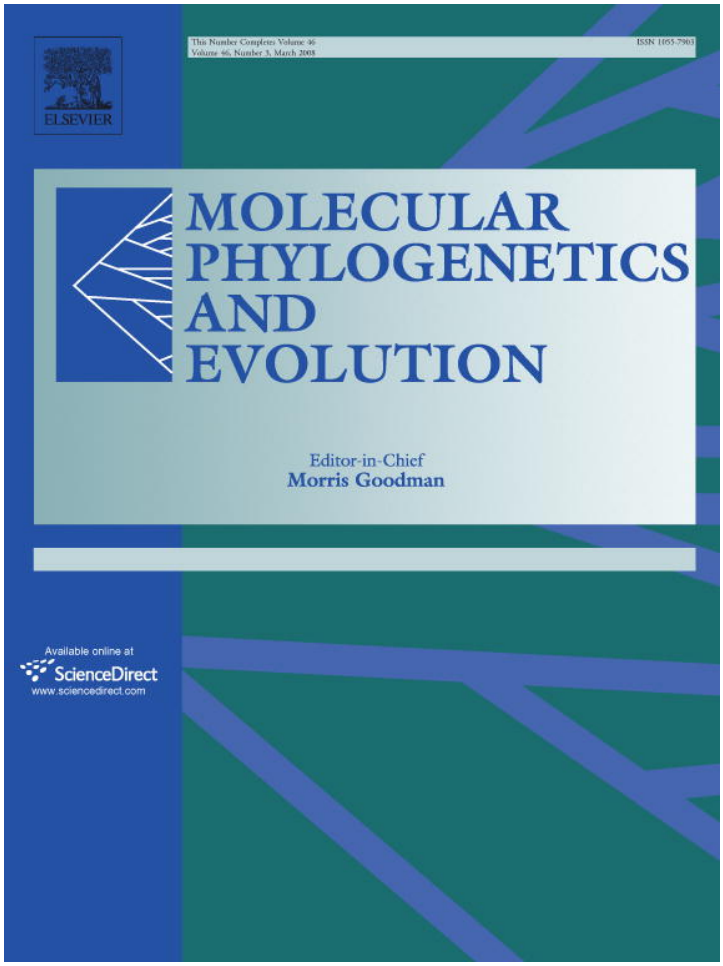

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Phylogenetics and biogeography of Mascarene angraecoid orchids (Vandeae, Orchidaceae)

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Abstract

The large angraecoid orchid clade (subtribe Angraecinae *sensu lato*) has undergone extensive radiation in the western Indian Ocean, which includes Africa, Madagascar, and a number of Indian Ocean islands, such as the Mascarene Archipelago. To investigate systematics and biogeography of these Mascarene orchids, phylogenetic relationships were inferred from four plastid DNA regions, *trnL* intron, *trnL-F* intergenic spacer, *matK* gene, and *rps16* intron. Parsimony and Bayesian analyses provided identical sets of relationships within the subtribe; the large genus *Angraecum* as currently circumscribed does not form an exclusive clade. *Bonniera*, an endemic genus to Reunion, is shown to be embedded in part of *Angraecum*. Evidence from our research supports the main origin of Mascarene Angraecinae from Madagascar, and although there were many independent colonizations, only a few of the lineages radiated within the Mascarene Archipelago.

Keywords: Angraecoid orchids; Biogeography; Mascarene Archipelago; Molecular phylogeny; Oceanic islands; Orchidaceae; Vandeae

1. Introduction

Oceanic islands have long played a major role in the understanding of biological diversity and evolutionary processes (Stuessy and Ono, 1998). In contrast to continental islands that share a biological heritage with the continents from which they separated, oceanic islands have never been connected to any landmass. For a species to establish a population in such isolated and reduced habitats may be difficult, and only immigrants with both high "disperser" and "founder" faculties can succeed (Carlquist, 1974). Consequently, such ecosystems have low species diversity compared with the mainland, but a high rate of endemism because species often evolve under ecological conditions radically different from their original area (Eliasson, 1995). Depending on the distance to the closest continent, the probability of colonization may be low (McArthur and Wilson, 1967), and endemic lineages have often been attributed to diversification from a single ancestral species (Darwin, 1859; Sakai et al., 1997), sometimes providing extraordinary examples of adaptive radiations (e.g. Carlquist, 1974, 1980; Wagner and Funk, 1995; Baldwin and Sanderson, 1998; Lindqvist and Albert, 2002). Although the history of the fauna and flora of the Hawaiian Archipelago and Galapagos Islands has been intensively investigated (e.g. Baldwin et al., 1991; Baldwin, 1998; Ballard and Sytsma, 2000; Lowrey et al., 2001), the Mascarene islands have received comparatively low levels of interest, and few studies on origins and establishment of biological

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diversity have been conducted thus far. The Mascarene Archipelago consists of three main islands, Reunion, Mauritius, and Rodriguez, situated in the western Indian Ocean, at 800, 1000, and 1500 km from the eastern coast of Madagascar, respectively. Reunion is the youngest island of the Archipelago, having emerged approximately three million years ago (McDougall, 1971). It is characterized by a high elevational gradient (to 3070 m asl) and represents the highest point in the Indian Ocean. The Mascarenes together with Madagascar and other nearby Indian Ocean islands belong to one of the 34 biodiversity hotspots (Myers et al., 2000; Mittermeier et al., 2005). On Reunion, approximately 30% of the original vegetation still remains (Strasberg et al., 2005), whereas less than one percent has been preserved on Mauritius (Strahm, 1993).

Although orchids are generally under represented on oceanic islands (e.g. only three native species have been recorded in Hawaii and one in the Juan Fernández Archipelago), they are numerous on the Mascarenes, especially Reunion. With approximately 130 species (du Petit-Thouars, 1822; de Cordemoy, 1895, 1899; Roberts, 2001; see also Jacquemyn et al., 2005), Orchidaceae are the largest family on the island, representing approximately 20% of the native vascular flora. On Reunion, angraecoid orchids (Angraecinae *sensu lato*, see below) are the most important groups of orchids, with 52 species (40.4% endemic) in 11 genera (*Aerangis*, *Aeranthes*, *Angraecopsis, Angraecum*, *Beclardia, Bonniera*, *Cryptopus*, *Jumellea*, *Oeonia*, *Oeoniella*, and *Solenangis*; Table 1).

According to the most recent orchid classification based largely on molecular data (Chase et al., 2003), angraecoid orchids are members of tribe Vandeae (subfamily Epidendroideae) along with Aeridinae (*c.* 98 genera, 1350 species) and Polystachyinae (*c.* 4 genera, 230 species; Chase, 2005). Historically, monopodial angraecoid orchids were referred to two subtribes, Angraecinae (*c.* 17 genera, 410 species) and Aerangidinae (*c.* 32 genera, 350 species) (e.g. Schlechter, 1926; Dressler, 1981, 1993; Chase et al., 2003), whereas recent insights from molecular phylogenetics of Vandeae have led to them being treated as a single subtribe (i.e Angraecinae *sensu lato*; Carlsward et al., 2006). However systematics of Old World angraecoid orchids remains understudied, especially Mascarene species for which nomenclatural confusion still exists. The most recent revisions date from the nineteenth century (du Petit-Thouars, 1822; de Cordemoy, 1895, 1899) and were based on herbarium specimens of Charles Frappier (1853–1895), which unfortunately have deteriorated badly.

Table 1
Subtribe Angraecinae *sensu lato* on the Mascarenes: number of species and geographical distribution on the three islands (named varieties and subspecies are not included)

Genera	REU	MAU	ROD	MS
Aerangis Rchb.f (1865)	1			1
Aeranthes Lindl. (1824)	5 (2)	1	1	5 (2)
Angraecopsis Kraenzl. (1900)	1	1		1
Angraecum Bory (1804)	28 (12)	17 (1)	1 (1)	30 (21)
Beclardia A.Rich. (1928)	1	1		1
Bonniera Cordem. (1839)	2 (2)			2 (2)
Cryptopus Lindl. (1824)	1	1		1
Jumellea Schltr. (1914)	9 (5)	4	1	9 (9)
Oeonia Lindl. (1824)	2	1		2
Oeoniella Schltr. (1918)	1	1	1 (1)	2 (1)
Solenangis Schltr. (1918)	1	1		1
Total	52 (21)	28 (1)	4 (2)	55 (35)
% Endemism	40.4	3.6	50	63.6

Parentheses show the number of endemic species (REU, Reunion; MAU, Mauritius; ROD, Rodriguez; MS, Mascarenes) (Check list based on du Petit-Thouars, 1822; de Cordemoy, 1895, 1899; Roberts, 2001; T. Pailler and J. Bosser, pers. com.; pers. obs.).

