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Multiple colonizations from Madagascar and converged acquisition of dioecy in the Mascarene Dombeyoideae (Malvaceae) as inferred from chloroplast and nuclear DNA sequence analyses

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• *Background and Aims* In the Mascarenes, a young oceanic archipelago composed of three main islands, the Dombeyoideae (Malvaceae) have diversified extensively with a high endemism rate. With the exception of the genus *Trochetia*, Mascarene Dombeyoideae are described as dioecious whereas Malagasy and African species are considered to be monocline, species with individuals bearing hermaphrodite/perfect flowers. In this study, the phylogenetic relationships were reconstructed to clarify the taxonomy, understand the phylogeographic pattern of relationships and infer the evolution of the breeding systems for the Mascarenes Dombeyoideae.
• *Methods* Parsimony and Bayesian analysis of four DNA markers (ITS, *rpl16* intron and two intergenic spacers *trnQ-rsp16* and *psbM-trnD*) was used. The molecular matrix comprised 2985 characters and 48 taxa. The Bayesian phylogeny was used to infer phylogeographical hypotheses and the evolution of breeding systems.
• *Key Results* Parsimony and Bayesian trees produced similar results. The Dombeyoideae from the Mascarenes are polyphyletic and distributed among four clades. Species of *Dombeya*, *Trochetia* and *Ruizia* are nested in the same clade, which implies the paraphyly of *Dombeya*. Additionally, it is shown that each of the four clades has an independent Malagasy origin. Two adaptive radiation events have occurred within two endemic lineages of the Mascarenes. The polyphyly of the Mascarene Dombeyoideae suggests at least three independent acquisitions of dioecy.
• *Conclusions* This molecular phylogeny highlights the taxonomic issues within the Dombeyoideae. Indeed, the limits and distinctions of the genera *Dombeya*, *Trochetia* and *Ruizia* should be reconsidered. The close phylogeographic relationships between the flora of the Mascarenes and Madagascar are confirmed. Despite their independent origins and a distinct evolutionary history, each endemic clade has developed a different breeding systems (dioecy) compared with the Malagasy Dombeyoideae. Sex separation appears as an evolutionary convergence and may be the consequence of selective pressures particular to insular environments.

Key words: Dombeyoideae, Mascarene archipelago, *Dombeya*, *Ruizia*, *Trochetia*, dioecy, Indian Ocean, biogeography, ITS, *rpl16* intron, *psbM-trnD*, *trnQ-rps16*.

INTRODUCTION

Since the observations of Darwin in the Galapagos, oceanic islands have been considered as ideal natural laboratories for the study of evolutionary processes. This is a direct consequence of their isolation from the continents, high geological dynamics, high habitat diversity, well-established flora and fauna, and the reduced gene flow between insular and continental populations (Emerson, 2002). In particular, oceanic islands and archipelago display the most striking examples of adaptive radiations (Baldwin, 1991; Seung-Chul *et al.*, 1996; Austin *et al.*, 2004). In comparison to Hawaii and the Galapagos, the evolutionary history of the Mascarene archipelago (also named Mascarenes) biodiversity has been overlooked, although it is included in a biodiversity hotspot

(Myers *et al.*, 2000). This archipelago is located 700 km east of Madagascar and comprises three young oceanic islands (Reunion island, 2500 km², 2·1 My; Mauritius, 1800 km², 7·2 My; Rodrigues, 110 km², 8–10 My or 1·2 My for the emerged part of the island; McDougall *et al.*, 1965; Giorgi and Borchiellini, 1998; Hanyu *et al.*, 2001; Sheth *et al.*, 2003). The largest island, La Réunion, possesses an active volcano and a high mountainous formation with the highest point at 3070 m a.s.l. As a consequence, the island shows numerous and diverse ecosystems (Strasberg *et al.*, 2005). Some angiosperm groups contain a high number of endemic species. For instance, the Dombeyoideae, subfamily of Malvaceae (ex-'Sterculiaceae') includes 23 species (22 Mascarene endemics, one indigenous but not endemic) distributed in four genera: *Trochetia* DC., *Ruizia* Cav., *Astiria*

Lind. – all restricted to the archipelago – and *Dombeya* Cav. (Friedmann, 1987). The subfamily is mainly distributed in Madagascar (with seven genera and 250 species out of 350 in total), but also in Africa, Australia and tropical Asia (Bayer and Kubitzki, 2003). In addition to their high specific diversity, Dombeyoideae play an important ecological role in Mascarene ecosystems (Cadet, 1980; Blanchard, 2000). The subfamily members occur in most of the habitats found on the islands. The most diverse genus *Dombeya* (14 species) forms the canopy of the tropical mountain cloud forests (hence the local name 'coloured tree forest', due to the large range of leaf coloration among different *Dombeya* species; Cadet, 1980). Despite their key ecological role, phylogenetic relationships among the Mascarene Dombeyoideae and species from other regions are poorly understood, and no molecular phylogenetic studies have been conducted on this subfamily so far. Several taxonomic studies have been carried out for this group of Mascarene species (Cavanilles, 1787; Baker, 1877; Jacob de Cordemoy, 1895; Arènes, 1959a; Friedmann, 1987), but major disagreements exist between some of the authors (Table 1). Recently, a phylogenetic study based on morphological characters (Le Péchon *et al.*, 2009) cast doubt on the validity of the genera *Dombeya*, *Astiria* and *Ruizia*.

Molecular phylogenetic relationships could potentially clarify the taxonomy of the Dombeyoideae genera endemic to the Mascarene Archipelago. Furthermore the molecular phylogenies can be used to understand both the phylogeographic history and character evolution of the Mascarene Dombeyoideae. The endemic Mascarene species of *Dombeya*, *Astiria* and *Ruizia* demonstrate specific breeding systems that have not been recorded in the Malagasy and continental African species. While bearing hermaphroditic flowers in these two regions, *Dombeya* species in the Mascarenes were described as dioecious by Friedmann (1987) and Jacob de Cordemoy (1895). These observations were confirmed and detailed by Humeau (1999) and Humeau *et al.* (1999a, b). Molecular phylogenies can be used to infer the evolution of dioecy acquisition in the Mascarene taxa and to determine whether sex separation within the Dombeyoideae was acquired by evolutionary convergence.

In this study, the first molecular phylogeny of Mascarene Dombeyoideae based on four DNA markers is presented. The main aims were to (*a*) compare molecular and morphological phylogenetic hypotheses (Le Péchon *et al.*, 2009); (*b*) clarify the taxonomy of the Dombeyoideae genera endemic to the Mascarenes; (*c*) construct a phylogeographic pattern for Mascarene Dombeyoideae; and finally (*d*) infer the evolution of breeding systems.

MATERIALS AND METHODS

Taxonomic sampling

With the purpose of reconstructing the relationships among the Dombeyoideae, taxa were chosen to represent both the morphological and ecological diversity of the subfamily. As the study is focused on the Mascarene species, the majority of the sampling is composed of taxa endemic to this archipelago. Some species are found at several localities in the Indian Ocean (in the Mascarenes as well as in Madagascar; Table 2). In those cases, a specimen from each island was included in the sampling. A total of 26 specimens from the Mascarenes, including one undescribed species from Mauritius (*Dombeya* sp. 143), was selected. To establish whether the Mascarene Dombeyoideae are monophyletic, numerous taxa from outside the three islands were also included. Most Dombeyoideae diversity is found on Madagascar. Several studies (Cadet, 1980; Blanchard, 2000; Malcomber, 2002; Plunkett *et al.*, 2004; Micheneau *et al.*, 2008) show important relationships between the Malagasy and the Mascarene floras. Accordingly, a maximum number (i.e. 16 species) of Malagasy species was included, which also represents the maximum number of subsections of *Dombeya* defined by Arènes (1959b), involving three unidentified species (*Dombeya* sp. 252, *Dombeya* sp. 277 and *Dombeya* sp. 310). Three African species, one Asian species and one species from Saint Helena Island were added. Depending on the authors (Arènes, 1959a; Le Péchon *et al.*, 2009), the taxonomic relationships between the genera *Trochetia*, *Helmiopsis* H.Perrier and *Dombeya* are conflicting. To test the phylogenetic relationships among these three

TABLE 1. *Comparison between the classification of* Arènes (1959a, b) *and* Friedmann (1987) *for the Mascarene taxa of Dombeyoideae*

	Family	Genus	Subgenus	Section	No. of taxa	% of endemicity
Arènes (1959a)	Sterculiaceae					
		Dombeya				
			Dombeya			
				Astrapea	1	0
				Assonia	1	100
				Trochetia	6	100
				Capricornua	3	33
				Dombeya	17	88
Friedmann (1987)	Sterculiaceae					
		Ruizia			1	100
		Astiria			1	100
		Trochetia			6	100
		Dombeya				
				Dombeya	14	92

TABLE 2. *List of the Dombeyoideae taxa included in the analyses and Genbank accession numbers of the sequences*

