cavaco, 1967; or to 26 m; *Rajemisa 19*, MO, n.v.). These taxa also differ in characteristics of the bark, which is peeling (reddish-brown) in *Euclinia*, but smooth and flaking (pale gray) in *Melanoxerus* (Salisbury, 1808; Cavaco, 1967; Hallé, 1970). Both genera have terminal, solitary flowers that are white with a corolla throat with red to purple spots (apparently the corolla exterior of *Euclinia* can also be red-purplish-brown; Good, 1926; Hutchinson & Dalziel, 1931). Corollas are tubular to funnel-shaped in *Euclinia* and funnel-shaped to campanulate in *Melanoxerus*. The calyx lobes of *Euclinia* are persistent in fruit, whereas in *Melanoxerus* they are caducous, albeit the truncate calyx tube is frequently persistent in mature fruits (Fig. 4H). Furthermore, the fruits of *Melanoxerus* are covered with conspicous gray lenticells and turn olive-green to pale brown at maturity, whereas fruits of *Euclinia* are smooth, and (at least in *E. longiflora*) turn orange-yellow at maturity.

In summary, morphological characteristics for the distinction of *Euclinia* and *Melanoxerus* can be found in the habit (±scandent shrubs vs. trees), bark (peeling vs. smooth and flaking), and fruits (smooth with persistent calyx lobes vs. lenticellate with caducous calyx lobes). Finally, the two taxa also differ in ecology, as *Euclinia* appears restricted to the undergrowth of dense lowland or semi-deciduous rainforests (Hallé, 1967), whereas *Melanoxerus* grows in subarid, dry, or subhumid forests as a dominant tree (Schatz, 2001; K. Kainulainen, pers. obs.).

■ TAXONOMIC SYNOPSIS

In order to render *Euclinia* monophyletic, we propose that the Malagasy species *Euclinia suavissima* should be recognized at generic level, under the name *Melanoxerus* (derivation: μέλας, "black"; ξηρός, "dry"; leaf, flower, and fruit material of this genus usually dries black).

Melanoxerus Kainul. & B.Bremer, **gen. nov.** – Type: ***M. suavissimus*** (Homolle ex Cavaco) Kainul. & B.Bremer, **comb. nov.** ≡ *Gardenia suavissima* Homolle ex Cavaco in Adansonia, sér. 2, 7: 177. 1967 ≡ *Euclinia suavissima* (Homolle ex Cavaco) J.-F.Leroy in Adansonia, sér. 2, 14: 52. 1974 – Holotype: MADAGASCAR. Androy, Ambovombe, 27 Oct 1924, *Decary 3357* (P barcode P00852562 [image!]).

Deciduous trees. Leaves petiolate, glossy, ovate to obovate. Stipules triangular, caducous. Flowers bisexual, solitary, terminal. Calyx short-tubular; lobes acute, caducous. Corollas infundibular or campanulate; lobes contorted in bud, overlapping to the left. Styles swollen, ribbed, with secondary pollen presentation. Pollen dispersed in tetrads. Fruits fleshy, globose to ovoid, covered with many lenticells. Seeds lenticular, horizontally inserted and immersed in pulp.

Melanoxerus is endemic to Madagascar, and occurs in the subarid forests of the southwest, and in dry to subhumid forests of the north. Specimens from the southern and northern parts of Madagascar appear morphologically distinct in a number of characters, perhaps warranting recognition at species level.

■ ACKNOWLEDGEMENTS

We thank Claes Persson, Richard Razakamalala, and Johan Rova for providing plant material and DNA samples, and the curator of the Herbarium of the Muséum national d'Histoire naturelle (P), Paris, France, for material and images. Photographs were provided by Olga M. Montiel, Peter B. Phillipson, Christian Puff, Johan Rova, Paul Rebmann, Edd Russell, Andreas Tervort, the Missouri Botanical Garden, Forschungsinstitut Senckenberg, and the Smithsonian Tropical Research Institute. We also thank Kjell Jansson for assistance with the SEM, Anna Petri, Anbar Khodabandeh, and Stefan Löfstrand for laboratory assistance, and Sylvain Razafimandimbison and two anonymous reviewers for helpful comments on the manuscript. This work was supported by the Swedish Research Council (A0529602) and the Knut and Alice Wallenberg Foundation to BB.