The Old World angraecoid orchids are famous for their spectacular flowers (i.e. white, nectariferous, long-spurred flowers) and highly specialized hawk-moth pollination (e.g. Darwin, 1862; Nilsson et al., 1985, 1987; Nilsson and Rabakonandrianina, 1988; Wasserthal, 1997; Luyt and Johnson, 2001). In contrast, oceanic islands are known for the paucity of their pollinator entomofauna. On the Hawaiian Islands, for example, only 15% of insect families are present (Barrett, 1996). Because of this lack of insects, insular floras mainly consist of unspecialized generalist flowers (mostly small and green or white), reflecting weakly specialized interactions with the insect fauna (Carlquist, 1974; Lloyd, 1985; Barrett, 1996; Anderson et al., 2001; Olesen et al., 2002; Lehnebach and Robertson, 2004). Insect scarcity is also likely to have led to the high frequency of wind-pollinated (e.g. Carlquist, 1974; Anderson et al., 2001) and/or bird-pollinated flowers often observed in insular floras (e.g. Feinsinger et al., 1982; Anderson et al., 2001; Anderson, 2003; Bernardello et al., 2004; Dupont et al., 2004; Micheneau et al., 2006). Contrary to general patterns observed on oceanic islands, Reunion hosts a rich insect fauna: 15 species of diurnal Lepidoptera and more than 450 species of Heterocera, including 13 species of native sphingids (Guillermet and Guillermet, 1986). In comparison, only six species of hawk-moth have been recorded in Hawaii and none in New Zealand (Carlquist, 1980; Barrett, 1996). Diptera and Hymenoptera are also well represented on Reunion, and together they have an extensive interaction with the native flora (e.g. Pailler and Thompson, 1997; Humeau et al., 1999). On the Mascarenes especially, the great diversity of highly pollinator-dependant angraecoid orchids offers the opportunity to study establishment and evolution of endemic island groups.

In this study, we present a phylogenetic analysis of Mascarene angraecoid orchids based on sequences of four plastid DNA regions. Especially for angraecoid orchids in which floral convergence toward moth pollination is expected (Garay, 1973), DNA sequences should provide useful data to elucidate phylogenetic relationships. The objectives of this study were to (1) clarify generic relationships within subtribe Angraecinae *sensu lato*, (2) evaluate the monophyly of *Angraecum* and its sections, and (3) reconstruct the biogeographic history of these orchids on

the Mascarene Islands. Although molecular phylogenetics may offer new perspectives on angraecoid orchid systematics, historical biogeography may provide unique insights into origins and establishment of epiphytic orchids on a tropical oceanic Archipelago.

Phylogenetic relationships were inferred using a combined matrix of four plastid DNA regions: *trnL* intron, *trnL-F* intergenic spacer, *matK* exon, and *rps16* intron. They were chosen either because sequences were already available for Neotropical Angraecinae (Carlsward et al., 2003; *trnL-F* intron, *trnL-F* intergenic spacer, and *matK* exon) or because these sequences revealed the highest variability among orchids among a set of previously examined plastid regions (*rps16* intron; Pillon et al., 2007).

2. Material and methods

2.1. Plant material and DNA purification

DNA was obtained from plants collected in the wild on Reunion (Appendix A). These were vouchered at the Herbarium of Reunion (REU; dried or preserved in 70% ethanol), and leaf or floral tissue was dried in silica gel for DNA extraction (Chase and Hills, 1991). Specimens from Madagascar, the Comoros, and Africa came from either the DNA Bank (http://www.rbgkew.org.uk/data/dnaBank/homepage.html) or living collection at the Royal Botanic Gardens (RBG), Kew, except for *Angraecum teres* (B. Kayota, National Museums of Kenya), *A. sacciferum* (DNA bank, Kirstenbosh Research Center, Cape Town), and *Jumellea anjouanensis* (J.-N. Labat, National Natural History Museum of Paris). Vouchers for the sampled taxa are indicated in the Appendix A. We included 48 of 55 Mascarene species (amplifications failed for most absent species, especially for species of *Oeoniella*): 1/1 *Aerangis*, 5/5 *Aeranthes* 1/1 *Angraecopsis*, 27/30 *Angraecum*, 1/1 *Beclardia*, 2/2 *Bonniera*, 1/1 *Cryptopus*, 8/9 *Jumellea*, 1/2 *Oeonia*, 0/2 *Oeoniella*, and 1/1 *Solenangis*. For species from Madagascar and Africa, we included as many species as were available.

Total DNA was extracted from fresh leaves (1 g) or silica-gel dried material (0.3 g) using a modified 2× CTAB (cetyltrimethyl-ammonium bromide) protocol (Doyle and Doyle, 1987). Proteins were removed with SEVAG (chloroform/isoamyl alcohol 24:1). DNA was precipitated with ethanol (−20 °C), resuspended in 1.55 g/mL cesium chloride/ethidium bromide and then purified using a density-dependant gradient. Ethidium was removed with butanol and cesium chloride by dialysis.

2.2. PCR amplification and DNA sequencing

All regions were amplified in a Gene Amp 9700 PCR system (ABI, Applied Biosystems Inc., Warrington, Cheshire, UK). The primers used for amplification and sequencing of each individual region were c/d for the *trnL* intron and e/f for the *trnL-F* intergeneric spacer (Taberlet et al., 1991), *rps16*-1F/*rps16*-2R for the *rps16* intron (Oxelman et al., 1997), and 19F (Molvray et al., 2000), 1326R, 390F (Cuénoud et al., 2002), and *trnK*-2R (Johnson and Soltis, 1994) for *matK*. The amplification mixture for *trnL-F* and *matK* consisted of 1–5 μL of template (of unknown concentration), 5 μL buffer (10×), 6 μL $MgCl_2$ (50 mM), 1 μL dNPTs (10 mM), 1.5 μL bovine serum albumin (BSA, 0.4%), 0.5 μL of each primer (100 ng/mL), 0.3 μL Taq polymerase (5 U/μL) and water to make a total volume of 50 μL. For the *rps16* intron, the mixture consisted of 42.5–45.5 μL of PCR mastermix with 0.5 mM $MgCl_2$ concentration (ABgene, Epsom, Surrey, UK), 1.5 μL BSA (0.4%), 0.5 μL of each primer (100 ng/mL), and 2–5 μL of template. The PCR amplification profiles used for the *trnL-F* region and *rps16* intron consisted of an initial denaturation at 94 °C for 3 min followed by 28 cycles of 1 min at 94 °C, 1 min at 48 °C, and 1 min at 72 °C, with a final extension at 72 °C for 7 min. For *matK*, we used the following program: 2.50 min at 94 °C followed by 29 cycles of 1 min at 94 °C, 45 s at 52 °C and 30 s at 72 °C, with a final extension at 72 °C for 7 min. PCR products were cleaned using QIAquick columns (Qiagen, Inc., East Crawley, UK) following the manufacturer's protocol. Samples were sequenced on an ABI 3100 capillary DNA sequencer using Big Dye terminator v3.1 chemistry following the manufacturer's protocols (ABI). For cleaning of cycle sequencing products, we used precipitation with ethanol.

Sequences were edited and assembled using Sequence Navigator and Autoassembler (ABI), respectively, and were aligned manually following the guidelines of Kelchner (2000). The *trnL-F* sequences (*trnL* intron and *trnL-F* intergeneric spacer) as well as *matK* sequences from Neotropical Angraecinae (*Campylocentrum* and *Dendrophylax*) and *Angraecum cultriforme* were previously published by Carlsward et al. (2003). As outgroup taxa, we chose four species of *Polystachya* (*P. concreta*, *P. fulvilabia*, *P. henrici*, *P. melliodora*) since subtribe Polystachyinae has been previously identified as sister to Vandeae (Chase et al., 2003; Freudenstein et al., 2004).

2.3. Parsimony analysis (PA)

Cladistic analysis using Fitch parsimony (Fitch, 1971) was performed using PAUP* 4.0b (Swofford, 2003). Heuristic searches were performed using tree bisection-reconnection (TBR) swapping with 1000 replicates of random-taxon addition, and saving only 10 trees per replicate to reduce time spent in swapping on large islands of trees. In a second round of analysis, we used all trees found in the tree-limited analysis as starting trees with a limit of 10,000 trees, which were then swapped to completion. In all analyses, gaps were coded as missing data. Levels of internal support were estimated using bootstrap percentages (Elfron, 1979; Felsenstein, 1985): 1000 bootstrap replicates with simple addition and TBR branch swapping, saving 10 trees per replicate (Salamin et al., 2003.) We

combined all plastid regions into one analysis without performing separate analyses because there is no reason to suspect incongruence for regions from a uniparentally inherited, non-recombining genome.

2.4. Bayesian analysis (BA)

Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on the combined matrix. We calculated the model of DNA substitution that best-fitted our data using MODELTEST 3.7 (Posada and Crandall, 1998). The best-fit maximum likelihood model was chosen using the Akaike information criterion (AIC; Akaike, 1974). ModelTest indicated TVM+*I*+*G* (transversional model) as the best-fit model for the combined dataset, but MrBayes cannot implement this model of evolution, characterized by a five-parameter, nucleotide substitution-rate matrix. The substitution model then selected for the Bayesian analysis was the next best-fitting model, the general time reversible model (GTR) (six-parameter nucleotide substitution-rate matrix) with a proportion of invariable sites (*I*) and γ-distributed rate variation across sites (*G*), run with no prior-parameter values. MrBayes was run with four randomly initiated Monte Carlo Markov chains (MCMC) for 5,000,000 generations, sampling trees every 100 generations. Trees were checked for $-\ln L$ value stability, which happened at around 170,000 generations (''burn-in phase''); therefore the first 1700 trees were deleted. The posterior probabilities were determined from the 48,301 remaining trees. The majority-rule consensus tree was constructed using the ''sumt'' option in MrBayes.

