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Systématique évolutive et Biogéographie de *Angraecum* (Orchidaceae, Angraecinae) à Madagascar

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Résumé

Le genre *Angraecum* est un groupe d'orchidées tropicales qui compte environ 221 espèces réparties en Afrique subsaharienne, dans l'ouest de l'Océan Indien, et au Sri Lanka. Plus de la moitié des espèces se trouvent à Madagascar, dont au moins 90% sont endémiques à l'île. L'étude systématique et taxonomique du genre *Angraecum* a toujours été problématique à cause de sa grande diversité morphologique. Pour faciliter la classification, des sections ont été établies dont la plus connue est celle de Garay (1973), qui regroupe les espèces sous 19 sections. Plusieurs analyses phylogénétiques avaient montré que le genre *Angraecum* et les sections de Garay ne sont pas monophylétiques. Cependant, aucune révision systématique n'a été apportée à cause du faible échantillonnage dans ces analyses. En incorporant un plus grand nombre d'espèces et en ajoutant d'autres caractères morphologiques dans l'analyse, nous avons apporté une plus grande résolution à la reconstruction phylogénétique du groupe. Cette résolution concerne surtout les nœuds plus profonds qui représentent les différents clades à l'intérieur d'*Angraecum*, qui correspondent à des sections naturelles. A partir de ces clades, nous avons redéfini 14 sections monophylétiques toute en reconnaissant cinq nouvelles.

Grâce à cette nouvelle phylogénie d'*Angraecum*, nous avons pu étudier la diversification du genre et de la sous-tribu Angraecinae en utilisant des méthodes macroévolutives, notamment les roles joués par les traits floraux dans la spéciation, tout en l'interprétant grâce aux histoires géologique et paléoclimatique. Le modèle de diversification chez les Angraecinae semble avoir été celui communément rencontré dans les forêts tropicales humides, c'est-à-dire une diversification par accumulation graduelle d'espèces à travers le temps et non pas une radiation adaptative rapide, comme souvent observée chez des lignées animales malgaches. Plusieurs caractères morphologiques jouent un rôle important dans la diversification des espèces d'*Angraecum*. Le début de la diversification d'*Angraecum* à Madagascar coïncide avec le mouvement progressif de l'île vers le nord, l'établissement de la mousson dans la partie nord de l'île durant le Miocène, et l'expansion de la forêt tropicale malgache pendant cette période.

Notre étude de l'histoire biogéographique des Angraecinae suggère une origine malgache de la sous-tribu et du genre *Angraecum*. On observe de la dispersion à longue distance à partir de Madagascar vers le reste du monde dans le genre *Angraecum*. La forêt tropicale humide du Nord Est de Madagascar est le point de départ de la diversification des espèces d'*Angraecum*. Le premier événement de dispersion a débuté à l'intérieur de l'île vers la fin du Miocène. Cet évènement est marqué par une migration du Nord Est vers le centre de Madagascar. Par ailleurs, la majorité des événements de dispersion à longue distance se sont produits durant le Pliocène-Pléistocène à partir soit du centre, soit du Nord Est de l'île. On assiste à des migrations indépendantes vers l'Afrique de l'est et les Comores d'une part, et vers les Mascareignes d'autre part. Un seul événement fondateur ayant conduit à l'apparition de la section *Hadrangis* est observé dans les Mascareignes. La saison cyclonique joue un rôle significatif dans la dispersion à longue distance des graines d'orchidées, comparée aux vents dominants qui soufflent dans la région ouest de l'Océan Indien, notamment l'alizé et la mousson. La similarité des niches écologiques a facilité l'expansion des espèces d'*Angraecum* dans les Comores et les Mascareignes.

Mots-clés: accumulation graduelle des lignées, *Angraecum*, Angraecinae, biogéographie, délimitation des sections, diversification, phylogénie, systématique, taxonomie

Abstract

Angraecum is a group of tropical orchids that includes ca. 221 species distributed in Sub-Saharan Africa, the western Indian Ocean region, and Sri Lanka. At least half of the species are found in Madagascar, 90% being endemic to the island. Taxonomic studies of *Angraecum* have always been problematic because of the great morphological diversity of the group. To facilitate classification, sections have been proposed, the best-known system to date being that of Garay (1973) that subdivides the genus into 19 sections. Previous phylogenetic studies had shown that genus *Angraecum* and Garay's sections are not monophyletic. However, no systematic review was made because of a reduced species sample in these analyses. Using a greater sampling from Madagascar and adding morphological characters to the analyses, we brought greater resolution to the phylogenetic reconstruction of the group. This resolution mainly concerns the deeper nodes representing different clades within *Angraecum*, which basically correspond to natural sections. By using these clades, we redefined 14 sections and recognized five new ones.

Using this phylogeny of *Angraecum*, we evaluated species diversification using macroevolutionary methods, essentially the effect of floral traits in speciation. The great diversity of *Angraecum* species in Madagascar, the high endemicity, and the geology and paleoclimate histories allowed us to evaluate diversification patterns within the genus as well as sub-tribe Angraecinae. The model of diversification in Angraecinae follows that of most tropical rain forest taxa, which results from the gradual accumulation of species through time and not from a rapid adaptive radiation, as is often the case for Malagasy fauna lineages. Several morphological characters are involved in the diversification of *Angraecum*. The beginning of *Angraecum* diversification in Madagascar coincided with the progressive movement of the island northwards, the establishment of a monsoon regime in the northern part of the island during the Miocene, and the expansion of the Malagasy rainforest during that period.

Our historical biogeographic study of Angraecinae suggests a Malagasy origin of the subtribe Angraecinae and *Angraecum*. We observed out-of Madagascar long-distance dispersal in *Angraecum*. The north-eastern Malagasy rainforest is the center of species radiation for the genus. The first dispersal event within the island started in the late Miocene. This event was a migration from the north to the central highland. The majority of long-distance dispersal events outside Madagascar occurred during the Pliocene-Pleistocene, originating from either the center or the North East of the island. There were multiple independent dispersals to East Africa and the Comoros, and to the Mascarenes. A single founder-effect event in section *Hadrangis* is observed in the Mascarenes. The cyclonic seasons play a significant role in long-distance dispersal of orchid seeds, as compared to prevailing winds in the western Indian Ocean region, essentially trade wind and monsoon. Ecological niche similarity favored the expansion of *Angraecum* species in the Comoros and Mascarene archipelagos.

Keywords: accumulation of lineages through time, *Angraecum*, Angraecinae, biogeography, delimitation of sections, diversification, phylogeny, systematic, taxonomy

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AIC: Akaike information criterion

alt: alternative

APG: Angiosperm phylogeny group classification

BA: Bayesian analyses

BAMM: Bayesian analysis of macroevolutionary mixtures

BEAST: Bayesian evolutionary analysis sampling trees

BioGeoBEARS: biogeography with bayesian and likelihood evolutionary analysis in R scripts

BiSSE: binary state speciation and extinction

BS: bootstrap support

BSA: bovine serum albumin

C_3: plant metabolism using C_3 carbon fixation

CAM: crassulacean acid metabolism

ChiSq: Chi-square

CI: consistency index

CITES: convention on international trade in endangered species of wild fauna and flora