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Appendix 1. Overview of taxon sampling, including GenBank accession numbers and voucher specimens for previously unpublished sequences (indicated with an asterisk).

Species, voucher specimen (herbarium), accession no.: ETS, *matK*, *rps16*, *rpl32*, *trnT-F*, *Xdh*

Aidia abeidii S.E.Dawson & Gereau, *Luke 8317* (UPS), –, KJ136866*, KJ136933*, KJ136902*, KJ136949*, KJ136964*; ***Alberta magna*** E.Mey., *Tonkin 200* (UPS), KJ136841*, KJ136865*, FM204701[1], KJ136901*, AJ620118[6], KJ136963*; ***Aoranthe penduliflora*** (K.Schum.) Somers, *Iversen & Steiner 86776* (UPS), –, KJ136867*, HM164191[2], KJ136903*, HM164307[2], KJ136965*; ***Atractocarpus heterophyllus*** (Montrouz.) Guillaumin & Beauvis., *Mouly 850* (P), –, KJ136868*, KJ136934*, KJ136904*, KJ136950*, KJ136966*; ***Aulacocalyx jasminiflora*** Hook.f., *Schmidt & al. 1672* (MO), KJ136842*, KJ136869*, EF205639[4], KJ136905*, EU817455[7], KJ136967*; ***Burchellia bubalina*** (L.f.) Sims, *Bremer 3129* (UPS), KJ136843*, KJ136870*, HM164198[2], KJ136906*, HM164314[2], KJ136968*; ***Calochone acuminata*** Keay, *De Forresta 999* (P), –, KJ136871*, KJ136935*, KJ136907*, KJ136951*, KJ136969*; ***Calochone redingii*** (De Wild.) Keay, *s.c. s.n.* 820315 [= coll. date?] (K), KJ136844*, KJ136872*, KJ136936*, KJ136908*, KF965172[9], KJ136970*; ***Casasia calophylla*** A.Rich., *Rova & al. 2259B* (S), KJ136845*, KJ136873*, KJ136937*, KJ136909*, KF965177[9], KJ136971*; ***Casasia clusiifolia*** (Jacq.) Urb., *Watson 544* (FTG), cult. FTG no. 67-859, KJ136846*, KJ136874*, KJ136938*, KJ136910*, KJ136952*, KJ136972*; ***Catunaregam nilotica*** (Stapf) Tirveng., *Luke 8336* (UPS), KJ136847*, KJ136875*, AM117289[3], KJ136911*, KJ136953*, KJ136973*; ***Coddia rudis*** (E.Mey. ex Harv.) Verdc., *Bremer 3764* (UPS), KJ136848*, KJ136876*, HM164202[2], KJ136912*, HM164317[2], KJ136974*; ***Euclinia longiflora*** Salisb. **1,** *De Block 27* (BR), –, KJ136877*, HM164206[2], KJ136913*, AJ847399[8], KJ136975*; ***Euclinia longiflora*** Salisb. **2,** *Jongkind & al. 1523* (UPS), KJ136849*, KJ136878*, KJ136939*, KJ136914*, KJ136954*, KJ136976*; ***Euclinia longiflora*** Salisb. **3,** *Kaji 84* (P), KJ136850*, KJ136879*, KJ136940*, –, KJ136955*, KJ136977*; ***Euclinia squamifera*** (R.D.Good) Keay, *Hallé 3609* (P), KJ136851*, KJ136880*, –, KJ136915*, –, –; ***Euclinia suavissima*** (Homolle ex Cavaco) J.