2.5. Historical biogeography: ancestral area

We applied the ancestral area analysis of Bremer (1992) to the Bayesian tree (Fig. 3) to infer the biogeographic origin of Mascarene angraecoid orchids based on our results. This cladistic procedure, based on tree topology only, allows approximation of ancestral areas without assumptions about mechanisms of speciation. Five areas of endemism were circumscribed according to the main geographic distribution of angraecoid orchids: Africa, Madagascar, Comoros, Mascarenes, and America. The areas inhabited by each taxon were treated as binary characters with two states, present or absent. Analyses were based on the whole tree and separately on clades I and II. Two assumptions have been made concerning the origin of the taxa with a widespread distribution: *assumption 0*: widespread taxa have been considered as having the most ancient origin (i.e. Africa > Madagascar > Mascarenes); *assumption 1*: no assumption has been made concerning the ancestral origin of widespread taxa (Africa = Madagascar = Mascarenes). The area characters were optimized on the Bayesian tree using either forward or reverse Camin-Sokal parsimony (irreversibility of character), and the number of gains (*G*) (forward) and losses (*L*) (reverse) were computed. The *G*/*L* ratio for each area was then estimated, and the area with the highest *G*/*L* ratio was hypothesized to be the ancestral area (AA).

3. Results

3.1. Combined matrix

The number of taxa included in each individual matrix was: 134 for the *trnL* intron and *trnL-F* intergenic spacer (*Aerangis macrocentra*, *Angraecum calceolus*, *A.* sp. TPCM81, and *Phalaenopsis cornucervi* failed to amplify for both regions); 129 for *matK* (*Aeranthes adenopoda*, *A. henrici*, *A. ramosa*, *Angraecum ramosum*, *A. striatum* × *A. bracteosum*, *A. teretifolium*, *Jumellea anjouanensis*, *J.* sp. JF85 and *Oeonia rosea* failed to amplify); and 120 for *rps16* intron (*Angraecum cadetii*, *A. caulescens*, *A. conchoglosum*, and taxa from Carlsward et al., 2003, were not available). The number of aligned characters contributed by each individual region (after exclusion of incomplete ends and 84 base pairs from the *trnL-F* intron due to difficulties with alignment) was: 1377 from *trnL* intron (228, 16.6% potentially parsimony-informative), 487 from *trnL-F* intergeneric spacer (113, 23.2% potentially parsimony-informative), 1896 from *matK* (306, 16.1% potentially parsimony-informative), and 987 from *rps16* (162, 16.4% potentially parsimony-informative). The combined matrix contained 138 accessions and 4747 aligned characters. Sequences that were unavailable (Neotropical taxa from GenBank) or failed to amplify were coded as missing data in the combined matrix.

3.2. Phylogenetic analysis

The parsimony analysis yielded 9050 equally most-parsimonious trees of 2552 steps, consistency index, CI = 0.64 and retention index, RI = 0.84. One of these (randomly selected) is shown in Figs. 1 and 2 with the nodes that collapse in the strict consensus tree marked by arrowheads. Bayesian analysis produced approximately the same tree topology as parsimony. The Bayesian majority-rule consensus is shown in Fig. 3. The angraecoid orchids consist of two well-supported clades (I and II, Figs. 1 and 3), with 98 and 100 bootstrap percentages (BP), respectively, and each receiving posterior probabilities (PP) of 1.0. Clade I contains a well-supported group (BP 84, PP 1.0), comprising American (*Dendrophylax* and *Campylocentrum*) and African genera previously included in Aerangidinae (*Aerangis*, *Angraecopsis*, *Mystacidium*, *Chamaeangis*, *Solenangis*), and *Angraecum* species found only in Africa (*A. moendense*, *A. infundibulare*, *A. birrimense*, *A. eichlerianum*, *A. doratophyllum*, *A. subulatum*, *A. distichum*, *A. aporoides*; Figs. 1 and 3). Within clade I, another clade was well-supported by Bayesian analyses (PP 1.0). It includes Malagasy genera (with representatives in Mascarenes), characterized by flowers with lobed lips (*Cryptopus*, *Oeonia*, *Beclardia*) and two other species, *Aeranthes henrici* and *Angraecum*

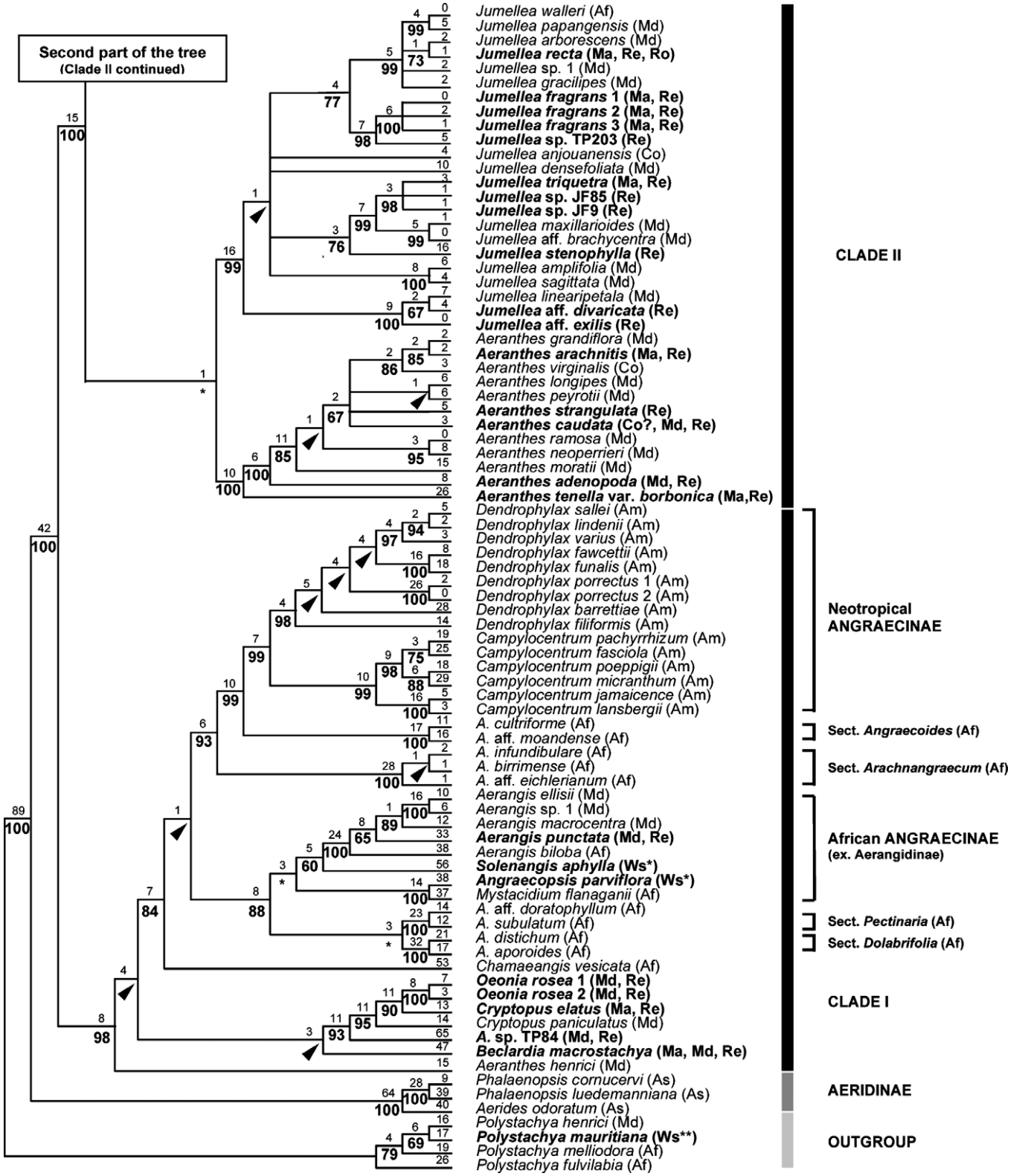

Fig. 1. First part of one of the 9050 most-parsimonious trees from the combined analysis. (length = 2552; consistency index, CI = 0.64; retention index, RI = 0.84). Branch lengths (DELTRAN optimization) are shown above and bootstrap percentages below branches. Clades that received less than 50% bootstrap are indicated with an asterisk. Arrowheads indicate nodes that collapse in the strict consensus tree. Bold, species present on the Mascarenes. Re, Reunion; Ma, Mauritius; Ro, Rodriguez; Co, Comoros; Se, Seychelles; Md, Madagascar; Af, Africa; Am, America; As, Asia; Ws*, widespread Af, Ma, Md, Re; Ws**, widespread Af, Co, Ma, Md, Re, Se. Sectional circumscription of *Angraecum* is according to Garay, 1973; *A.*, *Angraecum*.