CTAB: cetyl trimethylammonium bromide

DEC: dispersal-extinction-cladogenesis

DIVA: dispersal–vicariance

Df: degrees of freedom

DNA: deoxyribonucleic acid

e.g.: exempli gratia

etc.: et cætera

ExoSAP: exonuclease I and shrimp alkaline phosphatase

HPD: highest probability densities

IOI: Indian Ocean Islands

ITS: internal transcribed spacer

IUCN: international union for conservation of nature

K: Kew herbarium

lnLik: log likelihood

***mat*K**: chloroplast maturase K gene

MCCT: maximum clade credibility tree

MCMC: Markov chain Monte Carlo

ML: maximum likelihood

MP: maximum parsimony

MRCA: most recent common ancestor

MT: Marie-Victorin herbarium

MuSSE: multistate speciation and extinction

P: Paris herbarium

PAUP*: phylogenetic analysis using parsimony *and other methods

PBD: phylogenetic beta diversity

PCR: polymerase chain reaction

PP: posterior probability

Pr: probability

PVP: polyvinylpyrrolidone

RHS: Royal horticultural society

RI: retention index

***rps*16**: chloroplast ribosomal protein S16

s.l.: sensu lato

s.s.: sensu stricto

TAN: national herbarium of Madagascar

TBR: tree bisection–reconnection

***trn*L**: chloroplast transfer ribonucleic acid asparagine-like

WIOR: west Indian Ocean region

wt: weight

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Introduction

La famille des Orchidaceae, regroupant plus de 24000 espèces, est l'une des plus grandes familles de plantes vasculaires. Dans la sous-tribu des Angraecinae, le genre *Angraecum*, décrit comme un groupe polyphylétique (Carlsward et al., 2003; Carlsward et al., 2006; Micheneau et al., 2008a), contient près du tiers des espèces de la sous-tribu, soit 221 espèces sur les quelques 762 reconnues (Chase et al., 2015; Govaerts et al., 2015).

Angraecum Bory (1804), connu sous le nom « d'orchidée étoile » à cause de ses fleurs rappelant la forme d'une comète, est un genre restreint à l'Afrique continentale et à la région ouest de l'Océan Indien. Le centre de diversité du genre se situe à Madagascar (Hermans et al., 2007; Cribb et Hermans, 2009), où l'on dénombre 142 espèces dont 90 % sont endémiques à l'île (Govaerts et al., 2015). Ce genre comprend le fameux *A. sesquipedale* ou étoile de Darwin, connu pour son éperon de 30 cm de longueur et pour son papillon pollinisateur sphingidé à long proboscis, le *Xanthopan morganii praedicta*. Toutes les espèces appartenant à ce groupe sont ornementales et sont très convoitées en horticulture. Certaines espèces, comme *A. longicalcar*, sont aussi utilisées en industrie cosmétique pour leur fragrance. Mais, comme la plupart des orchidées tropicales, certaines espèces d'*Angraecum* se font rares en milieu naturel à cause de la perte d'habitat et diverses pressions anthropiques.

Alors que la découverte du genre *Angraecum* date de deux siècles, notre connaissance du groupe demeure limitée. Il faut d'abord comprendre que le grand nombre d'espèces et la diversité à l'intérieur d'*Angraecum* rend l'étude de ce groupe complexe (Garay, 1973; Stewart et al., 2006; Micheneau et al., 2008a). Les espèces ont été réparties en 19 sections (Garay, 1973) selon leurs ressemblances morphologiques, mais cette assignation n'est pas toujours évidente (Stewart et al., 2006). Par ailleurs, la délimitation même du genre pose problème à cause de l'homoplasie des caractères utilisés pour définir les genres dans la sous-tribu des Angraecinae. Jusqu'à maintenant les données moléculaires n'ont pas permis de résoudre entièrement la phylogénie faute d'un échantillonnage suffisant (Micheneau et al., 2008a), bien qu'elles aient semblé prometteuses. La sous-tribu des Angraecinae, à laquelle appartient le genre, paraît être un groupe polyphylétique (Carlsward et al., 2003; Carlsward et al., 2006) et le genre *Angraecum* lui-même pourrait aussi être artificiel (Micheneau et al., 2008a). Par ailleurs, les données moléculaires et morphologiques ne sont pas concordantes, ce qui remet en cause la fiabilité des sections et des genres. Enfin, la plus grande diversité d'*Angraecum* à Madagascar suscite des questions quant aux mécanismes de diversification des espèces, à l'origine du groupe et à sa biogéographie historique.

Afin de mieux comprendre l'évolution du genre *Angraecum*, notre étude a trois objectifs. Le premier consiste à évaluer la diversification du genre *Angraecum* à Madagascar, notamment afin de vérifier si la diversité morphologique est associée à la diversité des espèces. Le deuxième consiste à évaluer la taxonomie du genre *Angraecum*, défini de façon monophylétique, en combinant les données moléculaires et morphologiques. Enfin, le troisième objectif consiste à évaluer les hypothèses biogéographiques, notamment celle de l'origine malgache de l'ancêtre d'*Angraecum* sensu stricto.

1. Historique du genre *Angraecum*

Etymologiquement, le mot *Angraecum* vient du Malais 'angrek', qui signifie "orchidées épiphytes" en Asie du Sud-est (Stewart et al., 2006).

Le genre *Angraecum* Bory (1804), dont le spécimen type est *A. eburneum*, fut découvert par Jean-Baptiste Geneviève Marcellin Bory de Saint Vincent lors de son voyage à la Réunion et dans les iles voisines au tout début des années 1800. Louis Marie Aubert du Petit-Thouars, connu sous le nom de Petit-Thouars, a découvert à son tour une vingtaine d'autres espèces, dont *A. sesquipedale* Thouars, dans les deux décennies suivantes. L'ère de l'empire colonial a favorisé la recherche et la découverte d'orchidées dans les pays tropicaux comme Madagascar. En effet, les orchidées tropicales furent un centre d'intérêt pour la société bourgeoise européenne dans les années 1800 (Arditti et al., 2012). Peu de temps après la découverte du genre *Angraecum*, la commercialisation des espèces avait commencé et la demande s'accrut sur le marché. Au Royaume-Uni par exemple, une plante de *A. sesquipedale* se vendait à 20 £ (soit 31,75 $) vers les années 1860-1870, ce qui équivaut actuellement à 1460 £ (soit 2318,13 $) (Arditti et al., 2012). L'intérêt pour *Angraecum* n'a jamais diminué depuis. En horticulture, on compte plus de 45 hybrides interspécifiques et 28 hybrides intergénériques (RHS, 2015). Certaines espèces comme *A. longicalcar* sont maintenant utilisées dans l'industrie cosmétique à cause de leur fragrance.

Mais l'histoire la plus marquante sur le genre *Angraecum* reste celle de l'*A. sesquipedale* Thouars et de la prédiction fameuse faite par Darwin (1862) concernant son espèce pollinisatrice, et la théorie de coévolution qui en a découlé. En effet, après avoir reçu un spécimen de l'*A. sesquipedale* de son ami Bateman en 1862, Darwin fut émerveillé de la longueur de l'éperon de cette espèce, qui fait environ 30 cm de long. Il prédit que le papillon qui serait capable de polliniser *A. sesquipedale* devrait avoir une trompe aussi longue que l'éperon de la plante, alors qu'aucune espèce possédant cet attribut n'avait été découverte à l'époque. Ce n'est que 41 ans plus tard que le *Xanthopan morganii praedicta*, un papillon Sphingidé, fut découvert (Rothschild et Jordan, 1903), et 130 ans plus tard que preuve fut donnée de la pollinisation de l'*A. sesquipedale* par cet insecte (Wasserthal, 1993). Darwin avait conclu que l'*A. Sesquipedale* et son insecte pollinisateur (*X. morganii predicta*) seraient en coévolution, faisant allusion à la longueur de l'éperon de la plante qui concorde avec la longueur de la trompe de l'insecte. Cette théorie fut soutenue puis démontrée par Nilsson et al. (1985) après leurs études sur d'autres espèces d'*Angraecum, A. compactum* et *A. arachnites*, qui seraient en coévolution avec *Panogena lingense*. Cette théorie de coévolution reste tout de même un sujet de discussion pour les biologistes, pour la raison que ces insectes pollinisateurs visitent d'autres plantes et non seulement *Angraecum* (Nilsson et al, 1998; Jermy, 1999).

