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Historical biogeography of the coffee family (Rubiaceae, Gentianales) in Madagascar: case studies from the tribes Knoxieae, Naucleaeae, Paederieae and Vanguerieae

N. Wikström*, M. Avino, S. G. Razafimandimbison and B. Bremer

Bergius Foundation, Royal Swedish Academy of Sciences and Department of Botany, Stockholm University, SE-10691 Stockholm, Sweden

ABSTRACT

Aim In Madagascar the family Rubiaceae includes an estimated 650 species representing 95 genera. As many as 98% of the species and 30% of the genera are endemic. Several factors make the Rubiaceae a model system for developing an understanding of the origins of the Malagasy flora. Ancestral area distributions are explicitly reconstructed for four tribes (Knoxieae, Naucleaeae, Paederieae and Vanguerieae) with the aim of understanding how many times, and from where, these groups have originated in Madagascar.

Location Indian Ocean Basin, with a focus on Madagascar.

Methods Bayesian phylogenetic analyses are conducted on the four tribes. The results are used for reconstructing ancestral areas using dispersal–vicariance analyses. Phylogenetic uncertainties in the reconstructions are accounted for by conducting all analyses on the posterior distribution from the analyses.

Results Altogether, 11 arrivals in Madagascar (one in Paederieae, five in Knoxieae, three in Vanguerieae, and two in Naucleaeae) are reconstructed. The most common pattern is a dispersal event (followed by vicariance) from Eastern Tropical Africa. The Naucleaeae and Paederieae in Madagascar differ and originate from Asia. Numerous out-of-Madagascar dispersals, mainly in the dioecious Vanguerieae, are reconstructed.

Main conclusions The four tribes arrived several times in Madagascar via dispersal events from Eastern Tropical Africa, Southern Africa and Tropical Asia. The presence of monophyletic groups that include a number of species only found in Madagascar indicates that much endemism in the tribes results from speciation events occurring well after their arrival in Madagascar. Madagascar is the source of origin for almost all Rubiaceae found on the neighbouring islands of the Comoros, Mascarenes and Seychelles.

Keywords

Dispersal–vicariance analysis, historical biogeography, Knoxieae, long-distance dispersal, Madagascar, Naucleaeae, Paederieae, Rubiaceae, Vanguerieae.

*Correspondence: N. Wikström, Bergius Foundation, Royal Swedish Academy of Sciences and Department of Botany, Stockholm University, SE-10691 Stockholm, Sweden.
E-mail: niklas.wikstrom@bergianska.se

INTRODUCTION

The flora of Madagascar displays an almost unparalleled level of endemism and species diversity, and the island has been designated one of the world's most important biodiversity hotspots (Myers *et al.*, 2000; Groombridge & Jenkins, 2002). Although estimates indicate that no more than *c.* 10% of

primary vegetation remains on the island, counts of vascular plant species richness still indicate an impressive 10,000–12,000 species (Schatz *et al.*, 1996; Goodman & Benstead, 2003), 85–95% of which are endemic (Schatz, 2000; Goodman & Benstead, 2005). This puts Madagascar in the focus of conservation biologists, but from an evolutionary biology perspective we should also try to understand the underlying

mechanisms resulting in, and maintaining, Madagascar's outstanding diversity.

Interpretation of the origin of Madagascar's flora has changed several times over the years (see Yoder & Nowak, 2006, for a review), and there has been disagreement about the relative importance of different mechanisms of origin. Until recently, the dominant emphasis was on Gondwanan vicariance (Raven & Axelrod, 1974; Leroy, 1978; Grubb, 2003). High levels of both endemism and species richness in different groups of organisms were seen to indicate long and independent histories of these groups, and this was taken as support for a Gondwanan vicariance pattern (Leroy, 1978; Grubb, 2003). More recently, an increasing emphasis has been put onto oceanic dispersal as a mechanism of origin for elements of the Malagasy flora (Schatz, 1996; Yoder & Nowak, 2006). This partly results from a growing understanding that many plant groups with large Malagasy elements are too young to be affected by the break-up of Gondwana (Crane *et al.*, 1995; Magallón *et al.*, 1999; Wikström *et al.*, 2001), but also from a resurrection of dispersal as an important mechanism in historical biogeography (de Queiroz, 2005). Notwithstanding the controversy about mechanisms of origin, it is clear that much diversification has occurred on the island (Gautier & Goodman, 2003). Considerable diversity in ecological communities and extensive variation in soil types, topography and precipitation across the island, both now and in the past, have contributed to this diversification (Gautier & Goodman, 2003; Wells, 2003). However, a comprehensive hypothesis for the origin and evolution of the diversity and endemism seen in Madagascar has yet to be developed, and there are a number of questions that need to be addressed. From a biogeographical point of view we should resolve how, and from where, individual floral elements originated, and to what extent there are common biogeographical patterns on the island itself. This last question has rarely been addressed, but bears directly on the mechanistic diversification model developed for Madagascar's microendemic biota by Wilmé *et al.* (2006). We also need to infer at what geological times different floral elements arrived in Madagascar, if there are common temporal patterns across different groups, and if alternative temporal patterns can be associated with different vegetation types found on the island today.

Many elements of the Malagasy flora show affinities with continental Africa, especially with the Sudanian and Zambezian phytogeographical regions (*sensu* White, 1993). However, there are other affinities indicating a more complex history (Robbrecht, 1996; Gautier & Goodman, 2003). A large pantropical element, for example, is present, and there are elements, especially from eastern parts of the island, showing affinities with India, Australia and Malesia (Schatz, 1996). Analysing these oriental elements, Schatz (1996) identified three different vicariance/dispersal patterns depending on the inferred age of taxa: Cretaceous Gondwanan relicts, taxa showing an Eocene–Oligocene 'Lemurian stepping-stone' pattern, and more recent long-distance dispersals. The 'Lemurian stepping-stone' pattern refers to a distributional pattern

including Africa/Madagascar, the Seychelles, India/Sri Lanka and western Malesia (Schatz, 1996). Until recently, inferences about the origins and affinities of the Malagasy flora have been based on a taxonomic approach, focusing on taxa (species, genera, families) shared between geographical regions (Leroy, 1978; Robbrecht, 1996; Schatz, 1996). There have been few attempts to explicitly reconstruct from where, and at what times, different groups have arrived in Madagascar (Renner, 2004; Yuan *et al.*, 2005; Yoder & Nowak, 2006; Koopman & Baum, 2008; Micheneau *et al.*, 2008). Building a detailed and more comprehensive understanding of the origins of the Malagasy flora will require such reconstructions.

The coffee family (Rubiaceae) is essentially tropical and comprises an estimated 13,000 species in 600–700 genera, with *c.* 650 species and 95 genera found in Madagascar (Davis & Bridson, 2003b; Goodman & Benstead, 2005). Rubiaceae separated from remaining families in the Gentianales during the Late Cretaceous, *c.* 78 Ma (Bremer *et al.*, 2004), and this places any origin of Rubiaceae in Madagascar well after the final break-up of Gondwana (Storey *et al.*, 1995). Following recent tribal and subfamily classifications of the family (Bremer, 2009), Malagasy representatives are scattered across 30 tribes and all three subfamilies (Davis & Bridson, 2003b), and it is clear that there have been numerous introductions. Today, most species are found in evergreen humid forests, but the family occurs in almost every type of primary vegetation of the island, and is also well represented in dry deciduous forests in the west, south and north (Davis & Bridson, 2003b). The numerous introductions, their occurrence in various vegetation types of Madagascar and the large number of species found on the island make Rubiaceae a model system for developing a more comprehensive understanding of the origins of the Malagasy flora. In recent years, considerable effort has been put into constructing a phylogenetic framework for the family (see Bremer, 2009, for a review), and we have estimates for the origins of most tribes in the family (Bremer & Eriksson, 2009). Building on these efforts we initiate a more detailed analysis of the geographic origins of Rubiaceae in Madagascar through explicit reconstructions of ancestral area distributions using dispersal–vicariance analyses of four tribes of the family.

MATERIALS AND METHODS

Taxon sample

Groups of Rubiaceae for which phylogenetic analyses have been published were targeted and used in the present analyses. In total, data for four tribes were assembled. Paederieae (Backlund *et al.*, 2007) and Knoxieae (Kårehed & Bremer, 2007) from the subfamily Rubioideae, Vanguerieae (Lantz *et al.*, 2002; Lantz & Bremer, 2004, 2005; Razafimandimbison *et al.*, 2009) from the Ixoroideae, and Naucleaeae (Razafimandimbison & Bremer, 2001, 2002) from the Cinchonoideae. Data from these studies were complemented by additional sequence data and an extended taxon sample focusing on

representatives from Madagascar. The number of species included per genus/group and areas represented in the analyses are summarized in Table 1. Species names, GenBank accession numbers and voucher information for sequences published here are given for all included accessions in Appendix S1 in Supporting Information.