sp. TP84, the latter being the only Malagasy–Mascarene endemic *Angraecum* species found in clade I. This species is rare and poorly known; its identification remains to be clarified. In contrast, clade II comprises the bulk of genera

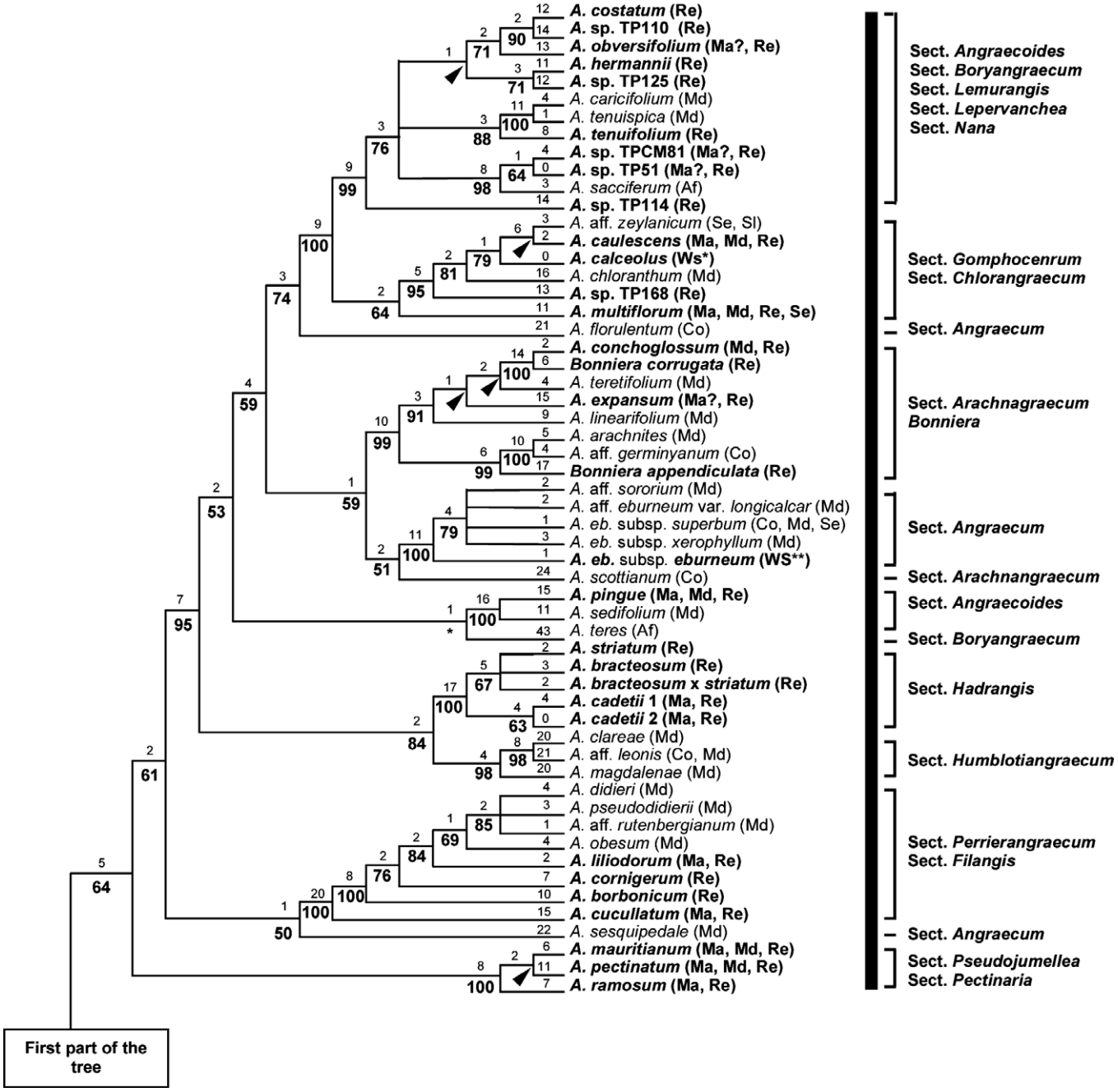

Fig. 2. Second part of the same tree shown in Fig. 1 (genera *Angraecum* and *Bonniera*). Branch lengths (DELTRAN optimization) are shown above and bootstrap percentages below branches. Clades that received less than 50% bootstrap are indicated with an asterisk. Arrowheads indicate nodes that collapse in the strict consensus tree. Bold, species present on the Mascarenes. Re, Reunion; Ma, Mauritius; Ro, Rodriguez; Co, Comoros; Se, Seychelles; Sl, Sri Lanka; Md, Madagascar; Af, Africa; Ws*, widespread Af, Co, Ma, Md, Re, Se; Ws**, widespread Co, Ma, Md, Re, Se. Sectional circumscription of *Angraecum* is according to Garay, 1973; *A.*, *Angraecum* and *eb.*, *eburneum*.

from Madagascar and the Mascarene Islands (the rest of *Angraecum*, *Bonniera*, *Jumellea*, and *Aeranthes*; Figs. 2 and 3). The large genus *Angraecum*, with species in both clades, is clearly polyphyletic: *Angraecum* species in clade II are mostly found in Madagascar or in nearby islands (Mascarenes, Comoros, Seychelles), except for two species exclusively found in Africa, *A. sacciferum* and *A. teres*, both of sect. *Boryangraecum* (small yellow–green flowers). All together, these *Angraecum* species form a weakly supported group in parsimony analysis (BP 64, Fig. 2), but they are strongly supported in the Bayesian analysis (PP 1.0, Fig. 3). There are no morphological characters or structural molecular traits that support the clade II *Angraecum* species. Some species, which belong to the same section (and are morphologically similar) could either be found in clade I or in clade II (e.g. sects. *Arachnangraecum*, *Angraecoides*). Within these *Angraecum* species, which are related to two monophyletic genera principally from Madagascar, *Jumellea* (BP 99, PP 1.0, Figs. 2 and 3) and *Aeranthes* (without *Aeranthes henrici*; BP 100, PP 1.0, Figs. 2 and

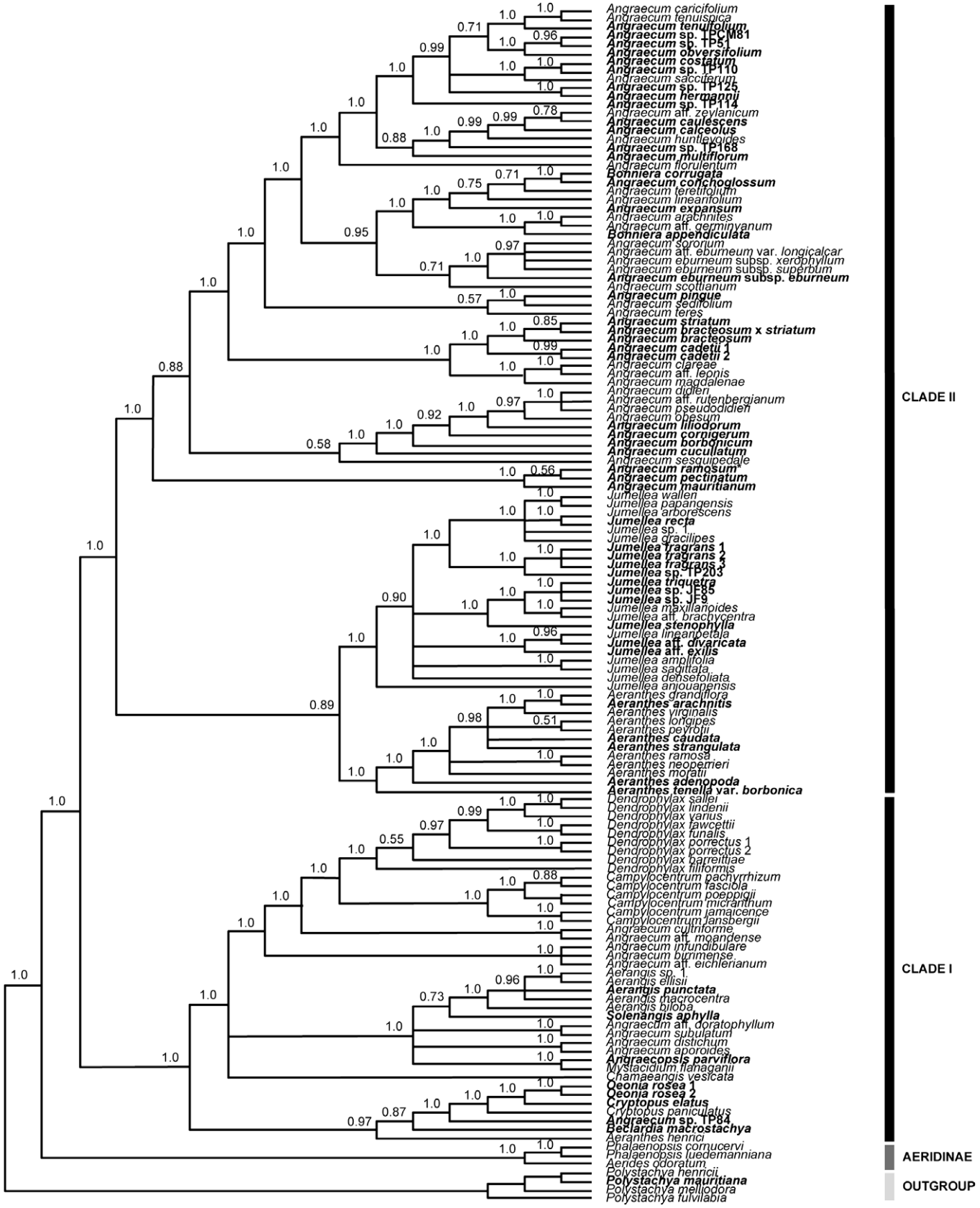

Fig. 3. Bayesian tree with posterior probabilities shown above the branches. Bold, species present on the Mascarenes (see Figs. 1 and 2 for species geographical distribution).