Sur le plan de la conservation et de la protection, le statut des espèces appartenant au genre *Angraecum* est mal connu faute d'investigation. Cependant, quatre espèces figurent sur la liste rouge de l'IUCN (2015): *A. longicalcar* (Bosser) Senghas, endémique du haut plateau de Madagascar, en danger critique; *A. rubellum* Bosser, endémique de la forêt humide de basse altitude de Madagascar, en danger critique; *A. sanfordii* P. J. Cribb et B.J. Pollard, endémique du Cameroun, en danger; et *A. pyriforme* Summerh., une espèce de l'Afrique de l'ouest, vulnérable. La collecte abusive pour la commercialisation est l'un des facteurs majeurs qui entraîne la rareté des espèces dans leur milieu naturel, malgré le fait que toutes les espèces d'orchidées sont classées à l'Annexe II de la CITES (2015). Mais les feux de brousse provoqués qui sévissent dans les forêts et prairies favorisent aussi la destruction, la fragmentation et la disparition de l'habitat et des espèces (Whitman et al. 2011; IUCN, 2015).

2. Classification et description d'*Angraecum*

Suivant l'APG III (2009), le genre *Angraecum* appartient à la famille des Orchidaceae Juss., sous-famille des Epidendroideae Lindl., tribu des Vandae Link, sous-tribu des Angraecinae Summerh.

Dressler (1993) avait subdivisé la tribu des Vandeae en quatre sous-tribus, Aeridinae, Aerangidinae, Angraecinae et Polystachyinae, qui regroupent 103, 36, 18 et 4 genres, respectivement. Cette tribu regroupe des espèces à croissance monopodiale (sauf les Polystachyinae qui ont une croissance polypodiale), avec des vélamens (Porembski et Barthlott, 1988; Arditii, 1992) et des graines de type Vanda (Dressler, 1993; Sin et al., 2002). Les Aeridinae et les Polystachyinae sont monophylétiques. Cependant, les Aerangidinae et les Angraecinae, qui forment le groupe informel des «angraecoides», sont tous deux polyphylétiques, mais forment ensemble un groupe naturel (Carlsward et al., 2006). Une forte ressemblance morphologique existe entre les deux groupes, leur différence se situant au niveau du gynostème (Dressler, 1993). Chez les Angraecinae, les deux pollinies sont portées par des stipes distincts attachés chacun sur un viscidium, alors que chez les Aerangidinae, les deux pollinies sont portées sur un seul viscidium (Fig. 1). Les récentes études phylogénétiques moléculaires sur la famille des Orchidaceae reconnaissent plutôt quatre sous-tribus à l'intérieur de la tribu des Vandeae: Aeridinae, Angraecinae (Aerangidinae + Angraecinae), Adrorhizinae, et Polystachyinae (Chase et al., 2015).

Le genre *Angraecum*, type de la sous-tribu des Angraecinae, est caractérisé par des plantes épiphytes, lithophytes, rarement terrestres; à racines aériennes généralement développées et longues; avec un port souvent dressé, pendant ou grimpant; des tiges courtes, allongées ou absentes; des feuilles distiques, généralement alternes, de forme et de longueur très variables; des entres nœud courts ou allongés; des feuilles minces, épaisses, ou charnues, de forme filiforme, lancéolée, ou ovale; inflorescences solitaires, racèmes ou épis; des fleurs petites à grandes, de couleur blanche, verte, jaune ou ocre; avec un éperon court à long; avec une anthère terminale, operculée avec des partitions réduites et deux pollinies (Dressler, 1993; Du Puy et al., 1999). Le nombre de chromosomes varie selon l'espèce et peut être $2n = 34$, 36, 38, 40, 42, 44, 46, 48 ou 50 (Dressler, 1993; Micheneau, 2005).

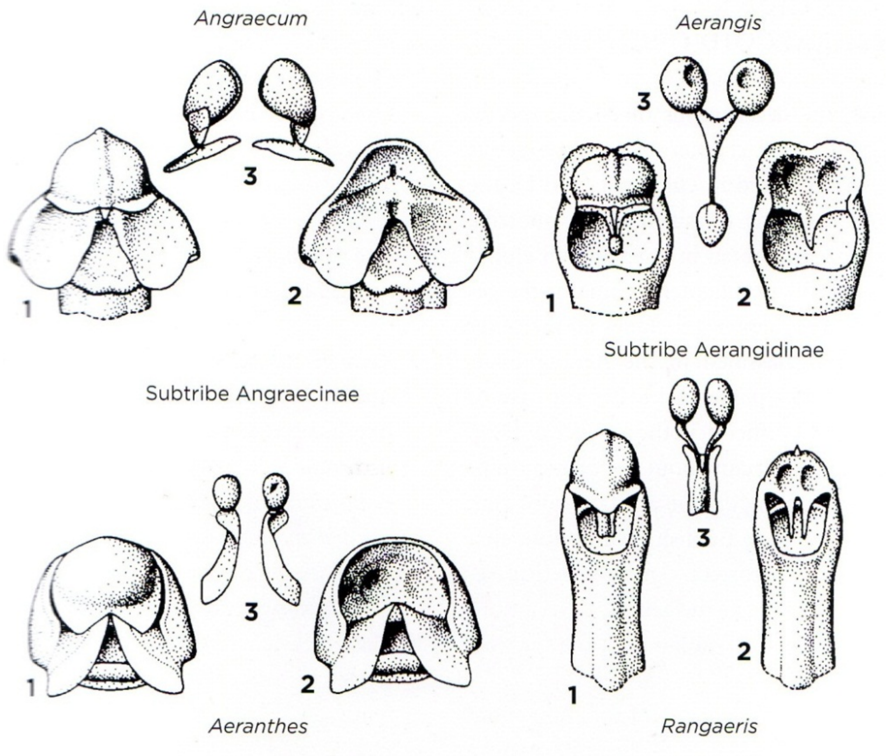


Fig. 1. Comparaison entre Angraecinae et Aerangidinae. 1: colonne avec anthère; 2: rostellum; 3: pollinies (Dressler, 1993).