-F.Leroy, *Razafimandimbison & Bremer 496* (UPS), KJ136856*, KJ136888*, KJ136945*, KJ136923*, KJ136959*, KJ136985*; ***Euclinia* sp. 1,** *Bremer & al. 5113* (S), KJ136855*, KJ136886*, KJ136943*, KJ136921*, KJ136957*, KJ136983*; ***Euclinia* sp. 2,** *Razakamalala RZK 6382* (TAN), –, KJ136887*, KJ136944*, KJ136922*, KJ136958*, KJ136984*; ***Gardenia volkensii*** K.Schum., *Luke 9043* (UPS), KJ136852*, KJ136881*, KJ136941*, KJ136916*, KJ136956*, KJ136978*; ***Genipa americana*** L., *Kiehn HBV sub RR-420* (WU), KJ136853*, KJ136882*, HM164209[2], KJ136917*, HM164322[2], KJ136979*; ***Hyperacanthus amoenus*** (Sims) Bridson, *Bremer & Bremer 3789* (UPS), –, KJ136883*, KJ136942*, KJ136918*, AM117364[3], KJ136980*; ***Macrosphyra longistyla*** (DC.) Hiern, *Ern 3120* (P), KJ136854*, KJ136884*, KJ186847*, KJ136919*, KF965221[9], KJ136981*; ***Massularia acuminata*** (G.Don) Bullock ex Hoyle, *De Block 57* (BR), –, KJ136885*, AF201005[5], KJ136920*, KF965224[9], KJ136982*; ***Morelia senegalensis*** A.Rich. ex DC., *Ern 2974* (P), –, KJ136889*, KF964962[9], KJ136924*, KF965229[9], –; ***Oligocodon cunliffeae*** (Wernham) Keay, *Coll. Unk. 348* (P), –, KJ136890*, AF201008[5], –, KF965232[9], KJ136986*; ***Pleiocoryne fernandensis*** (Hiern) Rauschert, *Hallé 204* (P), KJ136857*, KJ136891*, KF964974[9], –, KF965241[9], KJ136987*; ***Preussiodora sulphurea*** (K.Schum.) Keay, *De Foresta 1609* (P), KJ136858*, KJ136892*, KF964978[9], KJ136925*, KF965246[9], KJ136988*; ***Randia aculeata*** L., *Meagher 881* (FTG), cult. FTG no. 1-145, KJ136859*, KJ136893*, HM164221[2], KJ136926*, HM164334[2], KJ136989*; ***Randia armata*** (Sw.) DC., *Lorence 7953* (PTBG), –, KJ136894*, KJ136946*, KJ136927*, KJ136960*, KJ136990*; ***Randia carlosiana*** K.Krause, *Ståhl & Knudsen 1056* (GB), cult. Bergius Bot. Gard., KJ136860*, KJ136895*, KJ136947*, KJ136928*, KJ136961*, KJ136991*; ***Rosenbergiodendron longiflorum*** (Ruiz & Pav.) Fagerl., *Bremer 2740* (UPS), cult. Uppsala Bot. Garden, KJ136861*, KJ136896*, KJ136948*, KJ136929*, KJ136962*, KJ136992*; ***Rothmannia capensis*** Thunb., *Bremer & al. 4346* (S), KJ136862*, KJ136897*, AM117340[3], KJ136930*, AM117384[3], KJ136993*; ***Schumanniophyton magnificum*** (K.Schum.) Harms, *Sembé 64* (P), –, KJ136898*, KF964990[9], –, KF965258[9], KJ136994*; ***Sphinctanthus microphyllus*** K.Schum., *Persson & Gustafsson 353* (GB), KJ136863*, KJ136899*, AF201020[5], KJ136931*, KF965263[9], KJ136995*; ***Tocoyena pittieri*** (Standl.) Standl., *Rova & al. 2369* (GB), KJ136864*, KJ136900*, FM204738[1], KJ136932*, FM207145[1], KJ136996*.

Published sequences: 1, Kainulainen & al. (2009); 2, Lantz & Bremer (2004); 3, Kainulainen & al. (2013); 4, Mouly & al. (2007); 5, Mouly & al. (2009); 6, Bremer & Eriksson (2009); 7, Alejandro & al. (2005); 8, Persson (2000); 9, Mouly & al. (2014).