The tribe Paederieae s. str. is pantropical and includes *Paederia* (c. 30 species) and three Asian genera: *Spermadictyon* (1 species), *Leptodermis* (30 species) and *Serissa* (2 species) (Backlund *et al.*, 2007). Only the genus *Paederia* occurs in Madagascar, with 11 species (De Block, 2003; Govaerts *et al.*, 2008). Eight of these plus three unidentified accessions from Madagascar were included in the analyses. Recent analyses by Rydin *et al.* (2009) resolved *Saprosma* as sister to Paederieae (*sensu* Backlund *et al.*, 2007), with good support. Two species of *Saprosma* were therefore included in the analyses and used as outgroups in the Paederieae analyses. The tribe Knoxieae occurs in Africa, Madagascar, Arabia and Indomalaysia, and comprises 15 genera and more than 100 species (Kårehed & Bremer, 2007). All five genera (*Carphalea*, *Otiophora*, *Pentania*, *Phyllopentas*, *Triainolepis*) and 18 of the 25 species that are recognized by Govaerts *et al.* (2008) and occur in Madagascar were included in the analyses. Five unidentified accessions collected in Madagascar were also included. Vanguerieae is the most species-rich tribe in Madagascar and is represented in almost all naturally occurring forest types of the island (Davis & Bridson, 2003a). The group has a palaeotropical distribution and includes between 20 and 27 genera (Robbrecht, 1988; Verdcourt & Bridson, 1991; Lantz & Bremer, 2004) and 600–700 species (Razafimandimbison *et al.*, 2009). Six genera and up to 100 species are found in Madagascar. Twenty-six of the recognized Malagasy species, representing all but one of the genera (*Psydrax*), were included in the analyses. An additional 24 unidentified accessions from Madagascar were also included. The tribe Naucleaeae is a pantropical group including c. 27 genera and 180 species (Razafimandimbison, 2003). Most species occur in Asia (more than 120 species) and c. 24 species in five genera are found in Madagascar. Altogether 11 species from Madagascar, representing all five genera, were included in the analyses.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from silica dried material and/or herbarium specimens using a standard cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987), combined with the QIAquick® PCR cleaning kit (Qiagen, Hilden, Germany) following the protocol specified by the manufacturer. Amplification and sequencing of included regions were carried out using primers listed in Table 2. Amplification reactions were run on an Eppendorf® Mastercycler® gradient (Bergman & Beving Instrument, Stockholm, Sweden). Reactions were carried out in 50- μ L aliquots including: 5 μ L 10 \times Paq5000® reaction buffer, 5 μ L tetramethylammonium chloride (TMACl; Chevet *et al.*, 1995), 0.4 μ L 100 mM dNTP mix, 0.5 μ L Paq5000® DNA polymerase

(5 U μ L⁻¹), 0.5 μ L of each primer (20 μ M), 0.5 μ L bovine serum albumin (BSA; 1%), 1 μ L DNA template, and sterilized water. Amplification of the external transcribed spacer region (ETS) included the following steps: initial denaturation at 97 °C for 1 min; 40 cycles each of 97 °C for 10 s, 55 °C for 30 s and 72 °C for 20 s; and a final extension at 72 °C for 7 min. Amplification for the other markers included: initial denaturation at 95 °C for 2 min; 35 cycles each of 95 °C for 30 s, 55 °C for 1 min and 72 °C for 1.5 min; and a final extension at 72 °C for 7 min. Polymerase chain reaction (PCR) products were cleaned using the MultiScreen® Separations System (Millipore, Billerica, MA, USA), sequenced with the BigDye® terminator cycle sequencing kit and analysed on an ABI PRISM® 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The resulting sequences were assembled using the Staden package (Staden, 1996).

Phylogenetic analyses

New sequences were added to previously published data sets and aligned by eye. Three or four plastid and/or nuclear regions were included in each combined data set: Paederieae (*rbcL*, *rps16*, *trnT-F*); Knoxieae [*rps16*, *trnT-F*, internal transcribed spacer region (ITS)]; Vanguerieae (*trnT-F*, ITS, ETS); and Naucleaeae (*rbcL*, *trnT-F*, ITS, ETS). Phylogenetic analyses were conducted on the combined data sets for each group. We used Markov chain Monte Carlo (MCMC) methods (Larget & Simon, 1999) within a Bayesian framework to approximate the posterior distribution of trees using MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003). In the MCMC the data were partitioned and each region was allowed partition-specific parameters (Ronquist & Huelsenbeck, 2003; Nylander *et al.*, 2004). The nucleotide substitution model for each partition was selected based on the Akaike information criterion as calculated using MRAIC v.1.4.3 and PHYML v.2.4 (Guindon & Gascuel, 2003; Nylander, 2004). A summary of analysed data, giving details of included regions, selected substitution models and total number of characters in the alignments, is given in Table 3. For each tribe, two parallel chains were run for 10 million generations and sampled every 1000th generation. Convergence of the individual chains was confirmed by inspecting the average standard deviation of split frequencies, and the last 5000 generations in each run (representing the stationary phase of the chains) were pooled to obtain a final approximation of the posterior distribution of trees and parameter estimates.

Biogeographic analyses

Fourteen areas of geographical distribution were defined and used in the analysis (Table 4), and follow the Continental, Regional and Level 3 area schemes defined in the second edition of *World geographic scheme for recording plant distributions* (Brummit, 2001). Individual taxon distributions were obtained from the *World checklist of Rubiaceae* (Govaerts *et al.*, 2008). In a few cases a species name could not be assigned to

Table 1 Sampling density, with respect to number of species included and areas represented in the analyses, indicated for each of the four tribes of the Rubiaceae analysed. Unless stated otherwise, the number of species in each genus/group and areas in which they occur were obtained from the list of accepted names given by Govaerts *et al.* (2008). The number of species included in analyses indicates how many of those accepted names were included, but also if additional accessions were included (number given in parentheses) in the analyses. Area codes correspond to those defined in Table 4.

Tribe	Genus/group	Number of species in genus/group	Areas in which genus/group occurs	Number of species included in analyses	Areas represented in analyses
Paederieae	<i>Paederia</i>	30	b,d,e,f,g,h,l,m	16 (+3)	b,d,f,g,h,m
	<i>Leptodermis</i>	30	f,h	1	f
	<i>Serissa</i>	2	f	1	f
	<i>Spermadictyon</i>	1	f,h	1	f,h
Knoxieae	<i>Batopedina</i>	3	a,e	1	a
	<i>Carphalea</i> ¹	6	d	5	d
	<i>Chamaepentas</i>	6	a,b,e	4	a,b,e
	<i>Dirichletia</i>	5	b,e	4	b,e
	<i>Dolichopentas</i>	4	a,b,e	3	a,b,e
	<i>Knoxia</i>	13	a,f,h,n	4	a,f,h,n
	<i>Otiophora</i>	18	a,b,d,e	13 (+2)	a,b,d,e
	<i>Otomeria</i>	8	a,b,e	4	a,b,e
	<i>Parapentas</i>	3	a,b,e	3	a,b,e
	<i>Pentansia</i>	19	a,b,d,e	17	a,b,d,e
	<i>Pentas</i>	16	a,b,d,e,f,g	11	a,b,d,e,f,g
	<i>Phyllopentas</i>	13	a,b,d,e	11	a,b,d,e
	<i>Rhodopentas</i>	2	a,b,e	2	a,b,e
	<i>Triainolepis</i>	13	b,d,e,g,i	7 (+4)	b,d,e,g
Vanguerieae	<i>Afrocanthium</i>	17	a,b,e	8	a,b,e
	<i>Bullockia</i>	c. 8 ²	b,d,e	4 (+1)	b,d,e
	Spiny group ³	c. 45 ⁴	a,b,e,g,f,h	10	a,b,g,e,h
	' <i>Canthium</i> ' ⁵	c. 75	a,b,f,h,n	0	–
	<i>Cuviera</i>	23	a,b,e	1	a,e
	<i>Cyclophyllum</i>	38	h,n	2	h,n
	<i>Fadogia</i> – <i>Rytigynia</i> group	122 ⁶	a,b,d,e	31 (+6)	a,b,d,e
	<i>Keetia</i>	32	a,b,e	6	a,b,e
	<i>Multidentia</i>	9	a,b,e	4	a,b,e
	<i>Peponidium</i>	c. 45 ²	d,g,j	12 (+12)	d,g,h ⁷ ,j
	<i>Psydrax</i>	81	a,b,d,e,f,h,n	11 (+1)	a,b,e,f,h,n
	<i>Pygmaeothamnus</i>	2	a,b,e	2 (+1)	a,b,e
	<i>Pyrostria</i>	c. 80 ²	b,d,e,f,g,i,j	21 (+9)	b,d,e,f,g,i,j
	<i>Robynsia</i>	1	a	1	a
	<i>Vangueria</i>	61	a,b,d,e	26	a,b,d,e
	<i>Vangueriopsis</i>	3 ⁸	a,b	1	b
Naucleaeae	<i>Adina</i> ¹	3	f,h	2	f,h
	<i>Adinauclea</i>	1	h	1	h
	<i>Breonadia</i>	1	a,b,d,e,f	1	a,b,d,e,f
	<i>Breonia</i> ¹	20	d	7	d
	<i>Burttdavaya</i>	1	b,e	1	b,e
	<i>Cephalanthus</i>	6	b,e,f,h,l,m	3	b,e,l,m
	<i>Corynanthe</i>	1	a,e	1	a,e
	<i>Gyrostipula</i>	3	d,g	2	d,g
	<i>Haldina</i>	1	f,h	1	f,h
	<i>Janotia</i>	1	d	1	d
	<i>Ludekia</i>	2	h	1	h
	<i>Metadina</i>	1	h	1	h
	<i>Mitragyna</i>	8	a,b,e,f,h	3 (+2)	a,b,e,f,h
	<i>Myrmeconauclea</i>	4	h	1	h
	<i>Nauclea</i>	10	a,b,f,h,n	2	a,b,h,n
	<i>Neolamarckia</i>	2	h	1	h
	<i>Neonauclea</i>	68	f,h,n	3	h,n

Table 1 Continued

Tribe	Genus/group	Number of species in genus/group	Areas in which genus/group occurs	Number of species included in analyses	Areas represented in analyses
	<i>Ochreinauclea</i>	2	h	0	–
	<i>Pausinystalia</i> ¹	5	a,e	2	a
	<i>Pertusadina</i> ¹	4	f,h	3	f,h
	<i>Pseudocinchona</i>	2	a	2	a
	<i>Sarcocephalus</i>	2	a,b,e	1	a,b
	<i>Sinoadina</i>	1	f,h	1	f,h
	<i>Uncaria</i>	39	a,b,d,e,f,g,h,n,m	4	a,b,d,e,f,g,h,m

¹Genus is not resolved as monophyletic in the analyses.