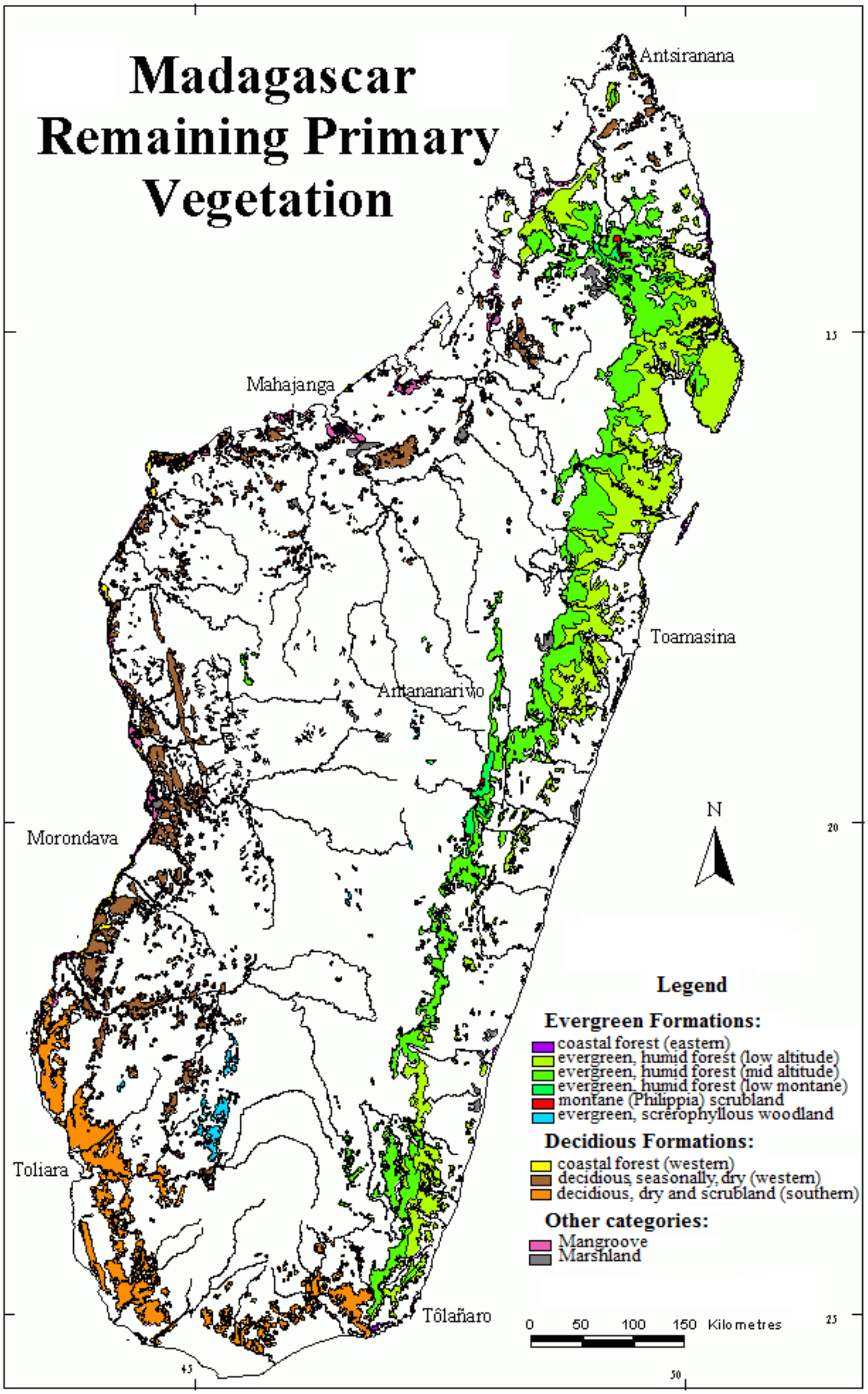
3. Biologie, écologie et répartition d'*Angraecum*

Le genre *Angraecum* s'étend de l'Afrique occidentale à l'Afrique orientale (subsaharienne), et dans l'Océan Indien à Madagascar, dans les Mascareignes (Réunion, Maurice, Rodrigues), dans les iles Comores, aux Seychelles et au Sri Lanka. Plus de 95% des espèces appartenant au genre sont épiphytes (Stewart et al., 2006; Hermans et al., 2007). Grace à leur système racinaire aérien et à leurs feuilles souvent charnues ou épaisses, les espèces présentent une plasticité et une tolérance au stress hydrique (Kerbauy et al., 2012), mais elles demeurent sensibles au feu (Whitman et al., 2011). D'après les études menées par Kluge et al. (1997), les espèces appartenant au genre *Angraecum* ont deux types de mécanismes photosynthétiques: CAM et C_3. Cette étude a montré que les espèces épiphytes sont de type CAM, ce qui est confirmé par les travaux de Kerbauy et al. (2012). Comme toutes les orchidées, *Angraecum* développe une relation symbiotique avec des champignons mycorhiziens (Arditti, 1992). Aucune étude sur la spécificité de ces microorganismes avec notre groupe n'a encore été faite, mais l'on sait qu'ils assurent un apport en carbone à la plante (Otero et Flanagan, 2006). Les espèces d'*Angraecum* croissent généralement à une altitude de 0 à 2000 mètres dans les régions tropicales humides, subhumides ou sèches (Du Puy et al., 1999; Stewart et al., 2006; Hermans et al., 2007). Elles abondent en forêt tropicale humide où la dénivellation est assez élevée. Des températures entre 10°C et 16°C, et une précipitation supérieure à 2000 mm par an sont considérées comme des conditions favorables au développement des orchidées épiphytes (Acharya et al., 2011).

Angraecum est considéré comme un genre sphingophile, c'est-à-dire présentant un mécanisme d'adaptation à la pollinisation par les papillons de nuit, de par la couleur claire des fleurs, des éperons nectarifères souvent longs et effilés, et l'émission de fragrance à la tombée de la nuit (Johnson et Steiner, 2000). Bien que des preuves de pollinisation par les papillons de nuit existent pour *A. sesquipedale* (Wasserthal, 1993, 1997), *A. arachnites* et *A. compactum* (Nilsson et al., 1985), la connaissance des espèces pollinisatrices d'*Angraecum* reste encore réduite. De récentes études avaient montré que des agents pollinisateurs autres que les papillons existent (Micheneau, 2005; Micheneau et al., 2008c, 2009, 2010). Ainsi, des observations sur la visite de l'*A. bracteosum* par l'oiseau *Zosterops borbonicus* et de l'*Angraecum cadetii* par le criquet *Glomeremus orchidophilus* ont été faites. Suite à ces observations, Micheneau et al. (2009) avaient conclu que l'adaptation de la plante suite à un changement d'habitat ou à la colonisation d'un nouvel habitat pourrait conduire à l'apparition de nouveaux agents pollinisateurs.

4. Choix du site d'étude et échantillonnage

Madagascar a été choisi comme site d'étude à cause de sa grande richesse en biodiversité, mais surtout parce que c'est le centre de diversité de notre groupe (Stewart et al., 2006). La diversité floristique de l'ile s'explique par les différentes formations géologiques qui constituent le territoire malgache (Du Puy et Moat, 1996). Mais chez les plantes épiphytes comme les orchidées, la diversité est aussi conditionnée par d'autres facteurs comme le gradient d'élévation et le climat (Acharya et al., 2011).



<u>Fig. 2.</u> Carte de végétation de Madagascar (Du Puy et Moat, 1996)

Humbert (1955) avait subdivisé Madagascar en sept zones de végétations: domaine de l'est (0 – 800 m), domaine du Sambirano (0 – 800 m), domaine du centre pentes orientales (800 – 2000 m), domaine du centre pentes occidentales (800 – 2000 m), domaine des hautes montagnes (2000 m et plus), domaine de l'ouest (0 – 800 m) et domaine du sud (800 – 2000 m). Plus tard, Du Puy et Moat (1996) ont raffiné les zones de végétation (Fig. 2) en se basant sur la description d'Humbert (1955) et celle de Faramalala (1995). La végétation primaire à Madagascar peut se résumer comme suit: à l'est, une forêt dense humide (littorale, basse et moyenne altitude, montagne), et à l'ouest, une forêt dense sèche caducifoliée, une savane et une forêt de palétuvier. Le sud est dominé par une forêt dense sèche et épineuse, alors que le centre est marqué par une forêt de montagne avec buisson éricoïde et une formation d'inselbergs. Le climat de l'île se subdivise comme suit: au centre, un climat tropical de montagne, généralement froid avec précipitation annuelle inférieure à 1500 mm; à l'est, un climat tropical humide avec précipitation annuelle supérieure à 2000 mm conditionné par le régime d'alizé sur toute la partie est et de mousson dans la partie nord de l'île; au sud, un climat semi-aride soumis au vent du sud (chaud et sec); enfin à l'ouest un climat tropical sec (Donque, 1975).