Taxon	Origin; voucher number*	Trnq-rps16	Rpl16	Psbm-trnD	ITS
Dombeya acutangula Cav.	Madagascar; Labat 3654 (P)	GU937945	–	GU937896	GU938038
Dombeya acutangula subsp. *acutangula* var. *acutangula* Arènes	Réunion; Le Péchon 58 (P)	GU937946	GU937994	GU937897	GU938039
Dombeya acutangula subsp. *acutangula* var. *acutangula*	Cult. CBNB; Le Péchon 131 (P)	GU937947	GU937995	GU937898	GU938040
Dombeya acutangula subsp. *rosea* Friedmann aff. var. *acutangula*	Mauritius; Le Péchon 153 (P)	GU937948	GU937996	GU937899	GU938041
Dombeya acutangula subsp. *rosea* aff. var. *palmata*	Mauritius; Le Péchon 154 (P)	GU937949	GU937997	GU937900	GU938042
Dombeya amaniensis Engl.	Tanzania; Phillipson 4834 (P)	GU937950	GU937998	GU937901	GU938043
Dombeya blattiolens Frapp. ex Cordem.	Réunion; Le Péchon 294 (P)	GU937951	GU937999	GU937902	GU938044
Dombeya brevistyla Arènes	Réunion; Labat 2863 (P)	GU937952	GU938000	GU937903	GU938045
Dombeya burgessiae Gerr. ex Harv. and Sond	Tropical greenhouses of MNHN Paris; Specimen 15936	GU937953	GU938001	GU937904	GU938046
Dombeya cacuminum Hochr.	Cult. CBNB; Le Péchon 133 (P)	GU937954	GU938002	GU937905	GU938047
Dombeya ciliata Cordem.	Réunion; Le Péchon 262 (P)	GU937955	GU938003	GU937906	GU938048
Dombeya delislei Arènes	Réunion; Le Péchon 114 (P)	GU937956	GU938004	GU937907	GU938049
Dombeya elegans Cordem. var. *elegans*	Réunion; Le Péchon 18 (P)	GU937957	GU938005	GU937908	GU938050
Dombeya elegans var. *virescens* Cordem.	Réunion; Le Péchon 272 (P)	GU937958	GU938006	GU937909	GU938051
Dombeya farafanganica Arènes subsp. *endrina*	Madagascar; Razakamalala *et al.* 2114 (MO)	GU937959	GU938007	GU937910	GU938052
Dombeya ferruginea Cav. subsp. *borbonica* Friedmann	Réunion; Le Péchon 22 (P)	GU937960	GU938008	GU937911	GU938053
Dombeya ferruginea subsp. *ferruginea*	Mauritius; Le Péchon 155 (P)	GU937961	GU938009	GU937912	GU938054
Dombeya ficulnea Baill.	Réunion; Le Péchon 3 (P)	GU937962	GU938010	GU937913	GU938055
Dombeya lucida Baill.	Madagascar; Ravelonarivo *et al.* 2011 (MO)	GU937963	GU938011	GU937914	GU938056
Dombeya macrantha Baker	Madagascar; Labat 3671 (P)	GU937964	GU938012	GU937915	GU938057
Dombeya mauritiana Friedmann	Mauritius; Le Péchon 129 (P)	GU937965	GU938013	GU937916	GU938058
Dombeya pilosa Cordem.	Réunion; Le Péchon 65 (P)	GU937966	GU938014	GU937917	GU938059
Dombeya populnea Baker	Mauritius; Le Péchon 141 (P)	GU937967	GU938015	GU937918	GU938060
Dombeya populnea	Réunion; Le Péchon 80 (P)	GU937968	GU938016	GU937919	GU938061
Dombeya punctata Cav.	Réunion; Le Péchon 1 (P)	GU937969	GU938017	GU937920	GU938062
Dombeya reclinata Cordem.	Réunion; Le Péchon 6 (P)	GU937970	GU938018	GU937921	GU938063
Dombeya rodriguesiana Friedmann	Rodriguez; Le Péchon; 160 (P)	GU937971	GU938019	GU937922	GU938064
Dombeya rottleroides Baill.	Madagascar; SW 60080 (P)	GU937972	GU938020	GU937923	GU938065
Dombeya sp. 143	Mauritius; Le Péchon 143 (P)	GU937973	GU938021	GU937924	GU938066
Dombeya sp. 252	Madagascar; Rakotonirina *et al.* 252 (MO)	GU937974	GU938022	GU937925	GU938067
Dombeya sp. 277	Madagascar; Rakotonirina *et al.* 277 (MO)	GU937975	GU938023	GU937926	GU938068
Dombeya sp. 310	Madagascar; Rakotonirina *et al.* 310 (MO)	GU937976	–	GU937927	GU938069
Dombeya superba Arènes	Madagascar; Rakotonirina *et al.* 293 (MO)	GU937977	GU938024	GU937928	GU938070
Dombeya tiliacea Planch.	South Africa; Phillipson 4834 (P)	GU937978	GU938025	GU937929	GU938071
Dombeya tremula Hochr.	Madagascar; Phillipson 5815 (P)	GU937979	–	GU937930	GU938072
Dombeya umbellata Cav.	Réunion; Le Péchon 115 (P)	GU937980	GU938026	GU937931	GU938073
Dombeya viburniflora Boj.	Madagascar; Labat 3771 (P)	GU937981	GU938027	GU937932	GU938074
Helmiopsis bernieri (Baill.) Arènes	Madagascar; Capuron 20937 (P)	GU937982	GU938028	GU937933	GU938075
Helmiopsis pseudopopulus (Baill.) Capuron	Madagascar; Capuron 27412 (P)	GU937983	–	GU937934	GU938076
Nesogordonia crassipes (Baill.) Capuron	Madagascar; Rakotonirina *et al.* 385 (MO)	GU937984	–	GU937935	GU938077
Nesogordonia suzannae Labat, Munzinger and O.Pascal	Mayotte; Berthelot 1327 (P)	GU937985	GU938029	GU937936	GU938078
Pterospermum heterophyllum Hance		–	–	–	AY083661
Pterospermum suberifolium (L.) Wild	Cult. Tropical greenhouses of MNHN Paris; Specimen 5616 (P)	GU937986	GU938030	GU937937	–
Ruizia cordata Cav.	Réunion; Le Péchon 81 (P)	GU937987	GU938031	GU937938	GU938079
Trochetia blackburniana Bojer ex Baker	Mauritius; Le Péchon 144 (P)	GU937988	GU938032	GU937939	GU938080
Trochetia boutoniana Friedmann	Mauritius; Le Péchon 147 (P)	GU937989	GU938033	GU937940	GU938081
Trochetia granulata Cordem.	Réunion; Le Péchon 101 (P)	GU937990	GU938034	GU937941	GU938082
Trochetia parviflora Boj.	Mauritius; Le Péchon 162 (P)	GU937991	GU938035	GU937942	GU938083
Trochetia triflora DC.	Mauritius; Le Péchon 162bis (P)	GU937992	GU938036	GU937943	GU938084
Trochetiopsis erythroxylon (G.Forst.) W.Marais	Cult. CBNB; Le Péchon 132 (P)	GU937993	GU938037	GU937944	GU938085

*CBNB, Conservatoire Botanique National de Brest.

genera with molecular markers, two species of *Helmiopsis* from Madagascar were included. Also included was *Trochetiopsis erythroxylon* endemic to Saint Helena Island, which appears morphologically close to *Dombeya* and *Trochetia* (*Trochetiopsis* was formerly considered to be a taxonomic synonym of *Trochetia*; Brodie *et al.*, 1998). *Nesogordonia* Baill. was chosen as an outgroup to root the inferred phylogenies according to Barnett (1987) and Nyffeler *et al.* (2005) who showed that this genus diverged earlier among the Dombeyoideae. Thus two species of *Nesogordonia* (i.e. *Nesogordonia crassipes* and *Nesogordonia suzannae*) were selected as outgroup members. To complete the outgroup selection, the Asian Dombeyoideae genus *Pterospermum* Schreb., which is morphologically and genetically distinct from *Dombeya* (Tang, 1992; Nyffeler *et al.*, 2005) was also added. The sample thus included a total of 50 specimens comprising 48 taxa. The sampling and voucher numbers are given in Table 2.

Extraction of DNA, amplification of molecular markers, and sequencing protocols

For DNA extraction, all samples originating from the Mascarene Archipelago were fresh leaf material collected from either wild specimens or from cultivated plants (Table 2), dehydrated with silica gel. The major part of the Malagasy species sampling came from herbarium specimens from which only small samples were taken. From this material, the DNA was extracted using the DNeasy Plant Mini (Qiagen) extraction kit, following the manufacturer's instructions.