²Species count from Razafimandimbison *et al.* (2009).

³The ‘spiny group’ *sensu* Lantz *et al.* (2002) corresponds to *Canthium* s. str. *sensu* Lantz & Bremer (2004).

⁴Species count is a rough estimation. Inclusiveness of the group is based on the phylogenetic analyses presented here and on Lantz & Bremer (2004) and Bridson (1987, 1992).

⁵*Canthium* as currently delimited in Govaerts *et al.* (2008) is a highly unnatural group. Some species are part of the ‘spiny group’ *sensu* Lantz & Bremer (2004), other are more closely related to *Cyclophyllum* (Razafimandimbison *et al.*, 2009), but many species, mainly from Asia, have never been properly investigated for their relationships. Our present species count is an approximation based on accepted *Canthium* species in Govaerts *et al.* (2008) minus species that are included either in the ‘spiny group’, in *Peponidium*, or in *Bullockia*.

⁶*Rytigynia mrimaensis* and *Rytigynia bygoyensis* are counted in the ‘spiny group’ and not here.

⁷A *Canthium* accession from New Guinea is resolved within *Peponidium* based on its external transcribed spacer region (ETS) sequence. This should be verified by additional sequence data but if correct would expand the distribution of *Peponidium* to also include Asia.

⁸*Vangueriopsis lanciflora* was excluded from the count and instead included in the count of *Vangueria*.

Table 2 Primers used for amplification and sequencing of new sequences in this study.

Region	Primer	Primer sequence from the 5′ end	Reference
rbcL	<i>rbcL_5′F</i>	ATG TCA CCA CAA ACA GAA ACT AAA GC	Bremer <i>et al.</i> (2002)
	<i>rbcL_bs427F</i>	GCT TAT ATT AAA ACC TTC CAA GGC CCG CC	Bremer <i>et al.</i> (2002)
	<i>rbcL_3′R</i>	CTT TTA GTA AAA GAT TGG GCC GAG	Bremer <i>et al.</i> (2002)
	<i>rbcL_Z895R</i>	ACC ATG ATT CTT CTG CCT ATC AAT AAC TGC	G. Zurawski, DNAX Research Institute (pers. comm.)
rps16	<i>rps16_F</i>	GTG GTA GAA AGC AAC GTG CGA CTT	Oxelman <i>et al.</i> (1997)
	<i>rps16_R2</i>	TCG GGA TCG AAC ATC AAT TGC AAC	Oxelman <i>et al.</i> (1997)
trnT–F	<i>trnT–F_a1F</i>	ACA AAT GCG ATG CTC TAA CC	Bremer <i>et al.</i> (2002)
	<i>trnT–F_cF</i>	CGA AAT CGG TAG ACG CTA CG	Taberlet <i>et al.</i> (1991)
	<i>trnT–F_iR</i>	CCA ACT CCA TTT GTT AGA AC	Bremer <i>et al.</i> (2002)
	<i>trnT–F_fR</i>	ATT TGA ACT GGT GAG ACG AG	Taberlet <i>et al.</i> (1991)
ITS	ITS_P17	CTA CCG ATT GAA TGG TCC GGT GAA	Popp & Oxelman (2001)
	ITS_P25	GGG TAG TCC CGC CTG ACC TG	Popp & Oxelman (2001)
	26S_82R	TCC CGG TTC GCT CGC CGT TAC TA	Popp & Oxelman (2001)
ETS	18S-E	GCA GGA TCA ACC AGG TAG CA	Baldwin & Markos (1998)
	ETS-HL	GAT CAC AGC CTG AGC GGT G	Razafimandimbison <i>et al.</i> (2009)
	ETS-Erit-F	CTT GTA TGG GTT GGT TGG A	Negrón-Ortiz & Watson (2002)

ITS, internal transcribed spacer region; ETS, external transcribed spacer region.

the accession. Distributions were in those cases scored based on the locality of the voucher specimen. The focus of the biogeographic analyses has been taxa from Madagascar and surrounding islands. Continental, Regional and Level 3 areas of Brummit (2001) were therefore generalized (merged and split) taking this into consideration. Scorings of distribution for all individual taxa are shown next to the taxon names in Figs 1–4.

Ancestral areas were reconstructed using dispersal–vicariance analysis (Ronquist, 1997), as implemented in *diva* v.1.2

(Ronquist, 2001). Recent developments of parametric methods for reconstructing ancestral distributions (Ree *et al.*, 2005; Ree & Smith, 2008; Sanmartín *et al.*, 2008) incorporate explicit models of geographic range evolution, and these can be seen as extensions or improvements of the ‘event-based’ approach to biogeography adopted by Ronquist (1997) and implemented in *diva* (Ronquist, 2001). Unlike dispersal–vicariance analysis, parametric approaches allow for stochastic events to be accounted for and time can be included in models of

Table 3 Data sets used in the phylogenetic analyses of Paederieae, Knoxieae, Vanguerieae, and Naucleaeae. The table indicates included markers, total number of characters in the alignments, and nucleotide substitution model (selected based on the Akaike information criterion as calculated by MRAIC v.1.4.2), for each marker in the data sets.

Data set	Markers included	Number of aligned characters	Substitution model selected
Paederieae	<i>rbcL</i>	1433	HKY + I + Γ
	<i>rps16</i>	867	GTR + Γ
	<i>trnT-F</i>	1855	GTR
Knoxieae	<i>rps16</i>	2042	GTR + Γ
	<i>trnT-F</i>	3204	GTR + Γ
	ITS	1314	GTR + Γ
Vanguerieae	<i>trnT-F</i>	2207	GTR + Γ
	ITS	878	GTR + I + Γ
	ETS	505	HKY + Γ
Naucleaeae	<i>rbcL</i>	1415	GTR + I + Γ
	<i>trnT-F</i>	1942	GTR + I + Γ
	ITS	622	GTR + Γ
	ETS	436	GTR + Γ

ITS, internal transcribed spacer region; ETS, external transcribed spacer region.

geographic range evolution. They also provide a statistical framework for evaluating the likelihood of alternative hypotheses (Ree & Sanmartín, 2009). However, this is associated with an increase in computational complexity, and analyses including a large number of taxa and areas are not feasible, especially if topological uncertainty is to be accounted for (Ree & Sanmartín, 2009).

A common problem in DIVA analyses is that the ancestral area optimizations are less reliable towards the root node (Ronquist, 1996), and that the root node distribution often

includes most areas occupied by the terminals. To avoid this one can include additional outgroups in the analyses. Alternatively, the analyses can be constrained by fixing the maximum number of allowed areas (maxareas) in the ancestral distributions (Ronquist, 1996). Assuming that the dispersal abilities of the ancestors were equivalent to those of their present-day descendants, one can justify setting maxareas equal to the number of areas seen in the most widespread extant representatives (Sanmartín, 2003; Nylander *et al.*, 2008). In Knoxieae and Naucleaeae the most widespread taxa occur in five areas, in Vanguerieae they occur in four areas, and in Paederieae they are found in no more than two areas and maxareas for each tribe was set to these numbers in our full analyses, i.e. the analyses of the entire posterior distribution of trees (see below). To evaluate the effect of enforcing these constraints, two additional sets of analyses were conducted, one employing no maximum area constraint and one constraining the number of areas in the ancestral distributions even more by setting maxareas to 2 for all tribes. These second sets of analyses were not run on the entire posterior distribution of trees. Instead they were run on a sample of trees (100 trees) randomly drawn from the posterior distributions of each tribe.

Phylogenetic uncertainty in the biogeographic analyses was accounted for by conducting the analyses on all 10,000 trees from the posterior distributions of the phylogenetic analysis. Frequencies of ancestral areas of clades were recorded and plotted as marginal distributions on the 50% majority rule consensus trees resulting from each of the phylogenetic analyses. If multiple ancestral areas were reconstructed for a clade in any single tree (e.g. a, b, ab), each possibility was recorded as a fraction (a:1/3, b:1/3, ab:1/3). The marginal distributions for alternative reconstructions at each node in the trees are therefore the product of the phylogenetic uncertainty (in the rest of the tree) and the mapping uncertainty in the

Table 4 Areas of distribution used in the biogeographic analysis of the Rubiaceae. Area definitions (names and numbers in column 3) follow those defined in the second edition of the *World geographic scheme for recording plant distributions* (Brummit, 2001).