Une série d'échantillonnages a été réalisée de 2007 à 2012 dans 12 sites différents de Madagascar incluant toutes les zones de végétations existantes à l'exception du centre pente occidentale et du sud car elles comptent peu d'espèces dont, de surcroît, les données de séquences sont déjà publiées (Carlsward et al., 2006; Micheneau et al., 2008). Les caractéristiques de chaque site sont présentées au Tableau 1. Les expéditions ont été faites en collaboration avec le Madagascar Biodiversity Partnership (une organisation non-gouvernementale) et l'Université d'Antananarivo. Une partie des feuilles (2 cm x 2 cm) a été collectée puis conservée dans du gel de silice (Chase et Hills, 1991) pour les études moléculaires. Chaque individu échantillonné a été photographié et ses coordonnées géographiques enregistrées. Des spécimens d'herbiers ont été collectés pour les sites qui n'avaient pas le statut d'aire protégée, en accord avec la politique de conservation et la gestion locale des aires protégées. Au total, nous avons récolté 727 échantillons issus d'une centaine d'espèces et 183 spécimens d'herbier. Les spécimens d'herbiers seront déposés à l'herbier de Tsimbazaza à Antananarivo (TAN) et à l'herbier Marie-Victorin (MT). Afin de compléter notre échantillonnage, nous avons utilisé les collections du Jardin Botanique de Montréal (don de M. Lecoufle).

Tableau 1. Liste et caractéristiques des sites d'étude.

Sites d'étude	date de l'expedition	région	type de formation	latitude longitude	altitude (m)
Ambohitantely	23 au 27 Avril 2012	Analamanga	Forêt humide de montagne	-18,19580556 47,28955556	1529 à 1639
Angavokely	18 au 22 Avril 2012	Analamanga	Forêt humide de montagne	-18,92172222 47,73122222	1569 à 1692
Anjozorobe	3 au 8 Mai 2012	Analamanga	Forêt humide de montagne	-18,39330556 47,93930556	1550 à 1612
Bemaraha	1 au 5 Déc 2007	Melaky	Forêt sèche caducifoliée	-19,04461111 44,78047222	45 à 162
Lakia	28 au 31 Janv 2008	Vatovavy-Fitovinany	Forêt humide de basse altitude	-21,49475000 47,89972222	193 à 444
Mananara Nord	14 au 22 Fév 2008	Analanjirofo	Forêt humide de basse altitude	-16,31261111 49,78536111	5 à 433
Manongarivo	10 au 19 Déc 2008	Diana	Forêt humide de moyenne altitude	-14,02302778 48,25522222	415 à 835
Mantadia	10 au 15 Janv 2008	Alaotra-Mangoro	Forêt humide de moyenne altitude	-18,82641667 48,44086111	980 à 1149
Marojejy	10 au 23 Mars 2009	Sava	Forêt humide de moyenne altitude	-32,98469444 92,00141667	779 à 865
Sangasanga	4 au 14 Sept 2009	Vatovavy-Fitovinany	Forêt humide de basse altitude	-21,37461111 47,86588889	106 à 404
Torotorofotsy	15 au 19 Déc 2007	Alaotra-Mangoro	Forêt humide de moyenne altitude	-18,78208333 48,43369444	925 à 1029
Vatovavy	15 au 30 Sept 2009	Vatovavy-Fitovinany	Forêt humide de basse altitude	-21,40777778 47,94100000	338 à 591

5. Systématique et phylogénie d’*Angraecum*

Les 19 sections de Garay (1973), basées sur des caractères morphologiques, servent présentement de référence pour l’étude du genre *Angraecum* (Tableau 2). En fait, le travail de Garay résulte de la révision systématique des travaux effectués par Schlechter (1918, 1925), Perrier de la Bathie (1941), et Summerhayes (1958). Stewart et al. (2006) ont essayé de déplacer certaines espèces d’une section à une autre en conservant les sections de Garay et en

Tableau 2. Liste des 19 sections de Garay (1973) avec leur répartition géographique; les chiffres donnent le nombre d'espèces de chaque région et ceux entre paranthèses les espèces endémiques (Govaerts et al., 2015). Les sous-espèces et variétés ne sont pas incluses. Abréviations: Af: Afrique; E: est; C: centrale; O: ouest; Com: Comores; Mad: Madagascar; Msc: Mascareignes; Sey: Seychelles; Sri: Sri Lanka.

Sections	**Af-O**	**Af-C**	**Af-E**	**Com**	**Mad**	**Msc**	**Sey**	**Sri**	**Total**
Acaulia					7(7)				**7**
Afrangraecum	18(10)	9(1)	9(5)						**24**
Angraecoides	1(1)				7(6)	3(2)			**10**
Angraecum			1	2	16(15)	2(1)	1		**17**
Arachnangraecum			1(1)	2(1)	9(7)	4(3)			**14**
Boryangraecum	1(1)		4(3)	1	10(9)	2(1)			**16**
Chlorangraecum					2(2)				**2**
Conchoglossum	1(1)		4(4)		3(3)				**8**
Dolabrifolia	3(3)								**3**
Filangis					7(7)	1(1)			**8**
Gomphocentrum			1(1)	1	15(13)	5(3)	3(1)	1	**19**
Hadrangis						4(4)			**4**
Humblotiangraecum				1	5(4)				**5**
Lemurangis			1(1)		5(4)	2(1)			**7**
Lepervenchea					6(6)	1(1)			**7**
Nana			4(4)		10(9)	6(5)			**19**
Pectinaria	5(3)	2			5(4)	1			**10**
Perrierangraecum			2(2)		30(30)	2(2)			**34**
Pseudojumellea					4(2)	3(1)			**5**
Inconnu					1(1)	1(1)			**2**
Total	**29(19)**	**11(1)**	**27(21)**	**7(1)**	**142(129)**	**37(27)**	**4(1)**	**1**	**221**

créant des sous-groupes au sein des sections, ce qui rejoint un peu la subdivision des sections de Perrier de la Bathie (1941). Toutefois, les différences morphologiques sont tellement grandes au sein des sections mêmes qu'il leur était difficile de faire consensus sur la section où ils allaient placer certaines espèces. D'un autre côté, les résultats phylogénétiques basés sur des données moléculaires ne reflètent pas les sections et il en ressort que le genre *Angraecum* est polyphylétique (Carlsward et al., 2003; Micheneau et al., 2008a; Simo-Droissart et al., 2013).

6. Diversification chez *Angraecum*

La particularité du genre *Angraecum* est sa grande diversité morphologique (Fig. 3). Les sections ont été établies en premier lieu sur la base des différences morphologiques et florales entre les espèces (e.g., Schlechter, 1925; Perrier de la Bathie 1941; Garay, 1973). Par exemple, la section *Nana* réunit toutes les petites plantes ayant de petites fleurs, comme *Angraecum nanum*, qui mesure à peine 3 cm de haut et produit des fleurs de quelques milimetres de diamètre. La section *Angraecum*, quant à elle, regroupe les grandes plantes pouvant atteindre jusqu'à 1 m de hauteur avec de grandes fleurs, comme chez *Angraecum longicalcar*. La taille des plantes est souvent corrélée avec celle des fleurs et la longueur de l'éperon, mais ceci n'est pas le cas pour toutes les espèces, ce qui pourrait créer une certaine ambiguité entre l'ancien système de classification basé sur la morphologie et celui basé sur les données moléculaires. Certes, la diversité morphologique chez *Angraecum* constitue un outil important pour évaluer la systématique du groupe, mais elle peut aussi être efficace pour évaluer leur histoire évolutive. Plusieurs études utilisent maintenant la diversification d'un groupe de taxons pour évaluer les mécanismes de spéciation (e.g. Janssen et al., 2008; Anthony et al., 2010; Jonsson et al., 2012; Christidis et al., 2014). Par exemple, Freudenstein et Chase (2015) ont démontré que le passage vers l'épiphytisme chez les orchidées est un caractère innovateur ayant conduit à la grande diversification de la sous famille des Epidendroidae.