To reconstruct the relationships among the Dombeyoideae, four molecular markers were selected: the nuclear ribosomal region of internal transcribed spacers (ITS), the chloroplastic spacers *trnQ-rps16* and *psbM-trnD*, and the chloroplastic intron *rpl16*. The primers used to amplify and sequence these regions are listed in Table 3. For the *rpl16* intron, two new internal primers were designed to facilitate the sequencing, as the presence of poly(T) residues led to sequencing difficulties. The amplifications were carried out in a final volume of 25 μL with $0.1 - 3$ μL of genomic DNA of unknown concentration, 2·5 μL of specific buffer for the *REDTaq* at a concentration of $\times 10$ and containing $MgCl_2$, 1 μL of dNTPs (a final concentration of 100 μM for each nucleotide), 1·25 μL of DMSO (5 % of the final volume), 1 μL of each primer (at 10 pmol μL^{-1}) and 0·15 μL of *REDTaq* DNA polymerase (Sigma-Aldrich); purified water was added to bring the total

quantity to 25 μL. The polymerase chain reactions (PCR) were carried out with a Biometra T3000 thermal cycler. The PCR conditions for the *rpl16* intron and the *trnQ-rsp16* intergenic spacer were: initial denaturation at 80 °C for 5 min followed by 35 cycles of 1 min at 95 °C, 1 min at 50 °C and 5 min at 65 °C, a final extension at 65 °C for 4 min. For the *trnD-psbM* spacer, the PCR conditions were: initial denaturation at 80 °C for 5 min followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 3·5 min at 72 °C, a final extension was carried out at 72 °C for 5 min. For the ITS, the following protocol was used: initial denaturation at 94 °C for 3 min followed by 35 cycles of 30 s at 94 °C, 1 min at 55 °C and 1 min at 72 °C, a final extension was carried out at 72 °C for 10 min. PCR results were checked by migrating 2 μL of the product on a 1·5 % agarose gel with ethidium bromide. The PCR purifications and sequencing were carried out at the Centre national de séquençage (Genoscope; http://www. genoscope.cns.fr/spip/).

Sequence alignment

The resulting sequences were edited, assembled and aligned visually using the program Bioedit (Hall, 1999). Proper sequence alignment required the introduction of many insertion/deletion events (indels). In the present analysis, the gaps were treated with ID coding (Barriel, 1994, 2004) which has proven its usefulness in several studies (Delarbre *et al.*, 2000, 2002; Raymundez *et al.*, 2002; Hennequin *et al.*, 2003; Rouhan *et al.*, 2004).

Phylogenetic analyses

Two different methods to reconstruct Dombeyoideae phylogenetic relationships were used: the maximum parsimony (MP) and the Bayesian inference (BI). For the MP analysis, the tree search was performed using PAUP* ver. 4·0b1·0 (Swofford, 2001) with a heuristic search including the following options: 1000 replicates of random addition sequence and tree bisection and reconnection branch swapping. A preliminary MP search that included only plastid markers failed to find all the most-parsimonious trees resulting in an almost completely unresolved strict consensus of the trees (data not shown). A second MP analysis including only ITS sequences was performed. The strict consensus tree (data not shown) resulting from this search was better resolved than the strict consensus of plastid markers. Nevertheless, it presented several important

TABLE 3. *Selected molecular markers and their primers used in this study*

Molecular marker	Primer name	Sequence (5′ → 3′)	Reference
trnQ-rsp16	trnQ[(uug)]	GCG TGG CCA AGY GGT AAG GC	Shaw *et al.*, 2007
	rps16x1	GTT GCT TTY TAC CAC ATC GTT T	Shaw *et al.*, 2007
rpl16	rpl16F71	GCT ATG CTT AGT GTG TGAC TCG TTG	Pfeil *et al.*, 2002
	rpl16R1516	CCC TTC ATT CTT CCT CTA TG TTG	Pfeil *et al.*, 2002
	rpl16F310	CCA ATA TGT AAG GTC TAT GAA GC	This study
	rpl16R613	TTT TCA ATA GAA TCC TCT GCA TTC	This study
psbM-trnD	psbMF	AGC AAT AAA TGC RAG AAT ATT TAC TTC CAT	Shaw *et al.*, 2005
	trnD(guc)R	GGG ATT GTA GYT CAA TTG GT	Shaw *et al.*, 2005
ITS	ITS.leu	GTC CAC TGA ACC TTA TCA TTT AG	Baum *et al.*, 1998
	ITS4	TCC TTC CGC TTA TTG ATA TGC	White *et al.*, 1990

polytomies. These two consensus trees (i.e. chloroplastic and nuclear ribosomal consensus) did not show major topological incongruence. Therefore, it was decided to combine the data from the different DNA regions to increase the resolution and the support values. The node support was estimated with a bootstrap analysis (BS) with the following search options: heuristic search with 1000 000 replicates without branch swapping and one random addition sequence per replicate. Clades having a BS of 85–100 % will be considered here as highly supported, and 75–84 % as moderately supported.

The BI analysis was performed using MrBayes 3 (Ronquist and Huelsenbeck, 2003). For each of the four markers, an appropriate model of evolution was selected with the help of MrModeltest ver. 2 (Nylander, 2004) implementing the Akaike criterion. For the four datasets, the GTR + Γ model selected by MrModeltest was applied. A fifth partition, corresponding to the insertion and deletion coded with the ID coding (Barriel, 1994, 2004), was treated using a binary evolution model.

The molecular data analysis with BI was carried out using the following options. Four Markov chains derived using the Monte-Carlo procedure were utilized, and the trees were sampled every 100 generations for a total of 10 000 000 generations. The number of trees sampled before the $-\ln L$ values stabilized ('burn-in phase') was determined graphically. As a result, the first 10 000 generations that were required to achieve Markov chain convergence were discarded. The remaining trees were then used to calculate the majority consensus tree that provided the posterior probability (PP) on each node. Clades are described here as highly supported when PP is above 0·94, and as moderately supported when the PP value is between 0·85 and 0·94.

Phylogeographic inference

Among the sample, seven different geographic regions were represented: continental Africa, tropical Asia, Madagascar (including the Comoro archipelago), Saint Helena and the Mascarene archipelago represented by Mauritius, La Réunion and Rodrigues. Considered as a non-additive multi-state character, the evolution of geographic distribution was inferred using parsimony on the BI majority consensus tree, using Mesquite (Maddison and Maddison, 2006).

Evolution of breeding systems

The endemic Dombeyoideae of the Mascarenes, apart from *Trochetia*, are described as dioecious (Table 4). The evolutionary history of breeding systems was inferred using the same method employed for the phylogeography. In case of ambiguous optimization, two types were assessed: AccTran optimization (i.e. accelerated transformations, favouring reversions) and DelTran (i.e. delayed transformations, favouring convergences).

RESULTS

Combined data analysis

Altogether, 49 sequences were obtained for each intergenic spacer (*trnQ-rps16* and *psbM-trnD*), 48 sequences for the ITS and 44 sequences for the *rpl16* intron.

TABLE 4. *Breeding systems [Malagasy species (Arènes, 1959), African species (Seyani, 1991), Mascarene species (Friedmann, 1987; Gigord et al., 1998; Humeau, 1999, Humeau et al., 1999a)] and geographical distribution of each taxon included in the analyses*

Taxon	Breeding system	Distribution
Dombeya acutangula subsp. *acutangula* var. *acutangula*	Hermaphroditic	Réunion
D. acutangula subsp. *acutangula* var. *acutangula*	Hermaphroditic	Rodrigues
D. acutangula subsp. *rosea* aff. var. *acutangula*	Hermaphroditic	Mauritius
D. acutangula subsp. *rosea* aff. var. *palmata*	Hermaphroditic	Mauritius
D. acutangula	Hermaphroditic	Madagascar
D. amaniensis	Hermaphroditic	Africa
D. blattiolens	Dioecious	Réunion
D. brevistyla	Hermaphroditic	Madagascar
D. burgessiae	Hermaphroditic	Africa
D. cacuminum	Hermaphroditic	Madagascar
D. ciliata	Dioecious	Réunion
D. delislei	Dioecious	Réunion
D. elegans var. *elegans*	Dioecious	Réunion
D. elegans var. *virescens*	Dioecious	Réunion
D. farafanganica subsp. *endrina*	Hermaphroditic	Madagascar
D. ferruginea subsp. *borbonica*	Dioecious	Réunion
D. ferruginea subsp. *ferruginea*	Dioecious	Mauritius
D. ficulnea	Dioecious	Réunion
D. lucida	Hermaphroditic	Madagascar
D. macrantha	Hermaphroditic	Madagascar
D. mauritiana	Dioecious	Réunion
D. pilosa	Dioecious	Réunion
D. populnea	Dioecious	Réunion
D. populnea	Dioecious	Mauritius
D. punctata	Dioecious	Réunion
D. reclinata	Dioecious	Réunion
D. rodriguesiana	Dioecious	Rodrigues
D. rottleroides	Hermaphroditic	Madagascar
D. sp. 143	?	Mauritius
D. sp. 252	?	Madagascar
D. sp. 277	?	Madagascar
D. sp. 310	?	Madagascar
D. superba	Hermaphroditic	Madagascar
D. tiliacea	Hermaphroditic	Africa
D. tremula	Hermaphroditic	Madagascar
D. umbellata	Dioecious	Réunion
D. viburnifolia	Hermaphroditic	Madagascar
Helmiopsis pseudopopulus	Hermaphroditic	Madagascar
H. bernieri	Hermaphroditic	Madagascar
Nesogordonia suzannae	Hermaphroditic	Madagascar
N. crassipes	Hermaphroditic	Madagascar
Pterospermum heterophyllum	Hermaphroditic	South-east Asia
P. suberifolium	Hermaphroditic	South-east Asia
Ruizia cordata	Dioecious	Réunion
Trochetia blackburniana	Hermaphroditic	Mauritius
T. boutoniana	Hermaphroditic	Mauritius
T. granulata	Hermaphroditic	Réunion
T. parviflora	Hermaphroditic	Mauritius
T. triflora	Hermaphroditic	Mauritius
T. erythroxylon	Hermaphroditic	Saint Helena

Despite a good ITS amplification for *Pterospermum suberifolium*, sequencing was unsuccessful. The sequence of the ITS for the closely related species *Pterospermum heterophyllum* was available in Genbank (Table 2). To represent the Asian genus *Pterospermum*, a chimeric sequence combining the

chloroplastic sequences of *P. suberifolium* and the nuclear sequence of *P. heterophyllum* was reconstructed.