Area	Name	Including area names and numbers as defined by Brummit (2001)
a	Western Tropical Africa	West Tropical Africa (22) + West Central Tropical Africa (23)
b	Eastern Tropical Africa	Northeast Tropical Africa (24) + East Tropical Africa (25)
c	Northern Africa	Northern Africa (20) + Macaronesia (21)
d	Madagascar	Madagascar (29 MDG)
e	Southern Africa	Southern Africa (27) + South Tropical Africa (26)
f	Temperate Asia	Asia Temperate (30–39)
g	Comoros	Comoros (29 COM)
h	Tropical Asia	Asia Tropical (40–49)
i	West Indian Ocean	West Indian Ocean (29 excluding 29 MDG, 29 COM, 29 SEY)
j	Seychelles	Seychelles (29 SEY)
k	Europe	Europe (10–19)
l	North America	Northern America (70–79)
m	South America	Southern America (80–89)
n	Australasia	Australasia (50–59) + Pacific (60–69)

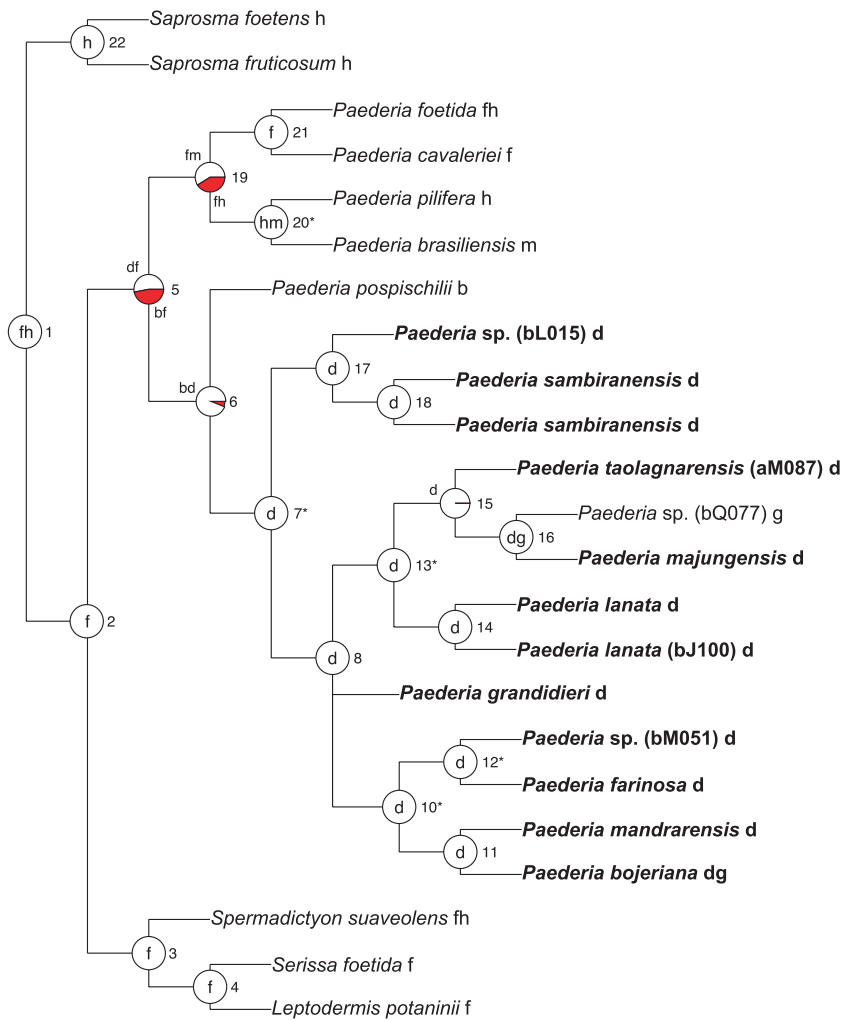


Figure 1 A summary of the dispersal–vicariance analyses for Paederieae. The tree is a 50% majority rule consensus tree of a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined data set of plastid *rbcL*, *rps16* and *trnT–F* DNA sequences. Pie charts at internal nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal–vicariance analysis (DIVA) while integrating over tree topologies using MCMC. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty (multiple equally parsimonious reconstructions) at each node, conditional on the node occurring. In the pie charts, areas are coloured according to their relative probability in the following order: white > red. Node numbers are given for each node and details of the reconstructed distributions are given in Appendix S2. An asterisk (*) in association with the node number indicates that the node is not well supported in the phylogenetic analyses (< 95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names following the one-letter codes of biogeographical regions given in Table 4. Taxa occurring on Madagascar are written in boldface.

biogeographic reconstruction (multiple equally parsimonious reconstructions), conditional on the node occurring.

RESULTS

Relationships indicated by the phylogenetic analyses are summarized as 50% majority rule consensus trees (Figs 1–4). Nodes with posterior probability values equal to or > 0.95 (e.g. the node appears in at least 95% of the sampled trees), are considered well supported (Alfaro et al., 2003). Details of Bayesian posterior probability values (BPP) for all nodes are given in Appendix S2. The relationships obtained generally agree with those obtained in previous analyses (Razafimanambison & Bremer, 2001, 2002; Lantz et al., 2002; Lantz & Bremer, 2004, 2005; Backlund et al., 2007; Kårehed & Bremer, 2007; Razafimanambison et al., 2009).

Ancestral area reconstructions are reported in the form of pie charts (Figs 1–4), representing marginal distributions for alternative reconstructions at each node in the trees. In Paederieae (Fig. 1) there is a single arrival in Madagascar, inferred as a vicariance event between Madagascar and Eastern Tropical Africa. This vicariance event followed a dispersal

event from Asia either directly to Madagascar or to Eastern Tropical Africa (node 2 to node 5), and an Asian ancestral distribution of Paederieae is indicated by the analyses. In Knoxiaceae a corresponding vicariance event occurred (node 15, Fig. 2a), following an unequivocal dispersal event from Eastern Tropical Africa to Madagascar (node 14–15). Four additional dispersal events out of Africa into Madagascar are reconstructed in Knoxiaceae. Two from Eastern Tropical Africa (node 87–89 Fig. 2a and node 71 to *Pentas micrantha*, Fig. 2b), one from Southern Africa (node 28 to ancestor of *Pentanisia veronicoides*, Fig. 2b), and one event which is less clear and concerns *Otiophora* (Fig. 2b). Although there are alternative reconstructions for *Otiophora*, they all imply a dispersal event out of Africa. In Vanguerieae there are at least three arrivals in Madagascar. One is resolved as a vicariance event with Eastern Tropical Africa (*Rytigynia*, Fig. 3c), following an unequivocal dispersal from Eastern Tropical Africa to Madagascar (node 147–148). In addition, there is one dispersal event from Southern Africa (*Vangueria madagascariensis* and *Vangueria edulis*, Fig. 3c), and one vicariance event with Australasia (dioecious group, Fig. 3b). The entire dioecious group is indicated as being present in Madagascar, and there are

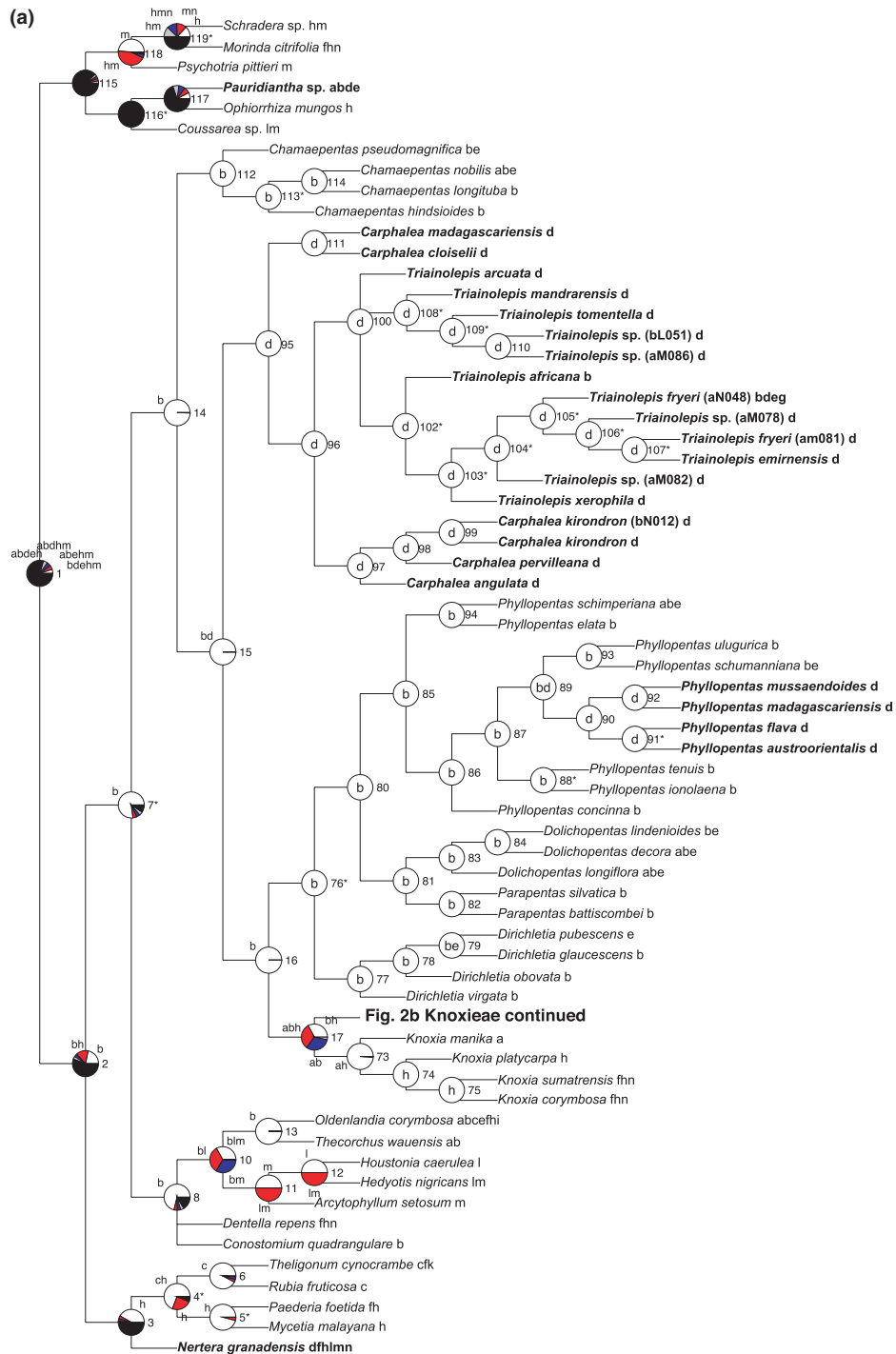


Figure 2 A summary of the dispersal–vicariance analyses for (a) Knoxieae; (b) Knoxieae continued. The tree is a 50% majority rule consensus tree of a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined data set of plastid *rbcl*, *rps16* and *trnT–F* DNA sequences. Pie charts at internal nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal–vicariance analysis (drva) while integrating over tree topologies using MCMC. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty (multiple equally parsimonious reconstructions) at each node, conditional on the node occurring. In the pie charts, the first four areas with highest probability are coloured according to relative probability in the following order: white > red > blue > grey. Any remaining areas are collectively shown in black. Node numbers are given for each node and details of the reconstructed distributions are given in Appendix S2. An asterisk (*) in association with the node number indicates that the node is not well supported in the phylogenetic analyses (< 95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names following the one-letter codes of biogeographical regions given in Table 4. Taxa occurring on Madagascar are written in boldface.