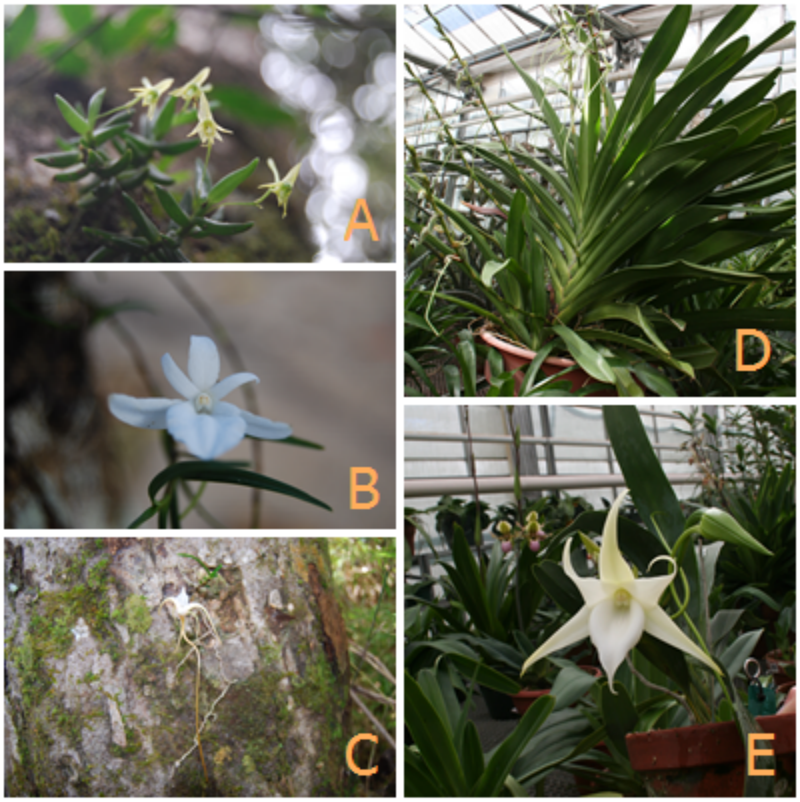


Fig. 3. Diversification du genre *Angraecum*. (A) *A. sedifolium*, (B) *A. danguyanum*, (C) *A. mirabile*, (D) *A. longicalcar*, (E) *A. sesquipedale* (Photo: Andriananjamanantsoa H.).

7. Biogéographie historique d'*Angraecum*

La biogéographie « étudie les organismes vivants, les plantes et les animaux, à la surface du globe, dans leur répartition, dans leur groupement et dans leurs relations avec les autres éléments du monde physique et humain » (Elhaï, 1968; Galochet, 2008). D'une manière simplifiée, la biogéographie est l'étude de la répartition géographique des organismes (Crisci, 2001). Le botaniste suisse de Candolle (1820) fut le premier à distinguer entre biogéographie écologique et historique. Selon lui, la biogéographie écologique s'intéresse aux processus écologiques qui se produisent sur une courte période et selon une petite échelle spatiale, tandis que la biogéographie historique s'intéresse aux processus évolutifs au cours des millions d'années à une grande échelle, souvent mondiale.

En biogéographie historique, trois types de processus qui modifient la disposition géographique et spatiale des organismes ont été identifiés: l'extinction, la dispersion et la vicariance. Ces processus sont maintenant évalués suivant trois éléments majeurs considérés comme influents dans l'étude de la biogéographie historique: la tectonique, la cladistique et la perception des biologistes de la biogéographie (Crisci, 2001). Pour l'étude de la biogéographie historique de notre groupe, nous allons essayer de trouver l'origine et les causes de la répartition des taxons en utilisant les données géologiques, écologiques, biologiques, et phylogénétiques.

D'après les données géologiques, Madagascar se serait séparé de l'Afrique à partir du bassin somalien du côté nord-ouest, puis du bassin du Mozambique du côté sud-ouest. Cette fracturation aurait eu lieu il y a environ 120 Ma (Eagles et Konig, 2008). La séparation entre Madagascar et l'Inde se serait produite il y a environ 80 Ma (Fig. 4), et la séparation entre l'Antarctique et l'Australie entre 40-55 Ma (Aslanian et Moulin, 2010; Torsvik et al., 2010). Cette séparation est aussi corroborée par des données biologiques, notamment fossiles (Ali et Krause, 2011). L'hypothèse de ponts reliant le bloc Indo-Madagascar avec le reste du Gondwana pendant le mi-Aptien a été avancée (Ali et Aitchison, 2009), mais cela reste controversé (Ali et Krause, 2011). L'ordre chronologique de la fragmentation du bloc de Gondwana-Est (Madagascar, Inde, Australie, Antarctique) reste un débat ouvert (Aslanian et Moulin, 2010; Torsvik et al., 2010).

Deux hypothèses majeures sont avancées sur l'origine des espèces à Madagascar, la dispersion transocéanique (Schatz, 1996; Yoder et Nowak, 2006) et la vicariance gondwanienne (Leroy, 1978; Grubb, 2003; Yoder et Nowak, 2006). L'origine vicariante des espèces malgaches est discutable à cause de l'âge géologique du pays (Bremer et al. 2004; Janssen et Bremer, 2004; Anderson et al., 2005; Davis et al., 2005). La colonisation par des espèces africaines pendant le Cénozoïque est l'hypothèse la plus soutenue (Yoder et Nowak, 2006). Une telle hypothèse est appuyée par Strijk et al. (2012) dans leur étude sur la dispersion de *Psiadia* (Asteraceae). Par ailleurs, de recentes études soutiennent une hypothèse sur l'origine Asiatique de plusieurs lignées malgaches (Cheke et Hume, 2008; Warren et al., 2010; Krüger et al., 2012; Buerki et al., 2013), remettant en question l'origine africaine des lignées malgaches et suggérant d'autes possibilités de dispersion à longue distance en direction est-ouest.

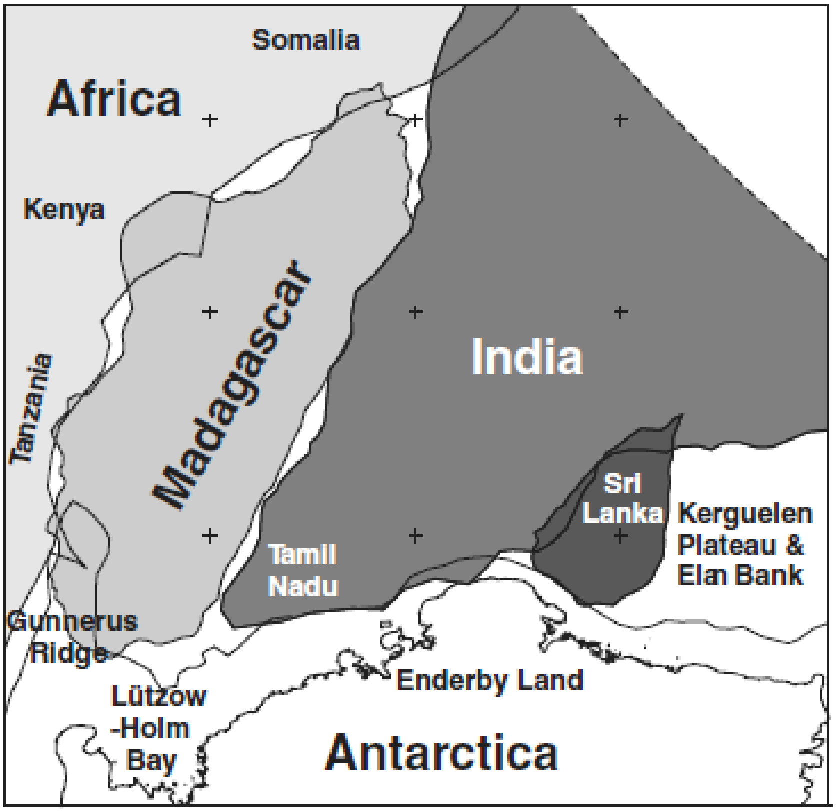


Fig. 4. Position approximative de Madagascar dans le bloc du Gondwana-Est au Crétacé. Marges continentales interprétées à partir des données gravimétriques satellitaires (Eagles et Konig, 2008).