Table 2
Bremer's ancestral areas method (1992) applied to the Bayesian topology (Fig. 3)

Area	*Assumption 0*				*Assumption 1*			
	G	*L*	*G*/*L*	AA	*G*	*L*	*G*/*L*	AA
Whole tree								
Africa	15	30	0.50	0.63	15	30	0.50	0.54
Madagascar	36	45	0.80	1.00	39	42	0.93	1.00
Mascarenes	24	40	0.60	0.75	37	43	0.86	0.92
Comoros	5	21	0.24	0.30	11	31	0.35	0.38
Americas	1	8	0.13	0.16	1	8	0.13	0.14
Clade I								
Africa	8	3	2.67	1.00	8	3	2.67	1.00
Madagascar	6	8	0.75	0.28	8	7	1.14	0.43
Mascarenes	1	6	0.17	0.06	8	22	0.36	0.13
Comoros	0	1	0.00	0.00	0	1	0.00	0.00
Americas	1	6	0.17	0.06	1	6	0.17	0.06
Clade II								
Africa	4	25	0.16	0.18	4	25	0.16	0.16
Madagascar	29	33	0.88	1.00	30	32	0.94	0.81
Mascarenes	23	32	0.72	0.81	29	30	1.03	1.00
Comoros	5	18	0.28	0.32	10	26	0.38	0.32

Assumption 0: widespread taxa are considered to be the oldest (i.e. Africa > Madagascar > Mascarenes). *Assumption 1:* no assumption has been made concerning the origins of widespread taxa (Africa = Madagascar = Mascarenes). *G*, gains; *L*, loss; AA, most probable ancestral area.

3), the Reunion endemic genus *Bonniera* is biphyletic. Few sections of *Angraecum* species from clade II are monophyletic (Fig. 2). However, some clades are well-supported, such as (1) the group of *Angraecum* with characteristic small yellow–green hyaline flowers (sects. *Angraecoides*, *Boryangraecum*, *Lemurangis*, *Lepervanchea*, *Nana*, *Gomphocentrum*, and *Chlorangraecum*; BP 100, PP 1.0, Figs. 2 and 3), (2) the group of species with solitary large white flowers, most with a prominent spur (sects. *Perrierangraecum* and *Filangis*; BP 100, PP 1.0, Figs. 2 and 3), (3) the Mascarene endemic section *Hadrangis* (BP 100, PP 1.0, Figs. 2 and 3) and (4) its sister Malagasy section *Humblotiangraecum* (BP 98, PP 1.0, Figs. 2 and 3).

3.3. Historical biogeography: ancestral area

The method of Bremer (1992) applied to the whole tree showed that Madagascar has the highest probability of being the ancestral area of angraecoid orchids; applying this method separately to clade I and clade II suggested that Africa would be the ancestral area of clade I, and Madagascar (*assumption 0*) and the Mascarenes (*assumption 1*) the ancestral areas of clade II (Table 2).

4. Discussion

4.1. Angraecoid orchid systematics

Our phylogenetic analyses confirm polyphyly of the large genus *Angraecum* as well as the unnaturalness of several of its sections. African species of *Angraecum* are intermixed with other genera in clade I (Figs. 1 and 3), but Malagasy and Indian Ocean island members of the genus form a natural group (clade II, BP 64, PP 1.0; Figs. 2 and 3), including *Bonniera*, a genus with just two species restricted to Reunion; these are clearly not monophyletic within this well-supported clade II (BP 99, PP 1.0; Figs. 2 and 3; Appendix B). The Malagasy *Angreacum* clade is represented by few species in our trees, but the radiation of *Angraecum* species was huge in Madagascar (*c* more than 130 species). A better sampling would provide better evidence of the monophyly of this group, as well as species establishment patterns in Madagascar.

Schlechter (1925) and Garay (1973) previously pointed out the morphological similarity between *Bonniera* and some species in *Angraecum* sect. *Arachnangraecum*. After examination of *Bonniera corrugata*, Garay (1973) reported that this species could be a peloric form of *Angraecum conchoglossum*, which is its sister species in our analyses (BP 100, PP 1.0; Figs. 2 and 3). There are a number of differences in these sequences, which support the idea that *B. corrugata* is a distinct species. Recently, Hermans and Cribb (2005) came to the same morphological conclusion about *B. appendiculata* and *A. arachnites* (i.e. the former is a peloric form of the latter). Our molecular analyses also corroborate this observation: *B. appendiculata* is closely related to *A. arachnites* and *A. germinyanum* (BP 99, PP 1.0, Figs. 2 and 3). The main differences between *Bonniera* species and their close relatives are (1) the lip, which is similar in shape to the other petals, (2) the spur, which is reduced or absent, and (3) the floral scent, which is also reduced or absent. Within Orchidaceae, this sort of peloria is rare, particularly in continental areas. Lip shape and spur reduction (and loss of floral fragrance) appear to be convergent in these insular taxa. Selection for such peculiar peloric forms could have been connected with the absence of hawk-moths in the Mascarenes (i.e. no appropriate pollinator fauna for ancestral colonists), but this speculative hypothesis needs to be further studied.

The large genus *Angraecum* has been divided into 19 sections (Table 3) according to floral characters (e.g. inflorescence, flower size and color, and spur length; Garay, 1973). Sixteen of these sections are represented in our analyses; we have no representatives of sects. *Acaulia* (6 species, Madagascar), *Afrangraecum* (10 species, Africa), and *Conchoglossum* (7 species, Madagascar and Africa; note that recently Stewart et al., 2006, transferred all *Angraecum* sect. *Angraecoides* species from Africa to sect. *Conchoglossum*, and all species of sect. *Conchoglossum* from Madagascar to sect. *Angraecoides*). In this study, we have followed the sectional system of Garay (1973), except for species later described and *A. hermannii*, which we have moved from sect. *Pectinaria* to sect. *Lemurangis* because its morphological characters indicate that is was misplaced. On the basis of our molecular results, few sections appear to be natural. For example, sects. *Pectinaria*, *Arachnangraecum*, and *Angraecoides* have representatives in both clades I and II (Figs. 1 and 2), and no less than seven sections

Table 3
Number of *Angraecum* species (%) per geographical locality according to section description (varieties not included)

Section description	Number of species (%)			
	AF	MD	MS	Total
Only white or partly white flowers, rather fleshy, small to large[a]	16 (33%)	70 (53%)	15 (50%)	98
Only yellowish to green flowers, thin, small to medium-size[b]	6 (13%)	41 (31%)	10 (33%)	54
White or colored flowers, rather thin, small to medium-size[c]				
White	5 (10%)	9 (7%)	1 (3.5%)	14
Colored	19 (40%)	11 (8%)	4 (13.5%)	34
Unknown color	2 (4%)			2
Green or yellowish green flowers, fleshy, medium-size[d]		2 (1%)		2
Total	48	133	30	204

AF, Africa; MD, Madagascar; MS, Mascarenes.
[a] Sects. *Angraecum*, *Arachnangraecum*, *Dolabrifolia*, *Filangis*, *Hadrangis, Humblotiangraecum*, *Pectinaria*, *Perrierangraecum*, *Pseudojumellea*.
[b] Sects. *Acaulia*, *Boryangraecum*, *Gomphocentrum*, *Lemurangis*, *Lepervanchea*.
[c] Sects. *Afrangraecum*, *Angraecoides*, *Conchoglossum*, *Nana*.
[d] Sect. *Chlorangraecum*.

represent the bulk of the well-supported group composed of species with characteristic yellow–green, small flowers (BP 100, PP 1.0, Figs. 2, 3, and 4A). Garay (1973), Stewart (1980), and more recently Stewart et al. (2006) reported difficulties in assigning a position for some species of *Angraecum*, as well as ambiguous morphological distinctions between some sections, which are often differentiated by only a few characters. Our results provide further support for changing the system of sectional delimitation. The current circumscription is not corroborated by molecular data, and it is not ideal for morphological use (the number of sections has to be reduced). However, better sampling would be desirable before a new sectional nomenclatural revision is appropriate.