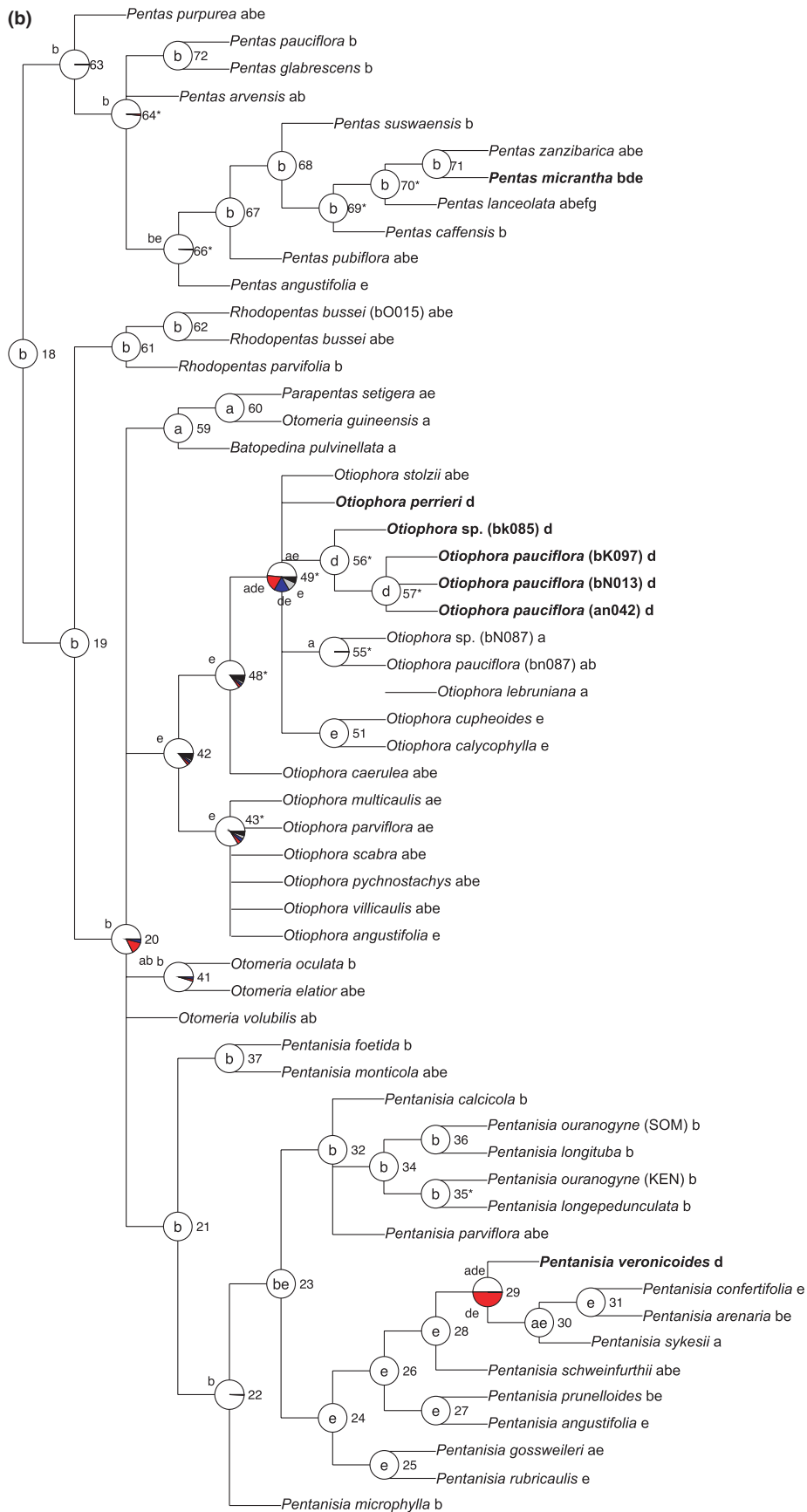


Figure 2 Continued

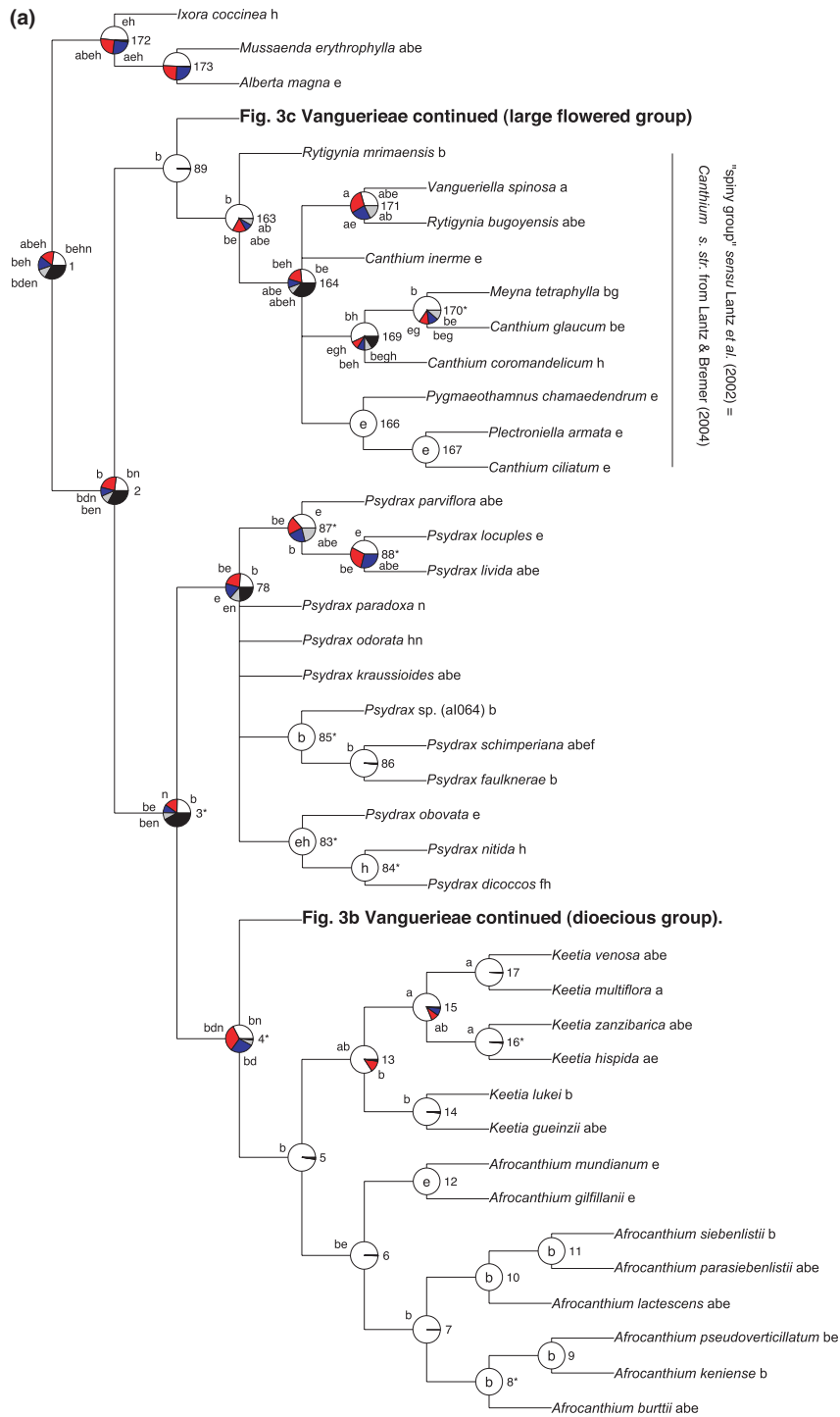
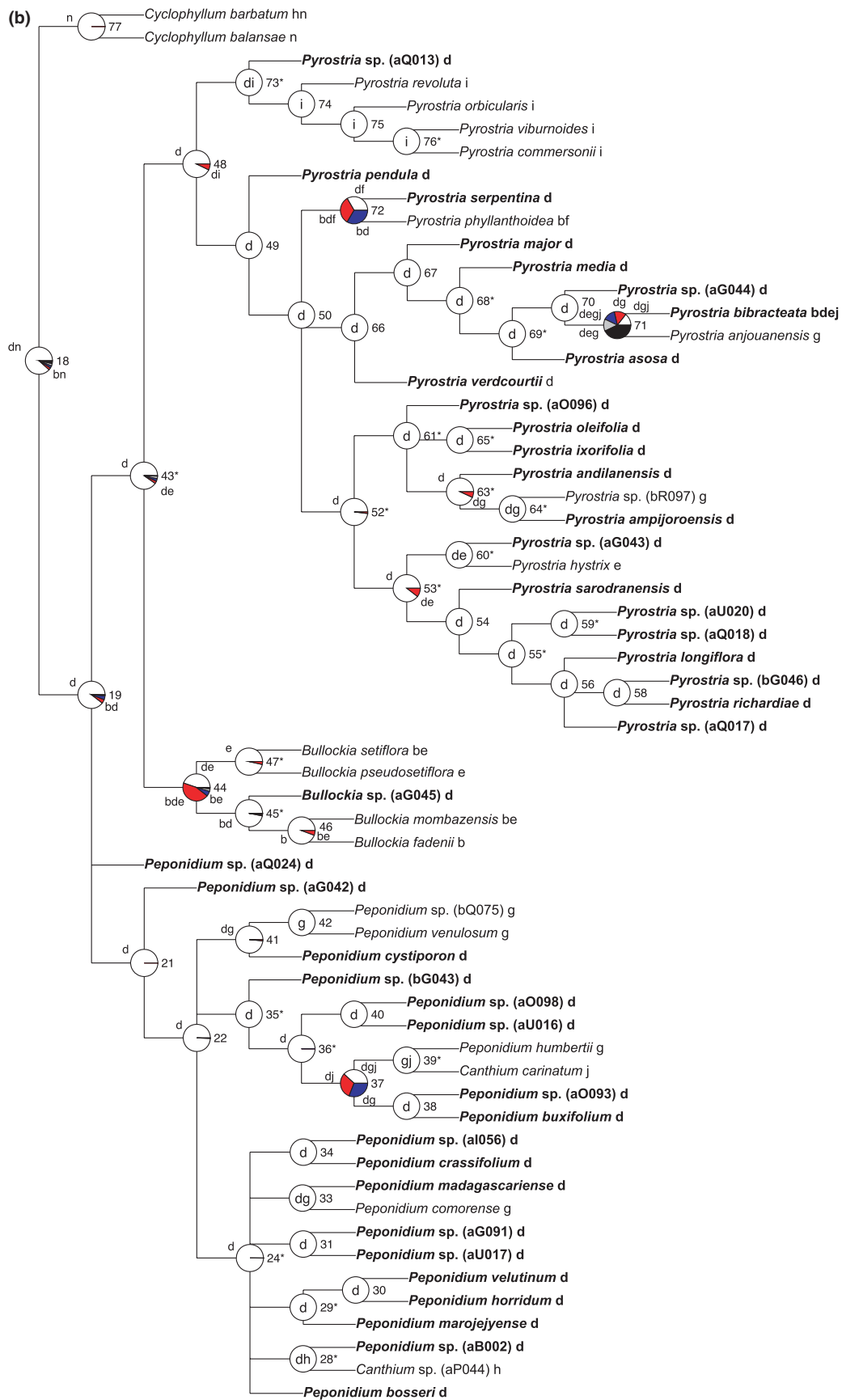