Pour la biogéographie historique d'*Angraecum*, seuls Micheneau et al. (2008a) ont émis une hypothèse sur l'origine biogéographique des espèces dans leurs travaux sur la phylogénie et la biogéographie des angraecoides des Mascareignes. Cette étude, basée sur l'analyse des aires ancestrales de Bremer (1992) proposait une origine malgache des Angraecinae (Micheneau et al., 2008a) qui serait apparue il y a environ 21 Ma (Micheneau et al., 2010). Plusieurs auteurs ont avancé différents âges pour la famille des Orchidaceae, pour certains elle serait jeune à 26 Ma (Wikstrom et al., 2001) ou 40 Ma (Bremer, 2000), alors que pour d'autres elle aurait apparue il y a environ 110 Ma (Janssen et Bremer, 2004). Ce n'est que récemment que deux fossiles appartenant à la sous-famille des Orchidoideae et à la sous-famille des Epidendroideae ont été découverts en Nouvelle-Zélande (Conran et al., 2009), ayant permis d'estimer l'âge de la famille à environ 76–84 Ma (Ramirez et al., 2007). Ceci a été par la suite validé par Gustafsson et al. (2010) après avoir integré d'autres fossiles d'orchidées dans leurs analyses.

8. Problématiques, objectifs et hypothèses de recherche

Compte tenu des études antérieures sur la systématique d'*Angraecum* (Carlswarde et al., 2006; Micheneau et al., 2008; Simo-Droissart et al., 2013), plusieurs questions demeurent concernant la monophylie du genre et des sections sensu Garay (1973), et la position taxonomique des espèces (délimitation du genre). La résolution partielle de la phylogénie, potentiellement due à un faible échantillonnage (Micheneau et al., 2008), limite d'autres études, comme celle de l'évolution et de la diversification des espèces ou encore celle de l'histoire biogéographique qui nécessitent une phylogénie entièrement résolue. Ces études pourraient fournir des éléments essentiels qui permettent d'évaluer le statut de conservation des espèces dans le but de mieux établir une politique de conservation basée sur le principe de nécessité et de priorité.

Face à ces problématiques, mon projet de doctorat comporte quatre objectifs majeurs relatifs à la systématique évolutive et la biogéographie. Le premier objectif (chapitre 1) consiste à reconstruire la phylogénie d'*Angraecum* en augmentant le nombre d'espèces provenant de Madagascar qui manquaient dans les études antérieures et en ajoutant des caractères morphologiques aux analyses. Avec les 727 échantillons que nous avons collectés, nous espérons accroître la résolution de la phylogénie. Le deuxième objectif (chapitre 2) consiste à faire une révision systématique du genre en se basant sur les résultats obtenus dans le premier chapitre. Le troisième objectif (chapitre 1 et 3), consiste à évaluer la diversification d'*Angraecum* et de la sous-tribu des Angraecinae, notament l'effet des traits floraux (chapitre 1) et la stratification forestière (chapitre 3) sur la diversification des espèces, en utilisant la phylogénie moléculaire et les caractères morphologiques. Le quatrième objectif (chapitre 3) consiste à évaluer l'histoire biogéographie d'*Angraecum* et des Angraecinae afin de comprendre l'origine, le sens de la migration ou encore les mécanismes de dispersion des taxons.

Afin de répondre à nos objectifs, cinq hypothèses sont avancées:

Hypothèse 1: un *Angraecum* monophylétique devrait exclure les sections africaines;

Hypothèse 2: la phylogénie infirme la classification sectionnelle de Garay (1973) pour *Angraecum*;

Hypothèse 3: certains caractères morphologiques utilisés en taxonomie, notamment floraux, furent impliqués dans la diversification d'*Angraecum*;

Hypothèse 4: la diversification d'*Angraecum* s'est faite par radiation adaptative rapide;

Hypothèse 5: l'ancêtre d'*Angraecum* s. s. est malgache, et la dispersion s'est effectuée de Madagascar vers d'autres régions.

Chapitre 1: Diversification of *Angraecum* (Orchidaceae, Vandeae) in Madagascar: species accumulation through time rather than rapid radiation

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Contribution respective des auteurs:

Tahiana Andriananjamanantsoa: Conception du projet, collecte des données sur terrain, traitement et analyse des données au laboratoire, interprétation et rédaction

Shannon Engberg: Supervision en laboratoire, conseils et recommandations

Edward Louis Jr.: Supervision sur terrain, conseils, recommandations et co-direction

Luc Brouillet: Supervision, conception du projet, conseils, recommandations, corrections et direction générale

1.1. Résumé/ Abstract

Résumé

Madagascar fait partie des 34 points chauds de biodiversité dans le monde. Les îles comme Madagascar sont souvent utilisées pour étudier les processus de diversification en raison de leur forte endémicité et de leur isolement. *Angraecum* est le plus grand genre de la sous-tribu Angraecinae (Orchidaceae) avec environ 221 espèces. Madagascar est le centre de diversité du genre avec ca. 142 espèces, dont 90% sont endémiques. La grande diversité morphologique associée à la diversification des espèces du genre à Madagascar offre des informations précieuses pour les études macroévolutives. Des phylogénies sur Angraecinae ont été publiées, mais le manque d'échantillonnage limite leur résolution, laissant des incertitudes taxonomiques. Nous présentons une nouvelle phylogénie d'*Angraecum* fondée sur des données de séquences chloroplastiques (*mat*K, *rps*16, *trn*L), nucléaire (ITS2) et 39 caractères morphologiques de 194 individus des Angraecinae dont 69 nouvellement échantillonnés, incluant 98 espèces du genre *Angraecum* et cinq extra groupes des sous-tribus Aeridinae et Polystachyinae. En utilisant cette phylogénie, nous avons évalué les sections d'*Angraecum* tel que défini par Garay et étudié les modèles de diversification des espèces au sein du genre. Nous avons utilisé la méthode de parcimonie et les analyses bayésiennes pour générer des arbres phylogénétiques et dater la phylogénie. Nous avons analysé les modèles de diversification au sein des Angraecinae et d'*Angraecum*, en nous basant sur quatre traits floraux (couleur et taille des fleurs, position du labellum, longueur de l'éperon), en utilisant des modèles macroévolutifs afin de trouver quels traits ou états de caractères seraient associés au taux de spéciation, et reconstruit les états ancestraux des caractères. L'analyse phylogénétique a montré la polyphylie du genre *Angraecum* s.l. et de toutes ses sections, à l'exception de la section *Hadrangis*, et la compatibilité de la morphologie avec la phylogénie. La position supérieure et inférieure du labelle est le caractère principal qui permet de délimiter des clades monophylétiques au sein d'*Angraecum* s.s. Ce caractère semble également influer sur le taux de spéciation chez *Angraecum*. Le model macroévolutif basé sur la phylogénie a échoué à détecter des changements de diversification pouvant être associés à la diversification morphologique. La diversification d'*Angraecum* résulte d'une accumulation progressive d'espèces à travers le temps plutôt qu'une radiation rapide, un modèle de diversification souvent rencontré dans les forêts tropicales humides.