After aligning and coding the indels, the number of characters used for the phylogenetic analyses was: 439 for *psbM-trnD* [with eight (1·8 %) informative characters for the ingroup and eight characters due to the gap handling], 690 for *trnQ-rsp16* [with 35 (5·07 %) informative characters for the ingroup and 40 characters due to the gap handling], 694 for the ITS [with 71 (10·23 %) informative characters for the ingroup and 38 characters due to the gap handling]. When aligning the chloroplastic intron *rpl16*, a particular region (position 438–530) was discovered that could not be aligned. The alignment issues in this intron-specific region were noted previously by Baum *et al.* (1998) and Pfeil *et al.* (2002). As a consequence, and to avoid any bias in the phylogeny reconstruction, the region of *rpl16* from position 438 to 530 was excluded. Finally, *rpl16* had a total of 1160 characters that were used for the analysis [with 52 (4·48 %)– informative characters for the ingroup and 55 characters due to the gap handling].

Following sequence alignment, exclusion of the ambiguous region of the *rpl16* intron, and treatment of the indels with ID coding, the final matrix contained 50 specimens included within 48 taxa and 2985 molecular characters. The unavailable sequences (due to amplification failure) were coded as missing data.

Phylogenetic analyses

A total of 9402 most-parsimonious trees were obtained. The strict consensus is given in Fig. 1. For the most part, the same relationships were retrieved with the BI. However, the BI majority consensus tree (Fig. 2) appears to be more resolved and shows more better-supported nodes.

In the two consensus trees shown in Figs 1 and 2, Clade A contains the *Dombeya* species, in addition to the taxa belonging to *Trochetia*, *Ruizia* and *Helmiopsis*, and is moderately supported (BS = 78 %, PP = 0·8). Within Clade A, all the Dombeyoideae from the Mascarenes are distributed into four subclades (referred to here as B, C, E and F).

Dombeya rodriguesiana is included in Clade B, which is weakly supported (BS = 50 %, PP = 0·8) and otherwise includes only taxa from Madagascar. The two *Helmiopsis* species (i.e. *H. pseudopopulus* and *H. bernieri*), which are endemic to Madagascar, constitute a subclade that is highly supported in both analyses (BS = 100 %, PP = 1). Clade B is the sister-group to a clade that contains all the other members of *Dombeya*, *Trochetia* and *Ruizia* (BS = 83 %, PP = 1).

Trochetia and *Ruizia*, in addition to six species of *Dombeya*, are included in Clade C, which is moderately supported by the MP (BS = 76 %), but more strongly supported by the BI (PP = 0·95). This clade contains only endemic species of the Mascarene archipelago. Because of the basal polytomy, the MP analysis does not allow the phylogenetic relationships between the species of this clade to be precisely established. Regarding the consensus obtained with BI analysis (Fig. 2), Clade C appears completely resolved and is divided into two monophyletic groups. A first subclade, the moderately supported Clade C_1 (PP = 0·9, Fig. 2), includes species of *Dombeya* in addition to the single known species of *Ruizia*,

which is endemic to La Reunion. Clade C_1 is the sister-group of Clade C_2, which is moderately supported (PP = 0·88, Fig. 2). This monophyletic group includes two subspecies of *Dombeya ferruginea* (PP = 0·99) and the species of *Trochetia*. The latter, endemic to the Mascarenes, appears to be monophyletic (PP = 0·86).

The sister-group of Clade C is the species *Dombeya tremula*, which is endemic to Madagascar. The monophyletic group (*D. tremula* + Clade C) is weakly supported by the MP analysis (BS = 63 %) but has a strong support in the BI analysis (PP = 1). This group (*D. tremula* + Clade C) is the sister-group of Clade D that only involves *Dombeya* species. Within Clade D, the endemic species of *Dombeya* from the Mascarenes are distributed between Clades E and F (Figs 1 and 2). Clade E includes all the subspecies of *D. acutangula*, which are endemic to the Mascarenes. However, this group appears weakly supported by the MP (BS = 64 %), but better supported by the BI analysis (PP = 0·95). The sister-group of Clade E is *D. acutangula* (BS = 80 %, PP = 1) from Madagascar, which supports the monophyly of this widespread species.

The other endemic species from the Mascarene archipelago are included in Clade F which is strongly supported only by the BI (BS = 57 %, PP = 1). All the species within this Clade F are endemic to La Réunion.

Phylogeographic inferences

The phylogeographic hypotheses inferred are shown on Figs 3 and 4. Madagascar appears to be the ancestral distribution for each clade containing Mascarene species and four colonization events of the Mascarenes can be inferred. A first colonization event from Madagascar to Rodrigues would have provided the lineage of *D. rodriguesiana* (Fig. 4B). A second event of dispersal from Madagascar to Mauritius explains the distribution of Clade C (Fig. 4A). Subsequently, four independent events of dispersal from Mauritius to La Réunion can explain the different geographic distributions for the lineages of *Ruizia cordata*, *Dombeya populnea*, *D. ferruginea* subsp. *borbonica* and *T. granulata*, respectively.

For Clade E, a dispersal event to the Mascarenes from Madagascar can be inferred (Fig. 4C). Clade F includes only the endemic species of La Réunion. This monophyletic group would also have originated from a dispersal event from Madagascar (Fig. 4B). These phylogeographic hypotheses assume that no extinction event has occurred in the Mascarenes.

Evolution of breeding systems

The evolution of breeding systems is shown on Fig. 5. Regardless of the optimization used, hermaphrodism unambiguously appears as the ancestral state and dioecy as the derived state. With Acctran as well as with Deltran, the gender separation was acquired once in Clade F and once for *D. rodriguesiana* (in Clade B). Within Clade C, two interpretations are possible according to the optimization. For the evolutionary scenario using Acctran, dioecy appears once in Clade C, then a reversion to hermaphrodism for the genus *Trochetia* (Fig. 5A). With Deltran, the acquisition of dioecy appears in Clade C_1, and for *D. ferruginea* in Clade C_2 (Fig. 5B).
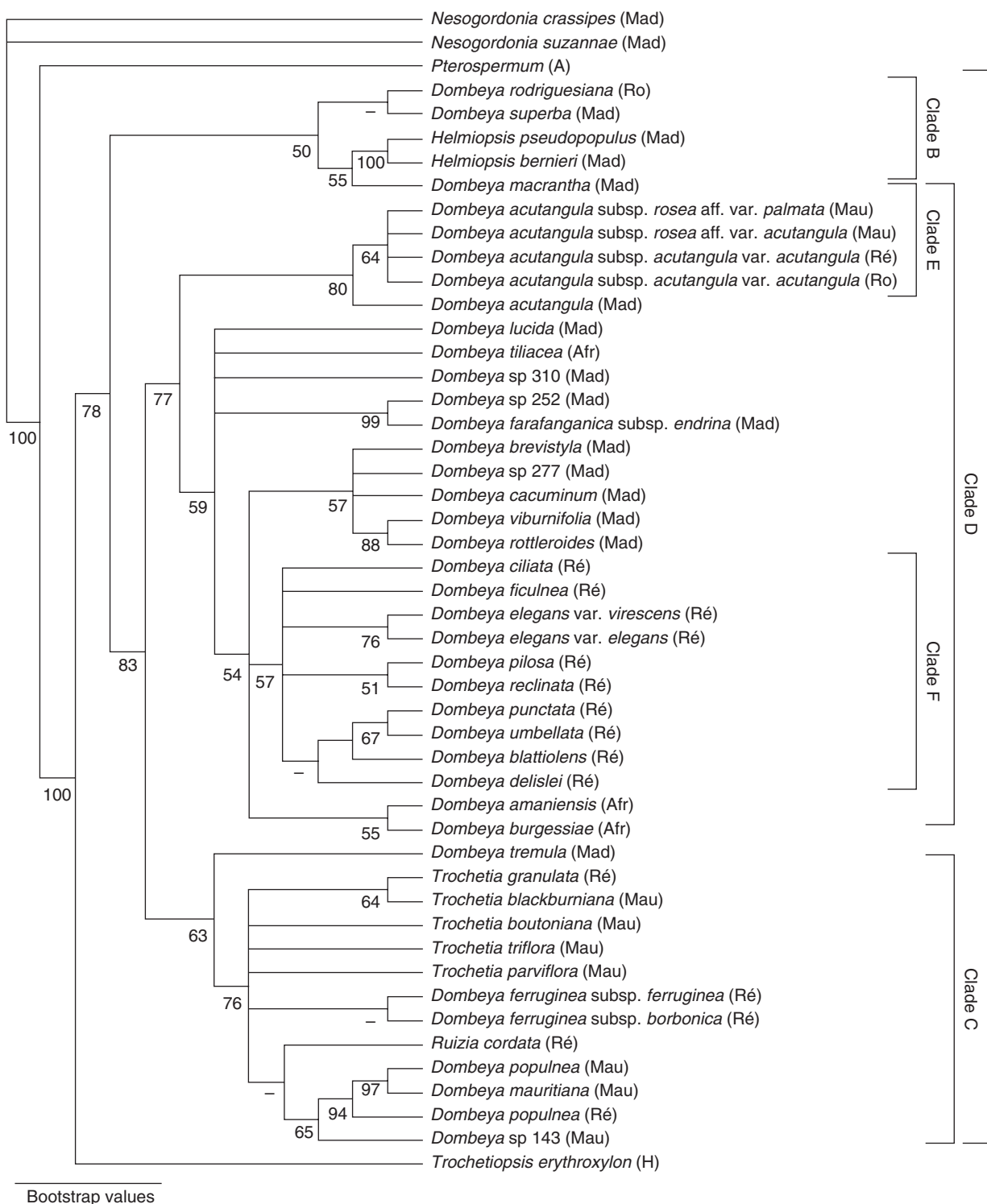
FIG. 1. Strict consensus of the 9402 most-parsimonious trees. Clades A–F correspond to the clades discussed in the text. BS values are indicated below the branches. Dashes indicates nodes that are not supported by the BS analysis (BS <50 %). Mad, Madagascar; Ré, Réunion; Ro, Rodrigues; Mau, Mauritius; As, Asia; Afr, Africa; H, Saint Helena.