Figure 3 A summary of the dispersal–vicariance analyses for (a) Vanguerieae; (b) Vanguerieae continued (dioecious clade); (c) Vanguerieae continued (large flowered group). The tree is a 50% majority rule consensus tree of a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined data set of plastid *rbcl*, *rps16* and *trnT–F* DNA sequences. Pie charts at internal nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal–vicariance analysis (DIVA) while integrating over tree topologies using MCMC. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty (multiple equally parsimonious reconstructions) at each node, conditional on the node occurring. In the pie charts, the first four areas with highest probability are coloured according to relative probability in the following order: white > red > blue > grey. Any remaining areas are collectively shown in black. Node numbers are given for each node and details of the reconstructed distributions are given in Appendix S2. An asterisk (*) in association with the node number indicates that the node is not well supported in the phylogenetic analyses (< 95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names following the one-letter codes of biogeographical regions given in Table 4. Taxa occurring on Madagascar are written in boldface.



Dioecious group (sensu Razafimandimbison et al., 2009)

Figure 3 Continued

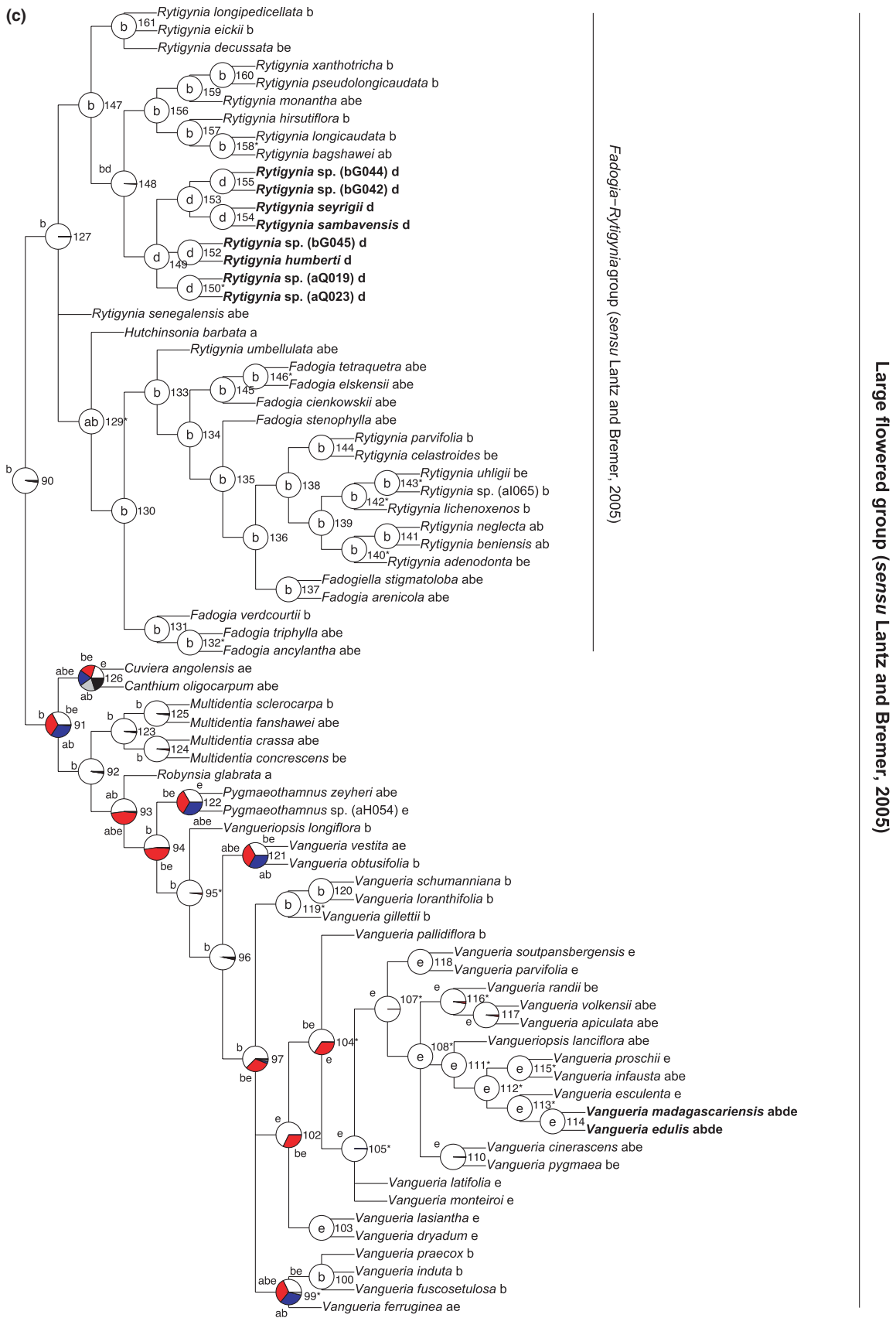


Figure 3 Continued

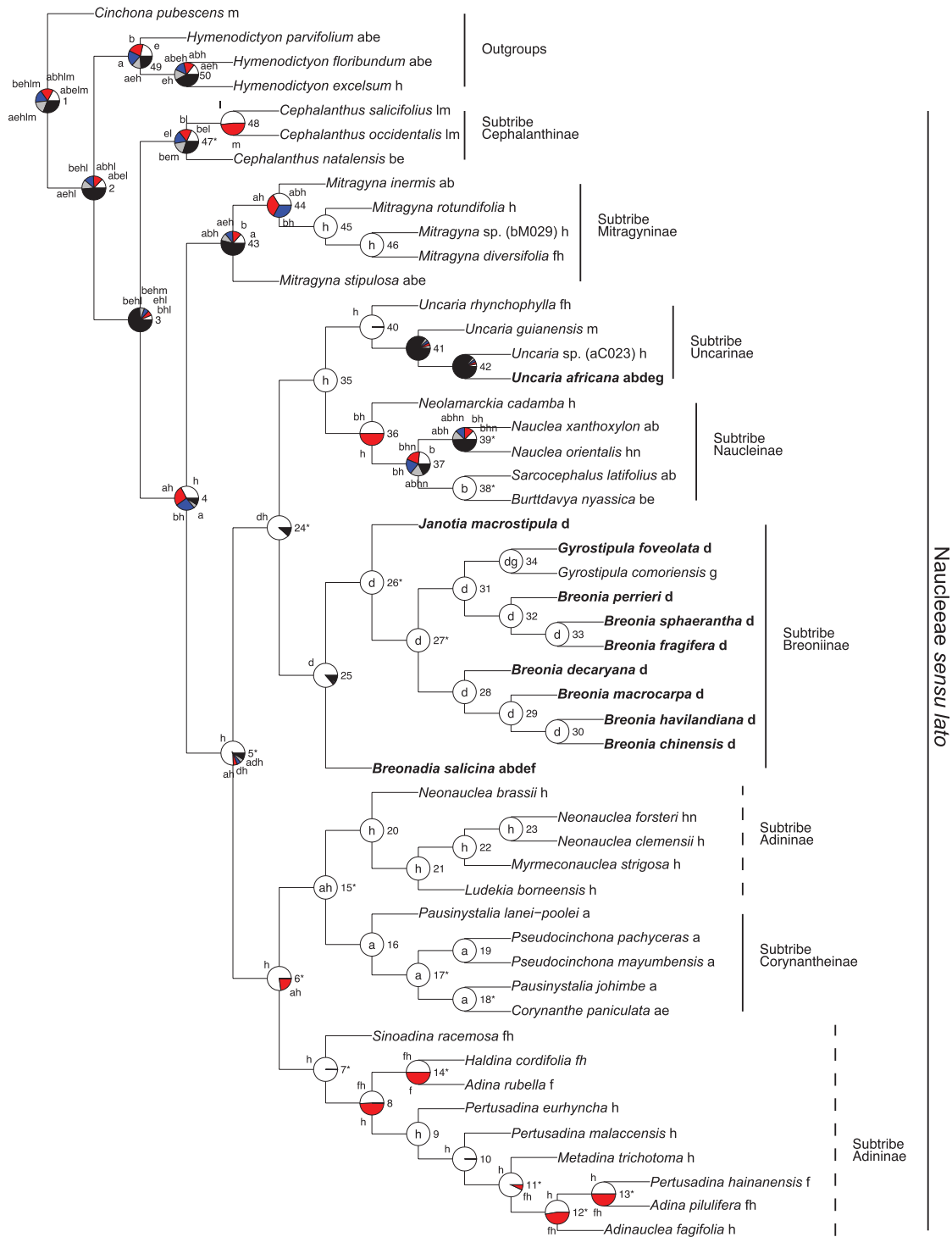


Figure 4 A summary of the dispersal–vicariance analyses for Naucleaceae. The tree is a 50% majority rule consensus tree of a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined data set of plastid *rbcL*, *rps16* and *trnT*–F DNA sequences. Pie charts at internal nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal–vicariance analysis (DIVA) while integrating over tree topologies using MCMC. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty (multiple equally parsimonious reconstructions) at each node, conditional on the node occurring. In the pie charts, the first four areas with highest probability are coloured according to relative probability in the following order: white > red > blue > grey. Any remaining areas are collectively shown in black. Node numbers are given for each node and details of the reconstructed distributions are given in Appendix S2. An asterisk (*) in association with the node number indicates that the node is not well supported in the phylogenetic analyses (< 95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names following the one-letter codes of biogeographical regions given in Table 4. Taxa occurring on Madagascar are written in boldface.

numerous out-of-Madagascar dispersal events reconstructed within this group (Fig. 3b). In Naucleaeae, two introductions in Madagascar are reconstructed, one vicariance event between Madagascar and Tropical Asia (node 24, Fig. 4) following a dispersal from Tropical Asia to Madagascar (node 5–24), and one dispersal event from Tropical Asia (*Uncaria africana*, Fig. 4).

DISCUSSION

As commonly observed in DIVA analyses (Ronquist, 1996; Sanmartín, 2003; Nylander *et al.*, 2008), our ancestral area reconstructions indicate wide distributions towards the root nodes in each of the four tribes. Despite these uncertainties, as well as uncertainties in the inferred relationships, the analyses generally provide clear indications about the geographical origins of the taxa occurring in Madagascar (Figs 1–4).

Altogether, 11 colonizations of Madagascar are inferred. As expected, different source areas are indicated, reflecting previously suggested phylogeographical connections (Robbrecht, 1996; Schatz, 1996; Grubb, 2003). Knoxieae, for example, have colonized Madagascar at least five times as the result of long-distance dispersal out of Africa (Fig. 2). A general lack of support for the phylogenetic relationships among the *Otiophora* species makes one of the colonization events unclear. Node 42 is the smallest supported group that includes all the Malagasy *Otiophora* species. This group is resolved as having a Southern African distribution, but the group includes species from three additional areas, and alternatives to a Southern African origin of the Malagasy *Otiophora* cannot be excluded. In fact, there is no support for monophyly of the species from Madagascar, and the possibility of more than one origin in Madagascar of *Otiophora* cannot be rejected. The main analyses of Knoxieae constrained the ancestral area distributions to include no more than five areas, but enforcing this constraint had no effect on the inferences for the Malagasy groups. Relaxing the constraint to allow any number of areas in the ancestral distributions resulted in the same resolution of the Malagasy origins, and if fewer areas are allowed (max-areas = 2) the support for the results seen in the main analyses is reinforced.

Vanguerieae arrived in Madagascar three times (Fig. 3). Two of the inferred events, the dispersals from Eastern Tropical Africa (*Rytigynia*, node 147–148) and from Southern Africa (*Vangueria madagascariensis*, *Vangueria edulis*), are unequivocally resolved, no matter how many areas are allowed in the ancestral distributions. The third arrival concerns the dioecious clade (*sensu* Razafimandimbison *et al.*, 2009). The main analyses restricted ancestral distributions to include no more than four areas and indicate a vicariance event between Madagascar and Australasia at the first node of this clade (node 18). However, it is unclear from where the group arrived in Madagascar, and changes in the number of areas allowed in the ancestral distributions affect the interpretation of this origin. In fact, the possibility that Madagascar is part of the ancestral distribution for Vanguerieae as a whole (Fig. 3)