Nonetheless, two clearly monophyletic sections do exist: the Malagasy sect. *Humblotiangraecum* (BP 98, PP 1.0) and the Mascarene sect. *Hadrangis* (BP 100, PP 1.0). Both sections, which are closely related (BP 84, PP 1.0), are morphologically similar (multi-flowered inflorescences, white to cream, fleshy flowers, spurs with a wide opening at the entrance; Garay, 1973). Contrary to species of sect. *Humblotiangraecum*, which have flowers that are strongly scented and long-spurred, the species of sect. *Hadrangis* have unscented, short-spurred flowers (see also Bosser, 1987 for description of sect. *Hadrangis*).

4.2. Origin and establishment of Mascarene angraecoid orchids

Our phylogenetic analyses support a Malagasy origin for the angraecoid orchids on the Mascarenes, obviously with many dispersal events (Figs. 1–3) (i.e. in the case of oceanic archipelagos, such as Mascarenes (isolated islands since their origin), biogeographic patterns exhibit solely long-dispersal rather than vicariance). Cadet (1977) suggested that the flora of Reunion has primarily a Malagasy or Afro-Malagasy origin (70–80% of native genera, e.g. *Mimusops*, *Sideroxylon*, Sapotaceae; *Nastus*, Poaceae; *Tabernaemontana*, Apocynaceae; *Pittosporum*, Pittosporaceae). More recent studies based on molecular data have added examples from *Polyscias*, Araliaceae (Plunkett et al. 2004) and *Gaertnera*, Rubiaceae (Malcomber, 2002). To reach the Archipelago from Madagascar, wind dispersal is most likely for Orchidaceae (see Section 4), and cyclonic systems could have played a major role in Mascarene colonization. The cyclonic period lasts from November to April, during which the Mascarenes are under the influence of low-pressure systems. These systems usually cross the Indian Ocean from east to west. However, atypical reverse cyclonic trajectories are not rare, arriving in the Mascarenes after having flirted with Madagascar (e.g. cyclones Felicie in 1971, Ines in 1975, and Hyacinthe in 1981; Cadet, 1977; Mayoka, 1998). Due to their dust-like seeds, orchids are capable of dispersal by wind over great distances (Arditti and Ghami, 2000). However, especially for Orchidaceae, presence of an appropriate mycorrhizal symbiont in the newly colonized habitat is necessary for seed germination and establishment (Dressler, 1981). Little is known about orchid–mycorrhizal partner specificity in tropical regions (Zettler et al., 2003; see however Clements, 1988; Otero et al., 2002), and even less in oceanic islands such as the Mascarenes. According to Otero et al. (2002), the degree of specificity in mycorrhizal interactions varies considerably among species. Due to the high diversity of orchids found on the Mascarenes (both terrestrial and epiphytic), it seems improbable that the fungal partner would have been a limiting factor in establishment of new colonists.

In cases of dispersal to islands, strong reproductive constraints may also create difficulties for establishment, e.g. scarcity or absence of an appropriate pollinator fauna. However in such cases, species that are self-compatible or able to reproduce by autonomous self-pollination (hereafter defined as auto-pollination, *sensu* Catling, 1990) would be favored on isolated islands (Baker, 1955, 1967; Stebbins, 1970). Following this pattern, a possible evolution of auto-pollination should not be excluded for explaining the successful colonization of the Mascarenes by angraecoid species, even if the capacity to reproduce without pollen

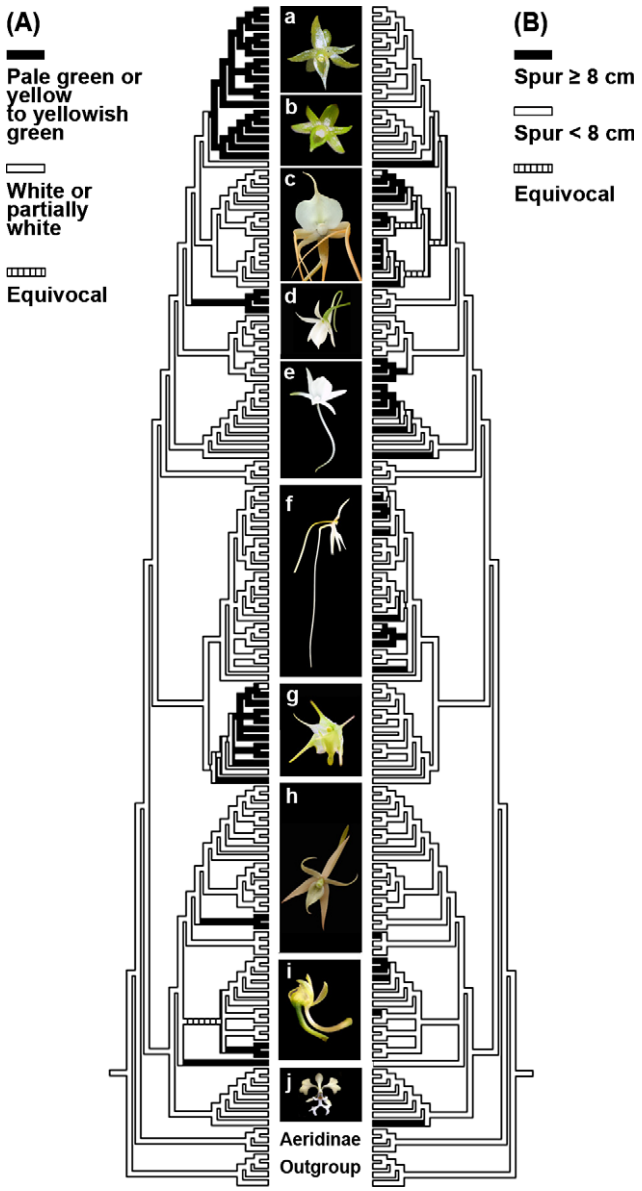

Fig. 4. Flower color (A) and spur length state (B) reconstruction of angraecoid orchids (a. *Angraecum costatum*, b. *Angraecum caulescens*, c. *Angraecum conchoglossum*, d. *Angraecum leonis*, e. *Angraecum borbonicum*, f. *Jumellea stenophylla*, g: *Aeranthes arachnitis*, h. *Angraecum cultriforme*, i. *Angraecopsis parviflora*, j. *Cryptopus elatus*; tree from Fig. 3; color labels are not included for subtribes Aeridinae and Polystachyinae).

vectors was not *a priori* expected in this highly pollinator-dependant group (e.g. auto-pollination is thought to occur in only 0.3% of Vandeae; Dressler, 1993). On Reunion, for example, approximately 15–20% of angraecoid orchids can auto-pollinate, and this rate reaches 30–40% for species exclusively restricted to Reunion (against 6% for species occurring also in Madagascar; Jacquemyn et al., 2005; Micheneau, 2005; see also Micheneau et al., in press).

In contrast to the great number of species found in the Mascarenes and their high level of endemicity, our results support five radiations that occurred principally on Reunion, and a maximum of three species is found in these Mascarene clades. The principle radiations are observed on Reunion, the youngest and largest island of the Mascarenes and closest to Madagascar (Table 4). It is also a "high" island (3070 m), in contrast to Mauritius (823 m) and Rodriguez (400 m): greater elevational gradients are expected to produce a greater diversity of both habitats and ecological niches and are more likely to offer favorable conditions for diversification, even if successful radiations are also governed by intrinsic features (genetic heritage). For example, the extraordinary radiation of Asteraceae on Hawaii has been attributed to a combination of genetic features: they are a large, widespread family with good dispersal ability, and they are evolutionarily labile (i.e. able to take on different growth forms; Baldwin et al., 1991; Baldwin, 1998; Baldwin and Sanderson, 1998). Similarly, Orchidaceae represent a large and widespread family, capable of long-distance dispersal. Orchid diversification has long been attributed to adaptive radiations driven by selection related to specific pollinators (Darwin, 1862; Johnson et al., 1998), but genetic drift related to the small number of individuals that really participate in reproduction could have played a role as important as natural selection in the extraordinary diversification of the family (Tremblay et al., 2005). Especially on islands, stochastic events may have large effects on small founder populations, which are highly subject to both genetic drift and selective differences imposed by pollinator shift or habitat alterations relative to their original conditions. Moreover pollinator pressures are expected to be highly consistent in epiphytic orchids, since pollination systems are likely to be more specialized (Ackerman, 1986; even if this is not always the case, Lehnebach and Robertson, 2004) and populations more dispersed than terrestrial ones (Ackerman, 1986). As a consequence, lineage diversification would be expected in those groups that have maintained interactions with pollinators. This seems to be the case for short-spurred *Jumellea* species and bird-pollinated *Angraecum* sect. *Hadrangis*. In contrast, auto-pollinating species, for which additional constraints such as low level of genetic variability could be expected (Catling, 1990; see also Tremblay et al., 2005), appear less favorable for adaptation to ecological variations and consequently less likely to be involved with adaptive radiations. Complementary studies addressing pollinator identity, genetic population structure, and species reproductive biology

Table 4
Geographical characteristics of the Mascarene islands (my, million years)

	Reunion	Mauritius	Rodriguez
Surface (km^2)	2500	1800	110
Elevation (m)	3070	823	400
Estimation of age (my)	<3	<8	?[a]
Distance to Madagascar (km)	800	1000	1500

[a] Rodriguez was long considered as the youngest island (McDougall and Compston, 1965), but it is certainly the oldest one.

are needed to infer more precisely the consequences of breeding systems and pollinator interactions on island radiations, especially for the understudied *Angraecum* species with small yellow–green flowers and in which regular discovery of new species has confused an already unclear taxonomy.