DISCUSSION

New insights into the systematics of Dombeyoideae in the Mascarene archipelago

In both types of analysis (MP and BI), all the Mascarene Dombeyoideae are included in Clade A. This monophyletic group also groups the species of *Dombeya*, *Helmiopsis*, *Trochetia* and *Ruizia*. The presence of all four genera within Clade A implies the paraphyly of the genus *Dombeya*, and supports the results from previous cladistic analyses based on morphological characters (Le Péchon *et al.*, 2009). Despite the existence of morphological homogeneity between the
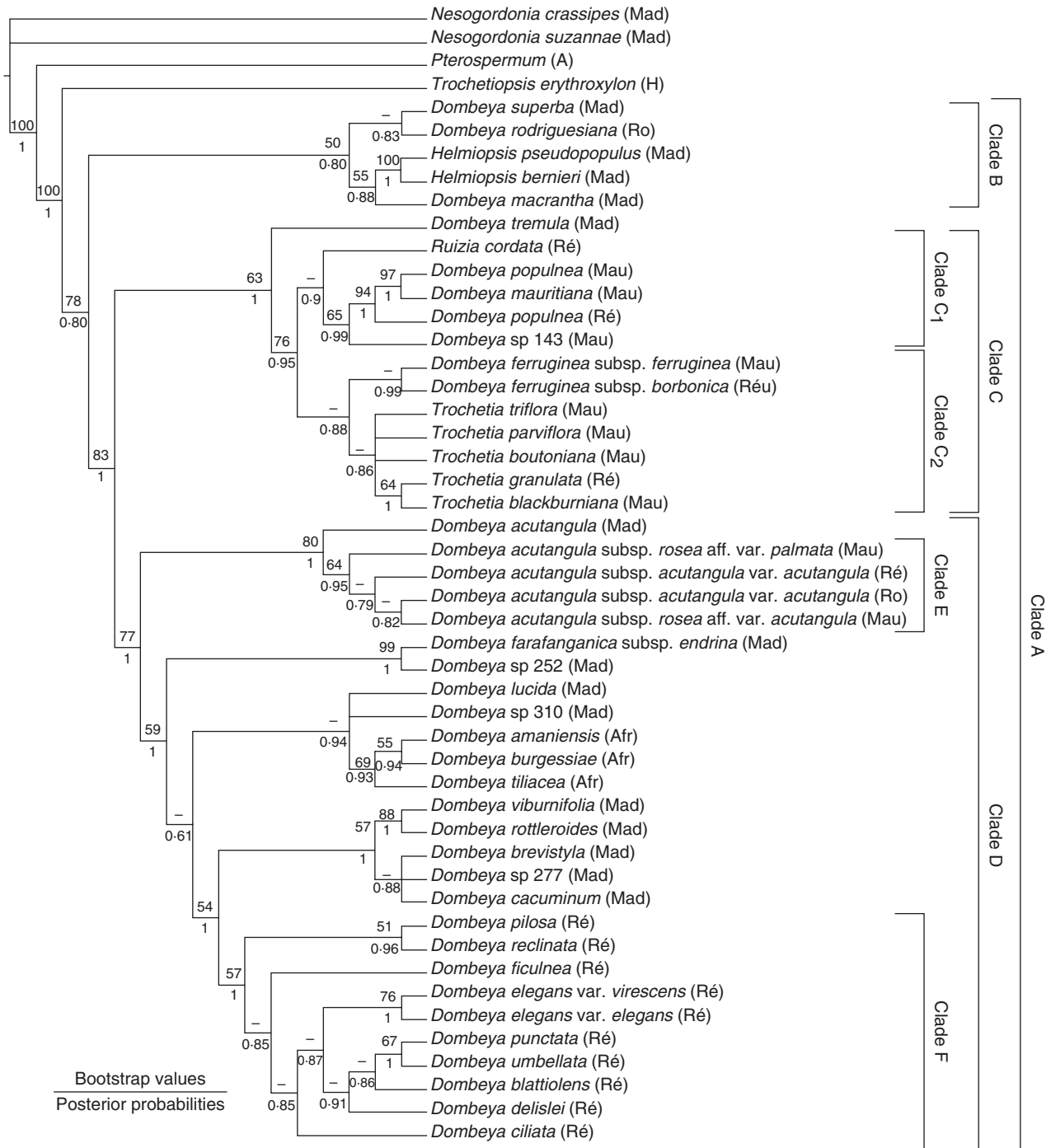
FIG. 2. Bayesian tree with BS (obtained from the MP analysis) and posterior probabilities (PP) values shown above and below the branches respectively. Clades A–F correspond to the clades discussed in the text (see Fig. 1 legend for abbreviations of species geographical distribution).

different taxa, showing their close relationships, it is difficult to find specific characters (i.e. synapomorphies) that define this clade. Within Clade A, the Mascarene Dombeyoideae are polyphyletic and are distributed into four distinct subclades.

Clade B includes the Rodrigues endemic: *D. rodriguesiana*. This species has a biparous pseudo-cyma synflorescence (as defined by Bayer, 1999), as it exhibits reduced staminodes