cannot be rejected with confidence if four or more areas are allowed in the ancestral distributions. If the number of areas allowed are constrained to include no more than 2, Madagascar is no longer part of the ancestral distribution for Vanguerieae as a whole, but the analyses still indicate alternative scenarios: one a dispersal from Asia and the other a dispersal out of Africa. This uncertainty is mainly caused by the poorly supported relationships of the dioecious group to other Vanguerieae, and the unclear ancestral distribution of *Psydrax* (node 78; Fig. 2a). *Psydrax* is a large group (Table 1) that includes species from Asia, Africa and Madagascar (Davis & Bridson, 2003b). However, the Malagasy representatives were not sampled in the present analyses. Expanding the sample of *Psydrax*, including additional representatives from both Africa and Asia, as well as representatives from Madagascar, should be a priority in future phylogenetic and biogeographic analyses of Vanguerieae. An expanded sample, together with additional sequence data, would help us understand the geographical origins of the dioecious Vanguerieae in Madagascar.

The Malagasy Naucleaeae and Paederieae are both indicated to have Asian origins, with two dispersal events inferred for Naucleaeae (Fig. 4) and one for Paederieae (Fig. 1). However, these inferences are affected by the maximum area constraints. If any number of areas are allowed in the ancestral distributions, Madagascar becomes part of the inferred ancestral distribution for Paederieae as a whole. In Naucleaeae the effects are more severe, and the origins in Madagascar become completely ambiguous if any number of areas are allowed in the ancestral distributions. One possible problem concerns the taxon sample (see Table 1). Extending the sample from subtribes not found in Madagascar should be a priority in future analyses. The ancestral distribution for subtribe Mitragyninae (node 43) is highly ambiguous in the main analyses, and the reconstruction for the ancestor of subtribes Uncarinae and Naucleinae (node 35) becomes equally ambiguous if any number of areas are allowed. This ambiguity affects the entire analysis and leads to the uncertainty seen in the unconstrained analyses. Restricting the number of allowed areas to two had marginal effects on the Naucleaeae analyses and simply reinforced the support for patterns seen in the main analyses (Fig. 4).

Out-of-Madagascar dispersals

Altogether, at least 13 out-of-Madagascar dispersal events are inferred by the analyses, and these were not affected by changes in the number of allowed areas in the ancestral distributions. One dispersal occurred in Paederieae and one in Naucleaeae, both from Madagascar to the Comoros islands (Figs 1 and 4). In Knoxieae three out-of-Madagascar dispersals are reconstructed. One to Eastern Tropical Africa, one to Africa and the Comoros in *Triainolepis* (Fig. 2a), and a possible third dispersal to Africa in *Otiophora* (Fig. 2b). In Vanguerieae there are as many as nine out-of-Madagascar dispersals, all in the dioecious *Bullockia–Peponidium–Pyrostria* group (Fig. 3b),

and at least six of these are inferred to have occurred from Madagascar to one of the neighbouring Indian Ocean islands. This dispersal pattern is similar to that documented for chameleons (Raxworthy *et al.*, 2002) and tree ferns (Janssen *et al.*, 2008), and is by far the most common one reconstructed in our analyses. Madagascar is, with few exceptions, the origin of the Rubiaceae found in the Comoros, the Mascarenes and the Seychelles. A Malagasy or an African–Malagasy origin was suggested for the flora of Reunion by Cadet (1977). Our results for the dioecious Vanguerieae from the Mascarene islands are consistent with this idea, and a corresponding pattern was recently documented for angraecoid orchids (Micheneau *et al.*, 2008). The pattern is not entirely unexpected, given the geographical position of the Mascarene Islands, but it is not easy to understand why almost all representatives from the Comoros also originate from Madagascar. The islands are located approximately halfway between continental Africa and Madagascar but only two unequivocal out-of-Africa origins are indicated by our analyses, one in Knoxieae (*Pentas lanceolata*, Fig. 2b) and one in Vanguerieae (*Meyna tetraphylla*, Fig. 3a). This asymmetry results mainly from the large number of out-of-Madagascar origins in the dioecious Vanguerieae (Fig. 3b). The group is characterized by having small drupaceous fruits that are potentially dispersed by a wide range of birds (see Renner, 2004, for a discussion of birds that regularly cross the Mozambique Channel). Whether the group truly displays an elevated out-of-Madagascar dispersal rate compared with other Rubiaceae is unclear. The pattern could result from a biased taxon sample in our analysis. Additional representatives from the Indian Ocean islands, from continental Africa and from Southeast Asia should be included in future analyses to investigate this pattern further.