The most famous example of *Angraecum* radiation on the Mascarenes involves the diversification of bird-pollinated sect. *Hadrangis*. Although the most closely related species in Madagascar fit the sphingophilous pollination syndrome, *Hadrangis* species are bird-pollinated and have short-spurred, scentless flowers (Micheneau et al., 2006). From our results, it is not possible to infer the ancestral morphology of *Hadrangis* species; sect. *Humblotiangraecum* may have colonized Reunion from Madagascar (making the presence of odor-producing, long-spurred flowers and sucrose-rich nectar plesiomorphic) or recolonized Madagascar from the Mascarenes (in which case odorless, short-spurred flowers, and dilute nectar are plesiomorphic). However, there is a biological explanation that supports the hypothesis of colonization from Madagascar: (1) bird-pollination is an evolutionary trend observed on islands (see references in Section 1) and is expected to be derived from insect-pollination (e.g. Stiles, 1981; Grant, 1993, 1994; Bradshaw and Schemske, 2003; Manning and Goldblatt, 2005); (2) volcanic islands are expected to be colonized from a nearby mainland (due to their youth, isolation, and sterility), even if the reverse is not impossible (e.g. Nicholson et al., 2005), and (3) long-spurred species, especially from Madagascar, have been recognized as ancestral in the angraecoid orchid group (Nilsson et al., 1985; see Fig. 4B for placement of long-spurred species on the tree).

4.3. Concluding remarks

This phylogenetic study provides further evidence to support recognition of a single subtribe, Angraecinae, which includes all angraecoid orchids. Further studies, including greater sample sizes and morphological, cytological, and molecular investigations, would be worthwhile to resolve problems still existing in our systematic understanding of the group, especially the large genus *Angraecum* (*c.* 210 species). From our results, there is a clear geographical distinction between groups of *Angraecum* species occurring exclusively in Africa and species from Indian Ocean islands (especially Madagascar). Our study also highlights (1) the Malagasy origin of Mascarene species, with many potential colonization events from Madagascar and only a few radiations in the Archipelago, and (2) different hypotheses that may have governed orchid establishment following long-distance dispersal to islands in a short geological time. The number and diversity of angraecoid orchids found in the primary forests of Reunion provides new evidence for the fascinating ability of orchids to develop different strategies to successfully colonize and become established in new habitats.

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Appendix A

Taxa sampled for molecular studies. Taxon names and author abbreviations follow World Checklist of Monocotyledons (The Board of Trustees of Royal Botanic Gardens Kew, 2007), except for Mascarene species. Herbarium abbreviations follow http://sciweb.nybg.org/science2/IndexHerbariorum.asp.

[a]Abbreviation "aff." was used for ambiguously name species (i.e. not fully verified at RBG Kew, or a Mascarene species that has to be determined or for which status has to be reconfirmed); [b]Unvouchered corresponds to samples from living collections from Reunion (REU) or RBG Kew (K); [c]Af, Africa; Am, America; Co, Comoros; Md, Madgascar; Ma, Mauritius; Ro, Rodriguez; Re, Reunion; Se, Seychelles; Sl, Sri Lanka; [d]Species from Carlsward et al. (2003), in *trnL-F* and *matK* matrices only; [e]Species in *rps16* matrix only.

Taxon[a]; Voucher[b] (location); Distribution[c]

Tribe Vandeae, subtribe Aeridinae

Aerides odoratum Reinw. ex Blume; *Chase 15081* (K); Asia.

Phalaenopsis cornu-cervi Blume & Rchb.f.; Chase O-1356 (K); Asia—*P. lueddemanniana* Rchb.f.; Chase 15803 (K); Asia.

Tribe Vandeae, subtribe Angraecinae (*sensu* Carlsward et al., 2006).

Aerangis biloba (Lindl.) Schltr.; *Chase 15215* (K); Af—*A. ellisii* (Rchb.f.) Schltr.; *Chase 15080* (K); Md—*A. macrocentra* (Schltr.) Schltr.; *Hermans 779* (K); Md—*A. punctata* J.Stewart; unvouchered (REU); Md, Re—*A.* sp. 1; unvouchered (K); Md.

Aeranthes adenopoda H.Perrier; *Fournel 83* (REU); Md, Re—*A. arachnitis* (Thouars) Lindl.; *Fournel 126* (REU); Ma, Re—*A. caudata* Rolfe; *Chase 17918* (K); Co?, Md, Re—*A. grandiflora* Lindl.; unvouchered (K); Md, Co—*A. henrici* Schltr.; unvouchered (K); Md—*A. longipes* Schltr.; *DuPuy 17* (K); Md—*A. moratii* Bosser; *Chase 17918* (K); Md—*A. neoperrieri* Toill.-Gen., Urs. & Bosser; unvouchered (K); Md—*A. peyrotii* Bosser; *Chase 14644* (K); Md—*A. ramosa* Rolfe; *Hermans 113* (K); Md—*A. vir-*

ginalis D.L.Roberts; *Chase 17901* (K); Co—*A. strangulata* Frapp. ex Cordem.; *Micheneau & Pailler 100* (REU); Re—*A. tenella* var. *borbonica* Bosser; *Pailler 137* (REU); Ma, Re.

Angraecopsis parviflora (Thouars) Schltr.; *Hermans 4363* (Personal); Af, Md, Ma, Re.

Angraecum Bory

sect. *Angraecoides* (Cordem.) Garay: *A. moandense* De Wild.; unvouchered (K) (referred as *A. chevalieri*); Af—*A. cultriforme* Summerh.[d]; *Carlsward 298* (FLAS); Af—*A. obversifolium* Frapp. ex Cordem; *Micheneau & Pailler 8* (REU); Ma? (maybe extinct), Re—*A. pingue* Frapp. ex Cordem.; unvouchered (REU); Ma, Md, Re—*A. sedifolium* Schltr.; *Hermans 3802* (K); Md.

sect. *Angraecum*: *A. eburneum* Bory—*A. eburneum* subsp. *eburneum* unvouchered (K); Co, Ma, Md, Re, Se—*A. eburneum* subsp. *superbum* (Thouars) H.Perrier; *Carter 761* (K); Co, Md, Se—*A. eburneum* subsp. *xerophilum* H.Perrier; unvouchered (K); Md—*A.* aff. *eburneum* var. *longicalcar* Bosser; unvouchered (K); Md—*A. florulentum* Rchb.f.; unvouchered (K); Co—*A. sesquipedale* Thouars; unvouchered (K); Md—*A.* aff. *sororium* Schltr.; unvouchered (K); Md.

sect. *Arachnangraecum* Schltr.: *A. birrimense* Rolfe; *Smith 531* (K); Af—*A.* aff. *eichlerianum* Kraenzl.; unvouchered (K); Af—*A. expansum* Thouars; *Micheneau 2* (REU); Ma? (maybe extinct), Re—*A.* aff. *germinyanum* Hook.f.; *Hermans 5540* (K); Co—*A. arachnites* Schltr.; *Hermans 4241* (K); Md—*A. conchoglossum* Schltr.; *Micheneau 5* (REU); Md, Re—*A. infundibulare* Lindl.; unvouchered (K); Af—*A. linearifolium* Garay; *Hermans 3528* (K); Md—*A. scottianum* Rchb.f.; unvouchered (K); Co—*A. teretifolium* Ridl.; unvouchered (K); Md.

sect. *Boryangraecum* Schltr.: *A. teres* Summerh.; *Bytebier 673* (EA); Af—*A. sacciferum* Lindl.; *Bytebier* 2226 (NBG); Af.

sect. *Chlorangraecum* Schltr: *A. huntleyoides* Schltr.; *Hermans 4248.1* (K) (referred as *A. chloranthum* Schltr.); Md.

sect. *Dolabrifolia* (Pfitz) Garay: *A. aporoides* Summerh.; *Wageningen 367* (K); Af—*A. distichum* Lindl.; *Smith 560* (K); Af.

sect. *Filangis* Garay: *A. cornigerum* Cordem.; *Pailler 176* (REU); Re.