and short red hairs on all the growing organs. All the other species of this clade are endemic to Madagascar. The general morphology of *D. rodriguesiana* seems radically different from the other species of Clade B, contrasting with *D. macrantha*, *D. superba* and *Helmiopsis bernieri* which are characterized by unifloral synflorescences (as defined by Bayer, 1999) and petaloid staminodes. From a morphological perspective, *D. rodriguesiana* appears closely related to
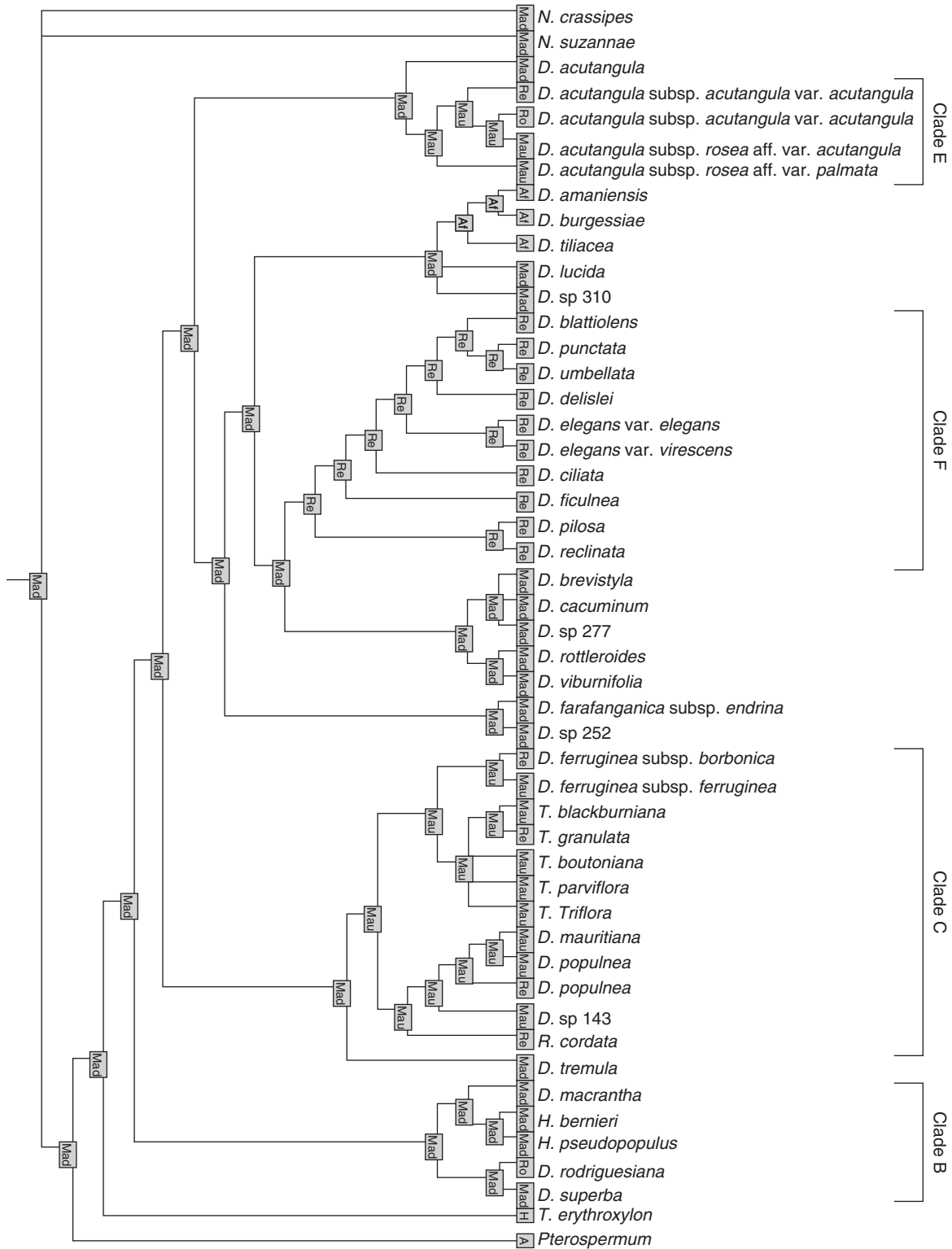
FIG. 3. Biogeographical hypotheses inferred on the Bayesian tree using a parsimony procedure. Abbreviations as in Figs 1 and 2.

another endemic species of Mascarenes, *Dombeya ferruginea* (Le Péchon *et al.*, 2009) from which it has been separated only recently by Friedmann (1981). The molecular phylogenetic position of *D. rodriguesiana* is thus unexpected.

Clade C includes all the species of *Trochetia*, *Ruizia* and four species of *Dombeya*. All these taxa are endemic to the Mascarenes. Except for the presence of *Trochetia*, the relationships among the endemic species of Dombeyoideae from the
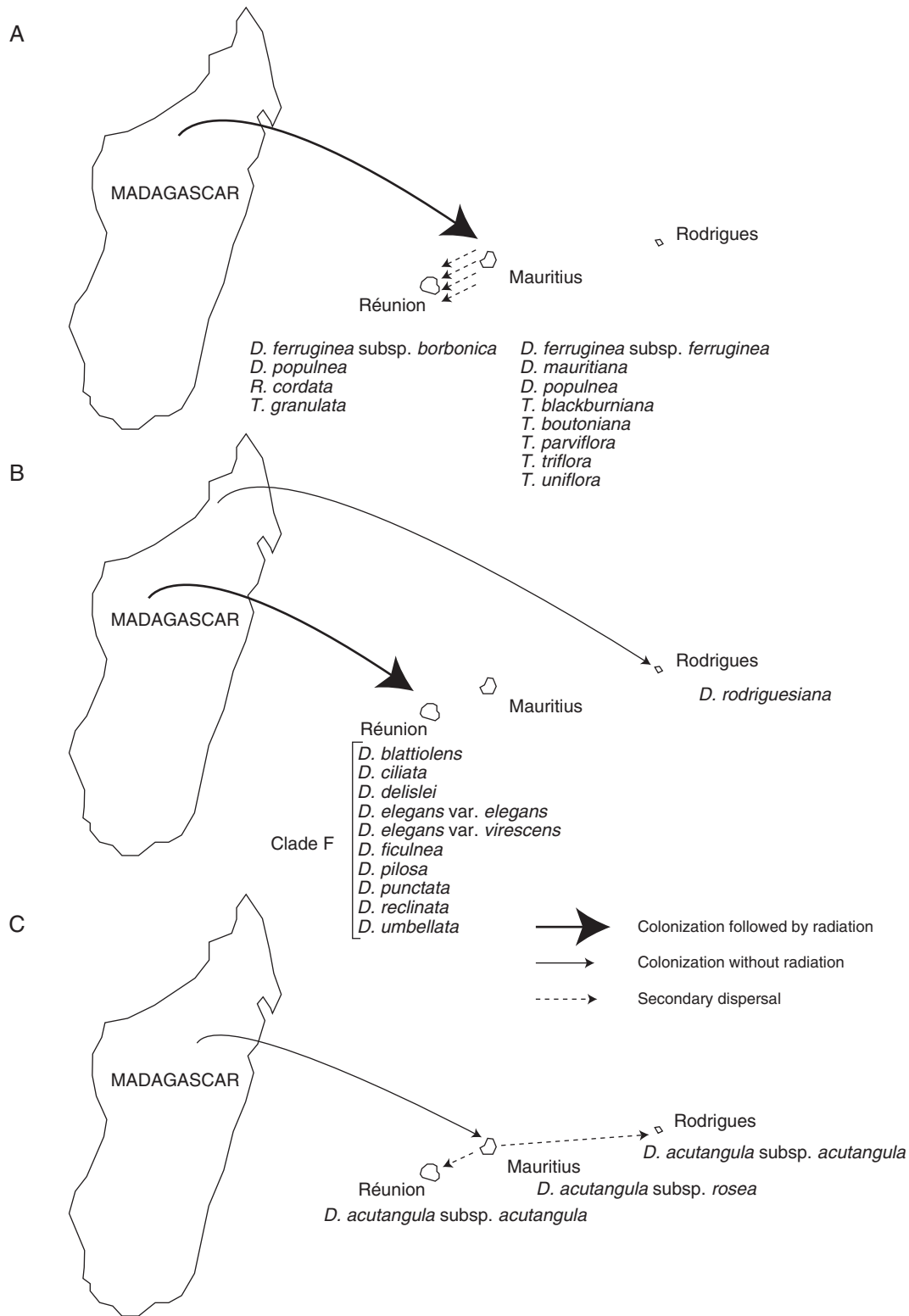
A

MADAGASCAR

Rodrigues

Mauritius

Réunion

D. ferruginea subsp. *borbonica*   *D. ferruginea* subsp. *ferruginea*
D. populnea   *D. mauritiana*
R. cordata   *D. populnea*
T. granulata   *T. blackburniana*
                                       T. boutoniana
                                       T. parviflora
                                       T. triflora
                                       T. uniflora

B

MADAGASCAR

Rodrigues

D. rodriguesiana

Mauritius

Réunion

Clade F
┌ *D. blattiolens*
│ *D. ciliata*
│ *D. delislei*
│ *D. elegans* var. *elegans*
│ *D. elegans* var. *virescens*
│ *D. ficulnea*
│ *D. pilosa*
│ *D. punctata*
│ *D. reclinata*
└ *D. umbellata*

➤ Colonization followed by radiation

→ Colonization without radiation

- - -➤ Secondary dispersal

C

MADAGASCAR

Rodrigues

D. acutangula subsp. *acutangula*

Mauritius

D. acutangula subsp. *rosea*

Réunion

D. acutangula subsp. *acutangula*

FIG. 4. Colonization hypotheses of the Mascarene archipelago (A) for clade B, (B) for clade F and *D. rodriguesiana* and (C) for clade E.