GEOGRAPHICAL ORIGINS AND NEOENDEMISM

Analysing patterns of vicariance and dispersal in plants, vertebrates and invertebrates, Yoder & Nowak (2006, p. 424) concluded that 'Madagascar is an island primarily comprised of neoendemics that are the descendants of Cenozoic waif dispersers', and that 'Africa appears by far to be the most important source of floral dispersal to Madagascar' (Yoder & Nowak, 2006, p. 416). Notwithstanding topological and mapping uncertainties in our analyses, it is clear that the four tribes arrived in Madagascar at least 11 times, and that they arrived via dispersal from both Africa and Asia. These results are highly consistent with the conclusions drawn by Yoder & Nowak (2006). Knoxieae and Vanguerieae have their origins in the Eastern Tropical and Southern Africa, although the origin of the dioecious clade in Vanguerieae is somewhat unclear. In contrast, both Paederieae and Naucleaeae are indicated to have Asian origins.

Schatz (1996) was among the first to emphasize dispersal as a mechanism of origin for elements of the Malagasy flora. Noting a 'remarkably high affinity with the Indo-Australo-Malesian floras far to the east', he identified two main modes of dispersal between these areas and Madagascar: (1) Eocene–

Oligocene 'Lemurian stepping-stones'; and (2) long-distance dispersal (Schatz, 1996). *Pyrostria* (Vanguerieae) was included in his list of 'possible examples of taxa' showing an Eocene–Oligocene 'Lemurian stepping-stone' distribution. This refers to a distribution including Africa/Madagascar, the Seychelles, India/Sri Lanka and western Malesia, and was originally named after van Steenis (1962) and his paper on the occurrence of a land bridge between Madagascar and Sri Lanka (Ceylon), a bridge that he gave the name of 'Lemuria' (Schatz, 1996). Modern understanding of the geological history of the Indian Ocean provides no support for the existence of a land bridge (McKenzie & Sclater, 1973), and this was fully acknowledged by Schatz (1996). However, noting that 'the distributional pattern still stands' for a large number of plant groups, he separated the 'Lemurian stepping-stone' distribution from one resulting from ordinary long-distance dispersal and discussed alternative explanations for the observed pattern. In particular he discussed the possibility that significant portions of the Chagos/Laccadive Plateau and the Mascarene Plateau (contiguous with the Seychelles at the time) were above sea level during the Eocene–Oligocene, and that these could have served as a stepping-stone dispersal track facilitating dispersal of Laurasian elements into Africa/Madagascar via India, Sri Lanka and the Seychelles (Schatz, 1996). This stepping-stone dispersal between Asia and Africa/Madagascar via emergent land areas in the Indian Ocean Basin has subsequently been considered both for other plant (Linder *et al.*, 1997; Alejandro *et al.*, 2005; Yuan *et al.*, 2005; Kulju *et al.*, 2007; Trénel *et al.*, 2007) and animal (Warren *et al.*, 2005; Klaus *et al.*, 2006) groups. Schatz (1996) correctly pointed out that the only difference between a stepping-stone dispersal and a long-distance dispersal is the distance parameter, which is just one of the factors influencing the probability of dispersal (Simpson, 1952). Nevertheless, immigration via long-distance dispersal has been deemed 'too improbable' for elements of the Malagasy biota, and land bridge hypotheses, including those of oceanic islands serving as stepping-stones, have commonly been invoked to explain the distribution of organisms around the Indian Ocean Basin (Rage, 1996; McCall, 1997; Case, 2002; Noonan & Chippindale, 2006; Voelker & Outlaw, 2008). Although there is evidence that some of these 'land bridges' existed, their role in shaping the present-day flora of Madagascar is poorly understood (see Yoder & Nowak, 2006).

If the Seychelles and/or the Mascarene Islands have served as stepping-stones as suggested by Schatz (1996), facilitating dispersal between Asia and Africa, no trace of this is seen in our reconstructions of ancestral distributions in any of the groups investigated here (Figs 1–4). If they had served as stepping-stones, we would expect the ancestral distributions to indicate a progression from Asia to Africa/Madagascar via the Seychelles or the Mascarene Islands (Whittaker & Fernández-Palacios, 2007). In Paederieae and Naucleaeae, two tribes that are indicated to have Asian origins, as well as in Knoxieae, several Asia/Africa dispersals are reconstructed (Figs 1, 2 & 4). However, today neither group is found on the Seychelles or the Mascarene Islands (Govaerts *et al.*, 2008), a pattern similar to

that documented for *Exacum* (Gentianaceae) by Yuan *et al.* (2005). In Vanguerieae there are at least four instances of dispersals between Africa/Madagascar and Asia. Inferred events concern *Canthium coromandelicum* and *Psydrax* (Fig. 3a), *Cyclophyllum*, *Pyrostria phyllanthoidea* and *Peponidium* (Fig. 3b). Although the analyses fail to resolve the exact location of these dispersal events, none include any taxa from the Seychelles or the Mascarene Islands. Two dispersals to the Seychelles (*Pyrostria bibracteata* and *Canthium carinatum*) and one to the Mascarene Islands (*Pyrostria*, node 48–73) are reconstructed for the dioecious Vanguerieae, but all three dispersals are indicated to have occurred from Madagascar, and none result in any further ‘stepping-stone’ hopping towards Asia or the Pacific (Fig. 3b).

Gaertnera, *Ixora* and *Psychotria* are additional Rubiaceae genera considered by Schatz (1996) to have reached Madagascar via a ‘stepping-stone’ type of dispersal from Asia. However, phylogenetic analyses of *Gaertnera* (Malcomber, 2002) and *Ixora* (Mouly *et al.*, 2009) do not provide unequivocal support for this idea, and neither of the analyses provided an explicit reconstruction of ancestral distributions. The analysis of *Gaertnera* suffered from a lack of resolution, and the relationships among species from Africa, Madagascar, Mauritius, Sri Lanka and Southeast Asia were not supported. The analysis by Mouly *et al.* (2009) resolved *Ixora* into two main lineages: an Asian-Pacific lineage and a lineage including African, Malagasy, Neotropical and Mascarene representatives. Although the Mascarene representatives were grouped as sister to all the African and Malagasy species, the pattern is more complex and both Neotropical, as well as an Asian species (*Ixora nigricans*), were resolved within this second lineage (Mouly *et al.*, 2009).

Given the well-documented long-standing geographical isolation of Madagascar (see Wells, 2003), the conclusion of Yoder & Nowak (2006), that endemism in Madagascar is primarily of recent origin, is somewhat surprising. In fact, the traditional interpretation has been to view this geographical isolation as the main contributing factor behind the high levels of endemism (Leroy, 1978; Grubb, 2003). Although no temporal analyses for our inferred origins are presented, recently published crown group age estimates for Rubiaceae tribes provide upper bounds on the ages (Bremer & Eriksson, 2009). These indicate that the dispersal events are Oligocene or younger, the only possible exception being the inferred dispersals in Paederieae. The crown group age of Paederieae was indicated as Eocene–Miocene by Bremer & Eriksson (2009), and Paederieae may have already been in Madagascar by the Eocene. Furthermore, some of the origins concern larger groups, each including a number of species only found in Madagascar, and taken together, this indicates that the endemism in the tribes result from comparatively recent speciation events that have occurred in Madagascar, well after the origins on the island. This corresponds well with the general pattern documented for plants by Yoder & Nowak (2006). If the long and isolated history of Madagascar is rejected as the main contributing factor behind the almost

unparalleled levels of endemism (Goodman & Benstead, 2005), other factors need to be considered. Quantifying geographic patterns of endemism-scaled richness, ‘endemism richness’ (Kier & Barthlott, 2001), across 90 terrestrial biogeographic regions, Kier *et al.* (2009) showed that oceanic islands exceed those of mainland areas by a factor of 9.5 for plants and 8.1 for vertebrates. Discussing this pattern of islands as global centres of endemism richness, and the underlying reasons, they identified two main types of islands that more or less correspond to the ‘Darwinian’ and ‘fragment’ types *sensu* Gillespie & Roderick (2002). In a rather traditional way, adaptive and rapid radiations were considered typical for more recently formed volcanic archipelagos (Darwinian islands) such as the Canary Islands or Hawaii, whereas ancient continental fragments, such as Madagascar, New Caledonia and New Zealand, were considered to harbour ancient palaeoendemic lineages (Kier *et al.*, 2009). For Madagascar we now know that this traditional view is poorly supported by the data (Yoder & Nowak, 2006).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List of investigated taxa and GenBank accession numbers for sequences used in the analyses.

Appendix S2 Bayesian posterior probabilities and reconstructed ancestral areas for all nodes.

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BIOSKETCH

The focus and interest of the research group are biodiversity studies of the large tropical plant family Rubiaceae. See http://www.bergianska.se/index_forskning.php for a comprehensive list of research interests for all members of the group.

Author contributions: B.B conceived the ideas; M.A. and S.G.R. collected the data; N.W. conducted all analyses and led the writing.

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