sect. *Gomphocentrum* (Benth.) Garay: *A. calceolus* Thouars; *Pailler 177* (REU); Af, Co, Ma, Md, Re, Se—*A. caulescens* Thouars; *Pailler 97* (REU); Md, Ma, Re—*A. multiflorum* Thouars.; *Pailler 154* (REU); Ma, Md, Re, Se—*A.* sp. TP168; *Pailler 168* (REU); Re—*A.* aff. *zeylanicum* Schltr.; unvouchered (K) (referred as *A. maheense*); Se, Sl.

sect. *Hadrangis Schltr.*: *A. bracteosum* Balf.f. & S.Moore; *Micheneau 7* (REU); Re—*A. bracteosum* × *A. striatum*; *Micheneau 3* (REU); Re—*A. cadetii* 1 Bosser; *Micheneau & Pailler 9* (REU); Ma, Re—*A. cadetti* 2; *Pailler 157*; Ma, Re—*A. striatum* Thouars; *Micheneau 4* (REU); Re.

sect. *Humblotiangraecum* Schltr.: *A. clareae* Hermans la Croix & P.J. Cribb; *Hermans 3788* (K); Md—*A.* aff. *leonis* Veitch; unvouchered (K); Co, Md—*A. magdalenae* Schltr. & H.Perrier; unvouchered (K); Md.

sect. *Lemurangis* Garay: *A. costatum* Frapp. ex Cordem.; *Micheneau & Pailler 174* (REU); Re—*A. hermannii* (Cordem.) Schltr.; unvouchered (REU); Re—*A.* sp. TP125; *Pailler 125* (REU); Re.

sect. *Lepervanchea* (Cordem.) Schltr.: *A. caricifolium* H.Perrier; unvouchered (K); Md—*A. tenuifolium* Frapp. ex Cordem.; *Pailler 116* (REU); Re—*A. tenuispica* Schltr.; unvouchered (K); Md.

sect. *Nana* (Cordem.) Garay: *A.* sp. TPCM81; *Micheneau & Pailler 81* (REU); Ma?, Re—*A.* sp. TP51 (=*A.* sp. TPCM81); *Pailler 51* (REU); Ma?, Re—*A.* sp. TP84; *Pailler 84* (REU); Md?, Re—*A.* sp. TP110; *Pailler 110* (REU); Re.—*A.* sp. TP114; *Pailler 114* (P); Re.

sect. *Pectinaria* (Benth.) Schltr.: *A. doratophyllum* Summerh.; unvouchered (K); Af—*A. pectinatum* Thouars; unvouchered (K); Ma, Md, Re—*A. subulatum* Lindl.; *Chase 12081* (K); Af.

sect. *Perrierangraecum* Schltr.: *A. borbonicum* Bosser; *Micheneau 1* (REU); Re—*A. cucullatum* Thouars; *Pailler 108* (REU); Ma, Re—*A. didieri* (Baill. ex Finnet) Schltr.; unvouchered (K); Md—*A. liliodorum* Frapp. ex Cordem.; unvouchered (REU); Ma, Re—*A. obesum* H.Perrier; *Hermans 2407* (K); Md—*A. pseudodidieri* H.Perrier; unvouchered (K); Md—*A. rutenbergianum* Kraenzl.; unvouchered (K); Md.

sect. *Pseudojumellea* Schltr.: *A. ramosum* Thouars; *Micheneau & Pailler 73*; Ma, Re—*A. mauritianum* Frapp. ex Cordem.; unvouchered (REU); Ma, Md, Re.

Beclardia macrostachya (Thouars) A.Rich.; *Fournel 276* (REU); Ma, Md, Re.

Bonniera appendiculata Frapp. ex Cordem.; Pailler 107 (REU); Re—*B. corrugata* Cordem.; Pailler 106 (REU); Re.

Campylocentrum fasciola (Lindl.) Cogn.[d]; *Carlsward 185* (FLAS); Am—*C. jamaicence* Benth. ex Rolfe[d]; *Whitten 1934* (FLAS); Am—*C. lansbergii* (Rchb.f.) Schltr.[d]; *Carlsward 272* (FLAS); Am—*C. micrantum* (Lindl.) Rofle[d]; *Carlsward 180* (FLAS); Am—*C. pachyrrhizum* (Rchb.f.) Rofle[d]; *Ackerman s.n.* (UPRRP); Am—*C. poeppigii* (Rchb.f.) Rofle[d]; *Carnevali 4507* (CICY); Am.

Chamaeangis vesicata (Lindl.) Schltr.; *Chase 14646* (K); Af.

Cryptopus elatus Thouars; *Micheneau 6* (REU); Ma, Re—*C. paniculatus* H.Perrier; *Hermans 5392* (K); Md.

Dendrophylax barrettiae Fawc. & Rendle[d]; *Carlsward 199* (FLAS); Am—*D. fawcetti* Rolfe[d]; *Whitten 1939* (FLAS); Am—*D. filiformis* (Sw.) Carlsward & Whitten[d]; *Whitten 1842* (FLAS); Am—*D. funalis* (Sw.) Benth. ex Rolfe[d]; *Carlsward 302* (FLAS); Am—*D. funalis*[e]; no data (K); Am—*D. porrectus* 1 (Rchb.f.) Carlsward & Whitten[d]; *Carlsward 329* (FLAS); Am—*D. porrectus* 2[d]; *Carlsward 184* (FLAS); Am—*D. sallei* (Rchb.f.) Benth. ex Rolfe[d]; *Whitten 1945* (JDSB); Am—*D. varius* (J.F.Gmel.) Urb.[d]; *Whitten 1960* (JDSB); Am.

Jumellea amplifolia Schltr.; unvouchered (K); Md—*J. anjouanensis* (Finet) H.Perrier; *Bryonnaud 62* (P); Co—*J. arborescens* H.Perrier; unvouchered (K); Md—*J. brachycentra* Schltr.; unvouchered (K); Md—*J. densefoliata*

Senghas; *Hermans 2809* (K); Md—*J.* aff. *divaricata* (Frapp.) Schltr.; unvouchered (REU); Re—*J.* aff. *exilis* (Cordem.) Schltr; *Pailler 25* (REU); Re—*J. fragrans* 1 (Thouars) Schltr.; *Fournel 90* (REU); Ma, Re—*J. fragrans* 2; *Fournel 92* (REU); Ma, Re—*J. fragrans* 3; *Micheneau & Pailler 10* (REU); Ma, Re—*J. gracilipes* Schltr.; unvouchered (K); Md—*J. linearipetala* H.Perrier; unvouchered (K); Md—*J. maxillarioides* (Ridl.) Schltr.; unvouchered (K); Md—*J. papangensis* H.Perrier; *Chase 17913* (K); Md—*J. recta* (Thouars) Schltr. (Reunion); *Fournel 114* (REU); Ma, Re, Ro—*J. sagittata* H.Perrier; *Chase 15221* (K); Md—*J.* sp. 1 (Thouars) Schltr.; unvouchered (K); Md (erroneously referred as *J.* aff. *recta*) –*J.* sp. JF9; *Fournel 9* (REU); Re—*J.* sp. JF85; *Fournel 85* (REU); Re—*J.* sp. TP203 (Frapp.) Schltr.; *Pailler 203* (REU); Re—*J. stenophylla* (Frapp. ex Cordem.) Schltr.; *Micheneau & Pailler 92* (REU); Re—*J. triquetra* (Thouars) Schltr.; *Micheneau & Pailler 11* (REU); Ma, Re—*J. walleri* (Rolfe) la Croix; unvouchered (K) (referred as *J. filicornoides*); Af.

Mystacidium flanaganii (Bolus) Bolus; *Hermans 5084* (K); Af.

Oeonia rosea 1 Rolfe; unvouchered (K); Md, Re—*Oeonia rosea* 2; *Hermans 3222* (K); Md, Re.

Solenangis aphylla (Thouars) Summerh.; *Hermans 2389* (K); Af, Ma, Md, Re.

Tribe Vandeae, subtribe Polystachyinae (outgroups)

Polystachya fulvilabia Schltr.; *Chase 17862* (K); Af—*P. henrici* Schltr.; *Chase 17856* (K); Md—*P. mauritiana* Spreng.; *Pailler 170* (REU); Af, Co, Ma, Md, Re, Se—*P. melliodora* P.J.Cribb; *Chase 17923* (K); Af.

Appendix B

Nomenclatural revision of the genus *Bonniera*

Angraecum appendiculatum Frapp. ex Cordem., Flore de l'île de La Réunion: 211, 1895.

Synonym *Bonniera appendiculata* (Frapp. ex Cordem.) Cordem., Revue Générale de Botanique 11: 416, 1899.

Angraecum corrugatum (Cordem.) Micheneau, comb. nov.

Basionym *Bonniera corrugata* Cordem., Revue Générale de Botanique 11: 416, 1899.

Angraecum appendiculatum was the first name given by de Cordemoy before he created a new genus, *Bonniera*, for both peculiar species (peloric forms) occurring only on Reunion. The results of our phylogenetic analyses support the transfer of *B. appendiculata* and *B. corrugata* to *Angraecum*. Both species belong to *A.* sect. *Arachnangraecum* (Figs. 2 and 3).

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