Mascarenes are consistent with previous morphological analyses (Le Péchon *et al.*, 2009). This monophyletic group is divided into two subclades (Clade C_1 and Clade C_2; see the BI tree in Fig. 2). Clade C_1 includes the genus *Ruizia*,

D. populnea (section *Assonia* Cordem.), *Dombeya mauritiana* and an undescribed Mauritius species (*D.* sp. 143). Using a morphological approach, *D. populnea*, *D. mauritiana* and *Ruizia cordata* can be characterized by their distinctive
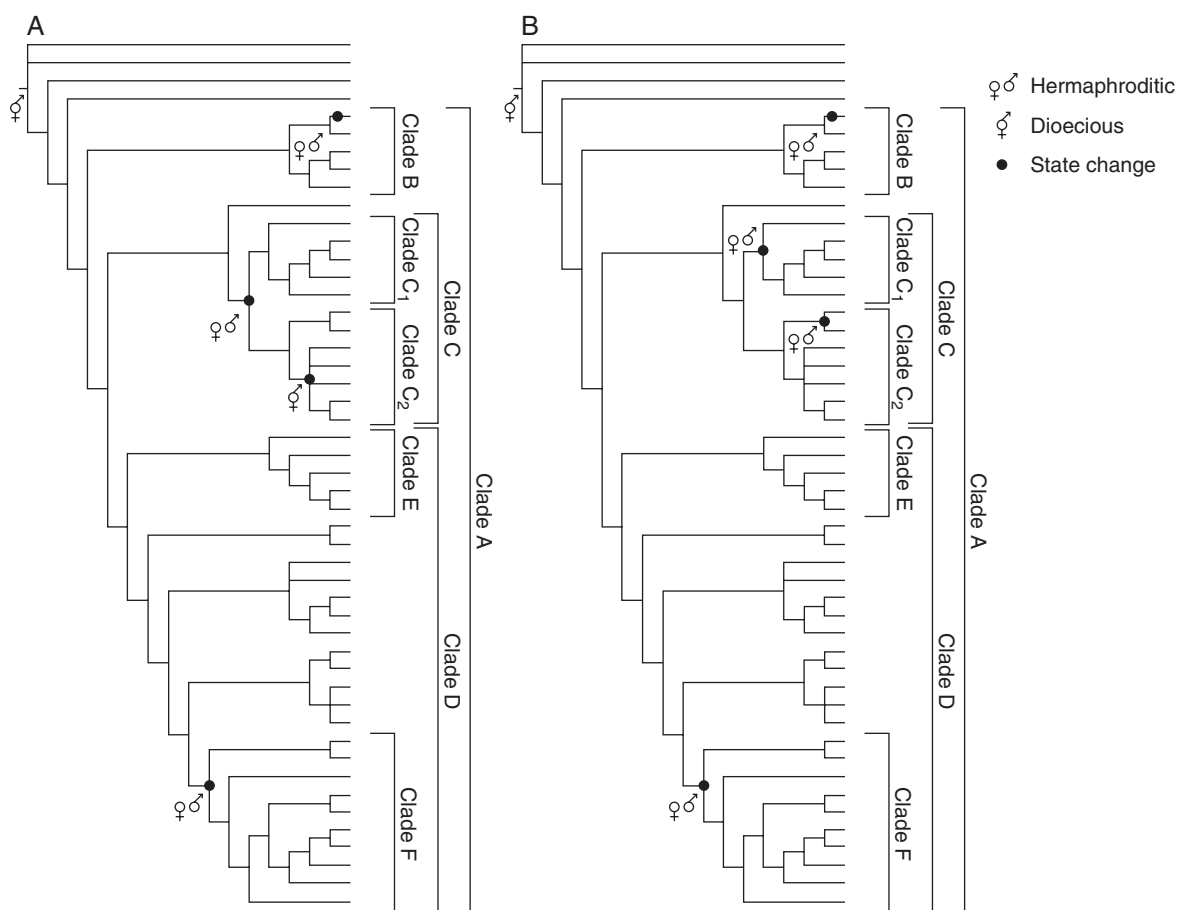
F<small>IG</small>. 5. Evolutionary reconstruction of breeding system pattern inferred on the Bayesian tree using a parsimony procedure: (A) AccTran optimization; (B) DelTran optimization. Clades A–F correspond to the clades discussed in the text.

heterophylly. In addition, the shape and structure of the epicalyx of these two *Dombeya* species are unique among the genus. The floral bracts are fused and the epicalyx comprises a single structure. In *D. populnea*, this structure is persistent and hairless in the specimens from La Réunion, whereas in Mauritius it is deciduous with stellate hairs. In the present phylogenetic analysis, *D. populnea* appears paraphyletic. The Mauritius specimen is more closely related to *D. mauritiana* than the specimen of *D. populnea* from La Réunion. The taxonomic delimitations between *D. mauritiana* and *D. populnea* have not been clearly established (Friedmann, 1987).

The phylogenetic relationships within Clade C_2 confirm the monophyly of *Trochetia* (Le Péchon *et al.*, 2009). Morphologically, *Trochetia* is homogenous and defined by the presence of a spathiform epicalyx, which encloses completely the young floral bud. In addition, the synflorescences (as defined by Bayer, 1999) of *Trochetia* are organized in uni- or tri-floral pseudo-cymes, which are unique in the Mascarenes. *Dombeya ferruginea*, the molecular sister-group of *Trochetia*, is characterized by the presence of biparous pseudo-cymes; an epicalyx composed of three subulate floral bracts; a reduced flower diameter; and sometimes the corolla is absent. Based on morphological characters, *Trochetia* might be considered more closely related to the Malagasy genus *Helmiopsis* (Le Péchon *et al.*, 2009). These two genera share ovoid fruit,

deciduous petals and scaly hairs. However, the latter synapomorphic character is distributed erratically among the Dombeyoideae and is present in several other genera (*Dombeya* Arènes, *Harmsia* K. Schum., *Cheirolaena* Benth. and *Paramelhania* Arènes; Jenny *et al.*, 1999). This suggests a potentially high level of homoplasy, especially for the highly plastic indument. *Trochetia* and *D. ferruginea* display strongly contrasting characters. Therefore, significant structural modifications could be rapidly acquired (especially in insular context; Baldwin *et al.*, 1991; Emerson, 2002). Further studies using both morphological and molecular data are needed to precisely determine the taxonomic position of *Trochetia* in Dombeyoideae.

Clade E includes all the endemic subspecies and varieties of *D. acutangula* from the Mascarenes. This species is the most widely distributed of the genus *Dombeya* [i.e. Continental Africa (Seyani, 1991), Madagascar (Hochreutiner, 1926; Arènes, 1959*b*) and Mascarenes (Arènes, 1959*a*; Friedmann, 1987; Seyani, 1991)] and presents such tremendous morphological variations among populations that Arènes (1959*a*, *b*) and Hochreutiner (1926) distinguished four different species on the basis of leaf shape (i.e. entire to deeply incised leaves) and on the length of the floral peduncle. All these species were placed in synonymy by Seyani (1991). In the Mascarenes, Friedmann (1987) separated two subspecies

according to petal coloration. In spite of this high intra-specific variability, the subspecies of *D. acutangula* appear monophyletic. Additionally, *D. acutangula* possesses biparous pseudo-cymes (*sensu* Bayer, 1999) composed of two divergent cincinni. This type of synflorescence is unique within *Dombeya* (Arènes, 1959*a*, *b*; Friedmann, 1987; Seyani, 1991).

Clade F is exclusively composed of *Dombeya* from La Réunion. This monophyletic group is also morphologically variable. Only the structure of the synflorescence (an umbellate pseudo-cyme with a single axis of flowering; Bayer, 1999) remains constant. However, this synflorescence architecture is not unique to species from La Réunion because *Dombeya brevistyla* (a species endemic to Madagascar) also exhibits this character. From an ecological point of view, the species of Clade F diversified in habitats at elevations from 800 to 2000 m. These types of ecosystems, which occupy a large area in La Réunion, are absent from Mauritius and Rodrigues islands. The heterogeneity associated with the topographic complexity of the island (i.e. presence of a windward zone and a leeward zone; Strasberg *et al.*, 2005) engendered the creation of multiple environmental conditions, and led to highly diverse ecosystems. The species of Clade F are mainly distributed in the mountain windward rainforests (e.g. *Dombeya pilosa*, *D. ficulnea*, *D. elegans*) but can also be found in the mountain leeward rainforests (e.g. *Dombeya delislei*, *D. punctata*). This diversification, both ecological and taxonomic, probably illustrates an important adaptive radiation event.

Hypotheses of colonization of the Mascarene archipelago

The Mascarenes is a relatively young oceanic archipelago (<10 My; Giorgi and Borchiellini, 1998; Hanyu *et al.*, 2001; Sheth *et al.*, 2003) resulting from a volcanic 'hotspot'. This means that the three islands have never been in contact with any other emerged land. The initial establishment of the fauna and flora could only be explained by long-distance dispersal and colonization events. The number of these events can be assessed from the phylogenetic relationships. Dispersal from Madagascar to Rodrigues Island can be inferred to explain the geographic distribution of *D. rodriguesiana* (Clade B). The phylogenetic history of Clade C is more complex. A single dispersal event from Madagascar to Mauritius can be inferred, followed by four dispersals of Mauritius to La Réunion (for *Ruizia cordata*, *Dombeya populnea*, *D. ferruginea* subsp. *borbonica* and *Trochetia granulata*). The majority of species are restricted to the dry lowland forests of Mauritius and La Réunion (e.g. *Ruizia cordata*, *Dombeya populnea*, *D. mauritiana*, *Trochetia boutoniana*, *T. uniflora*, *T. parviflora*). Although *Trochetia triflora*, *T. blackburniana*, *D.* sp. 143 and *D. ferruginea* subsp. *ferruginea* are located in moister environments of Mauritius. *Trochetia granulata* and *D. ferruginea* subsp. *borbonica* are distributed in leeward mountain rainforests, which are present in La Réunion but absent in the other two islands.

For Clade E, an initial colonization of Mauritius by *D. acutangula* can be inferred, followed by two separate dispersal events to La Réunion and Rodrigues.

Clades C and E include taxa distributed on the three islands of the Mascarenes. The ancestral distribution of these two clades is likely to be Mauritius Island. This phylogenetic inference is congruent with the geological data and the age of the islands.

According to the phylogeographic inference, a unique event of dispersal from Madagascar to La Réunion, followed by a high diversification can explain the diversity within Clade F. The species of this monophyletic group are mainly located in mountain windward rainforest of La Réunion.

Crisp *et al.* (2009) demonstrated that the distribution of many angiosperm groups in the southern hemisphere is to a large extent conserved phylogenetically and ecologically according to the theory of 'phylogenetic niche conservatism' (Wiens and Graham, 2005). This theory suggests that taxa evolving from a common ancestor are likely to diversify in the same habitat as their ancestor. Because the Malagasy sister-group of Clade F also involves taxa found in mountainous ecosystems, the diversification of the La Réunion species can illustrate such an example of niche conservatism. However, this hypothesis should be tested by further study of Malagasy taxa in order to confirm the observed relationship and the ecology of the retrieved sister-group of Clade F.

In the present study, the three Mascarene clades (Clade C, Clade E and Clade F) and *D. rodriguesiana* (in Clade B), each have a Malagasy origin. This phylogeographic pattern supports the close relationships between the Malagasy and the Mascarene archipelago hypothesized by Cadet (1980). In addition, numerous phylogenetic studies of other organisms obtained similar results. For example, studies within *Polyscias* (Araliaceae; Plunkett *et al.*, 2004), angraecoid orchids (Orchidaceae; Micheneau *et al.*, 2008), geckos (*Phelsuma*; Austin *et al.*, 2004), tree ferns (*Cyathea*; Janssen *et al.*, 2008), *Gaetnera* (Rubiaceae; Malcomber, 2002) demonstrated that all the Mascarene species probably have a Malagasy origin.

Evolution of breeding systems

The majority of Dombeyoideae species have been described to bear only hermaphroditic flowers (Bayer and Kubitzki, 2003). Certain genera, such as *Burretiodendron* Rehder and *Excentodendron* Hung T.Chang and R.H.Miao (Bayer and Kubitzki, 2003; Tang *et al.*, 2006, 2007*a*, *b*), along with *Astiria*, *Ruizia* and *Dombeya* (Jacob de Cordemoy, 1895; Friedmann, 1987; Humeau, 1999; Humeau *et al.*, 1999*a*, *b*; 2003; Bayer and Kubitzki, 2003), involve species described as monoecious or dioecious. Amongst *Dombeya*, only the endemic species from the Mascarenes are considered dioecious (Jacob de Cordemoy, 1895; Friedmann, 1987; Humeau, 1999; Humeau *et al.*, 1999*a*, *b*, 2003). This dioecy is cited as cryptic because the flowers appear morphologically bisexual; however, only one of the two sexes is functional (Humeau, 1999; Humeau *et al.*, 1999*a*, *b*). In some species (e.g. *Dombeya delislei*, *D. ciliata*, *D. pilosa*), functionally male specimens may carry fruits with a small proportion of fertile seeds. This phenomenon has been referred to 'leaky dioecy' (Humeau *et al.*, 1999*a*, *b*). The fact that the flowers are morphologically bisexual implies that this dioecy is derived compared with hermaphrodism (Tang *et al.*, 2006). The evolution of breeding systems inferred from the cladograms (Fig. 3) supports this hypothesis in accordance with Le Péchon *et al.*

(2009). Regardless of the evolutionary scenario, dioecy appears as homoplastic. In the present study, at least three acquisitions of this breeding system were distinguished in the Mascarenes. Dioecy is a functional feature that can be expressed in different ways. Thus, a number of different processes might explain the separation of sexes from hermaphroditic flowers. For instance, a male flower can be obtained by the reduction (or loss) of the stigmata (*D. ciliata*, *D. pilosa*), by loss (or sterility) of the ovules (*D. ficulnea*), or a combination of the two processes (Humeau, 1999). The same type of differential expression can be observed in female flowers in the absence of pollen (*D. ferruginea*; Friedmann, 1987) or in the presence of sterile pollen (*D. punctata*, *D. elegans*; Humeau, 1999). Therefore dioecy appears as a combination of several characters. The most recent studies of the genus *Dombeya* focused on the expression of dioecy within Clade F (Humeau, 1999; Humeau *et al.*, 1999*a*, *b*, 2003). However, despite the taxonomic descriptions of Friedmann (1987) and Jacob de Cordemoy (1895), very little information is currently available on the expression of dioecy within Clade C. The presence of dioecy within each of the endemic Mascarene clades can have emerged via a particular combination of characters.

The independent acquisition of sex separation raises several questions about the origin of the dioecy, particularly in the insular context. The frequency of dioecious taxa in island ecosystems is higher than on continents (Bawa, 1980). The Dombeyoideae from the Mascarene archipelago illustrate this phenomenon well. Several hypotheses might explain the acquisition of dioecy. The classic argument is that this breeding system is a mechanism to avoid selfing (Sakaï *et al.*, 1997), thus sex separation could be an adaptive advantage on islands. Indeed, genetic diversity is limited due to long-distance dispersal and loss of contact with the conspecific continental population. The lack of genetic diversity could represent a selective pressure to evolve a breeding system such as dioecy that implies obligatory outcrossing. Other hypotheses (i.e. resource allocation, ecological factors and fruit predation) have also been developed to explain the sex separation (Bawa, 1980; Thomson and Burnet, 1990). However, studies of the Hawaiian flora showed that the high frequency of dioecious taxa is the consequence of a number of dimorphic colonists as well as autochthonous evolution of sex separation (Sakaï *et al.*, 1995*a*, *b*). Arènes (1959*b*) described all Malagasy taxa as hermaphroditic but he noticed that some specimens may bear unisexual flowers amongst bisexual flowers. It is worth noting that Arènes (1959*a*, *b*) worked exclusively on herbarium specimens, and the cryptic dioecy makes the identification of dioecious species in dried specimens particularly difficult (Le Péchon *et al.*, 2009). Thus, each endemic dioecious clade from the Mascarenes might have a Malagasy dioecious group as sister-group. To conclude on the origin of dioecy in the Mascarene Dombeyoideae (i.e. autochonous versus phylogenetic origin), further ecological and taxonomical studies are necessary, especially for the Malagasy taxa.

Regardless the origin of dioecy, this breeding system seems to be favoured in an insular environment. The present study suggests that a functional dioecy has differentiated for each endemic clade. For instance, *D. acutangula*, the only hermaphroditic and native species of the archipelago, presents a highly functional and morphological variability among individuals within and among populations. Some produce more pollen, larger flowers and fewer fruits than others, which produce less pollen, smaller flowers and more fruits (L. D. B. Gigord, University of Lausanne, Switzerland, unpubl. res.), and this could therefore be interpreted as a sexual differentiation toward dioecy. This could be especially selected to avoid autogamy in small and scattered natural populations, which is often the case in an insular context. This hypothesis is especially true for species that possess a late-acting self-incompatibility system such as *D. pilosa*, *D. delislei* and *D. acutangula* (Humeau 1999; Gigord *et al.*, 1998). The expression of this incompatibility system involves a systematic loss of ovules. As a consequence, the reproductive success of such species tends to be extremely low, in particular in small populations. This strong selective pressure might explain the origin of the acquisition or the maintenance of functional dioecy in the Dombeyoideae of the Mascarenes.

Conclusions

This first molecular phylogeny highlights some taxonomic issues within the Dombeyoideae. Indeed, although the sampling was focused on the Mascarenes, the limits and distinctions of the genera *Dombeya*, *Trochetia* and *Ruizia* should be reconsidered. Determining the precise nature of such taxonomic and nomenclatural rearrangements requires stable and well-supported phylogenies. To that end a comprehensive work in phylogenetic systematics including both a more complete sampling of the members of Dombeyoideae and the application of new molecular markers, needs to be conducted in order to validate these results (i.e. the paraphyly of *Dombeya* and the positions of *Ruizia* and *Trochetia*). This first molecular historical framework clarifies the multiple origins of the Mascarene Dombeyoideae. Within the archipelago, this subfamily is divided into four distinct monophyletic groups originating independently by dispersal from Madagascar. The close phylogeographic relationships between the flora of the Mascarenes and Madagascar are confirmed. The phylogenetic pattern reveals two independent adaptive radiations within two main different ecosystems. Despite their independent origins and a distinct evolutionary history, each endemic clade has developed a different breeding system (dioecy) compared with the Malagasy Dombeyoideae which are considered as monocline. The present molecular phylogeny suggests at least three distinct acquisitions of this breeding system. This multiple acquisition of dioecy in the Mascarenes appears to be the result of evolutionary convergence, which may be the consequence of selective pressures particular to the insular environments. We think that the Dombeyoideae could be a valuable group for the study of evolutionary processes such as adaptive radiation linked to rapid speciation.

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