Mots-clés: accumulation des lignées, *Angraecum*, taux de diversification, macroévolution, Madagascar, phylogénie

Abstract

Madagascar is one of the 34 World biodiversity hotspots. Islands like Madagascar are often used to study diversification processes because of their high endemism and isolation. *Angraecum* is the largest genus of subtribe Angraecinae (Orchidaceae) with about 221 species. Madagascar is the center of the diversity for the genus with ca. 142 species, of which 90% are endemic. The great morphological diversity associated with species diversification in the genus on the island of Madagascar offers valuable insights for macroevolutionary studies. Phylogenies of the Angraecinae have been published but lack of sampling and the limited taxonomic resolution limit their uses for macroevolutionary studies. We present a new phylogeny of *Angraecum* based on chloroplast sequence data (*mat*k, *rps*16, *trn*L), nuclear ribosomal (ITS2) and 39 morphological characters from 194 Angraecinae specimens of which 69 were newly collected, including 98 *Angraecum* species and five outgroups from subtribes Aeridinae and Polystachyinae. Using this phylogeny, we evaluated the monophyly of the sections of *Angraecum* as defined by Garay and investigated the patterns of species diversification within the genus. We used maximum parsimony and bayesian analyses to generate phylogenetic trees and dated divergence times of the phylogeny. We analyzed diversification patterns within Angraecinae and *Angraecum* with an emphasis on four floral traits (flower color, flower size, labellum position, spur length) using macroevolutionary models to evaluate which traits or character states are associated to speciation rates, and inferred ancestral states of these characters. The phylogenetic analysis showed the polyphyly of *Angraecum* sensu lato and of all *Angraecum* sections except sect. *Hadrangis*, and that morphology is consistent with the phylogeny. The uppermost and lowermost position of the labellum was the main character helping to delimit clades within a monophyletic *Angraecum* sensu stricto. This character appeared also to be associated with speciation rates in

Angraecum. The macroevolutionary model-based phylogeny failed to detect shifts in diversification that could be associated directly with morphological diversification. Diversification in *Angraecum* resulted from gradual species accumulation through time rather than from rapid radiation, a diversification pattern often encountered in tropical rain forests.

Keywords: accumulation of lineages through time, *Angraecum*, diversification rate, macroevolutionary, Madagascar, phylogeny

1.2. Introduction

Madagascar is known for its biodiversity richness (Goodman and Benstead, 2005), and is a focus for evolutionary biologists who study the cause of species diversification. Many studies have been conducted using the high endemicity and diversity found in this isolated island as a model of diversification process in various taxa (Martin, 1972; Janssen et al., 2008; Townsend et al., 2009; Vences et al., 2009; Anthony et al., 2010; Jonsson et al., 2012; Reddy et al., 2012; Rakotoarinivo et al., 2013; Christidis et al., 2014). Most of these studies came to the conclusion that species diversification resulted from rapid radiation. High morphological variation associated with low genetic divergence appears to be a signature of the diversification processes operating on the island of Madagascar (Reddy et al., 2012) as well as on many other islands such as New Zealand, Hawaii, or Caribbean (Goldberg et al., 2014, Goodman et al., 2015, Lewis et al., 2015). With the improvement of methods for macroevolutionary studies (Pagel, 1994; Pybus and Harvey 2000; Maddison et al., 2007, Fitzjohn et al., 2009; Rabosky et al., 2014) it has been advocated that species diversification is not necessarily linked with adaptive radiations (Schluter, 2000), but could be a result of a gradual accumulation of ancestral lineages through time (Couvreur et al., 2011). The problems associated with studies on diversification are primarily due to insufficient data such as incomplete taxon samplings or phylogenetic uncertainties (Fitzjohn et al., 2009; Rabosky et al., 2013). Macroevolutionary studies require a good knowledge of paleontological events and eventually a fossil record to help calibrate the phylogenies, but most of the time these are lacking, making the interpretation of evolutionary histories controversial. Understanding the diversification processes of a group of organisms could be useful for biodiversity conservation

(Scantlebury et al., 2013), especially for hotspots like Madagascar (Myers et al., 2000) where priority should be given to the most valuable species due to lack of conservation funds.

Genus *Angraecum* is the second largest group of orchids in Madagascar; it has a high morphological diversity, which makes it a good candidate for macroevolutionary studies. *Angraecum* includes approximately 221 species (Chase et al., 2015; Govaerts et al., 2015) distributed from sub-Saharan Africa to Madagascar, the Indian Ocean Islands (IOI: Comoros, Mauritius, Reunion, Rodrigues, and Seychelles), and Sri Lanka. Madagascar is the center of diversity for the genus with ca. 142 species of which 90% are endemic. The majority of species are epiphytic, but some are lithophytic. Epiphytic plants are found in tropical rain forest across the eastern slope of the mountains or in mesic forests in the central highland, while lithophytic species are found on inselbergs or on limestone. Recent molecular phylogenetic work (Chase et al., 2015) placed *Angraecum* in subtribe Angraecinae, the latter sister to subtribe Aeridinae. The phylogenetic reconstructions of the tribe Vandeae (Carlsward et al., 2006) revealed the polyphyly of Angraecinae sensu Dressler (1993) and of many of its genera including *Angraecum* (Carlsward et al., 2006; Micheneau et al., 2008a). Accordingly, a new circumscription has been proposed by merging Angraecinae and Aerangidinae into a single subtribe Angraecinae (Micheneau et al., 2008a), which includes 47 genera and ca. 762 species (Chase et al., 2015). Even though revisions were made to resolve the polyphyly of several genera (e.g. Cribb et al., 2007; Cribb and Carlsward, 2012), similar attempts with *Angraecum* proved difficult because of limited sampling (Carlsward et al., 2006; Micheneau et al., 2008a). The study of *Angraecum* is complicated because of the large number of species associated with a great morphological diversity, and because of morphological similarities between many members of subtribe Angraecinae. Garay (1973) proposed 19 sections to accommodate the species according to morphological descriptions, essentially floral (e.g. flower color, flower size, spur length), as was done by previous authors (e.g., Schlechter, 1918, 1925; Perrier de la Bathie, 1941; Summerhayes, 1958). Recent molecular phylogenic work revealed that Garay's sections are polyphyletic (Micheneau et al., 2008a; Simo-Droissart et al., 2013). Some authors proposed to remove all species that cause the polyphyly with the aim of making *Angraecum* monophyletic (Szlachetko and Romowicz, 2007; Simo-Droissart et al., 2013; Szlachetko et al., 2013). All strictly African sections were removed from *Angraecum* sensu lato (Szlachetko et al., 2013) and elevated to generic rank (*Angraecoides*, *Dolabrifolia*, *Eichlerangraecum* and *Pectinariella*). Micheneau et al. (2008a) showed that the morphology-based classification did not reflect the molecular phylogeny, and concluded that most of the sections of Garay (1973) were non-monophyletic. This study was based on Mascarene species and was lacking samples from Madagascar.

Although, the evolution of pollinia, adaptation to epiphytic habitat, and development of crassulacean acid metabolism (CAM) photosynthesis was shown to have been involved in the diversification of the Orchidaceae (Givnish et al., 2015), little is known about the relationship between morphological variation and species diversification. With the morphological diversity observed in *Angraecum*, we were interested in whether the traits used to define the sections, essentially flower color, flower size and spur length, were also involved in species diversification. Questions arose also on how the diversification of the genus occured. The main objectives of the current study are to (1) reconstruct the phylogeny of *Angraecum* sensu Garay (1973) using a larger sample of Malagasy species and most available Angraecinae species; (2) test the monophyly of morphologically defined sections; and (3) evaluate whether species diversification corroborates with morphological diversification. Here, we present a comprehensive phylogenetic reconstruction of *Angraecum* sensu Garay based on molecular DNA sequence data and a more comprehensive sample of species. Using this phylogenetic framework combined with morphological data, we attempt to resolve the systematic problems existing at the sectional level, and try to understand the patterns of species diversification in *Angraecum*. To avoid confusion, we will use *Angraecum* to designate the sensu stricto genus, and *Angraecum* sensu lato to identify the widest concept of the genus as defined by Garay.

1.3. Material and methods

1.3.1. Taxon sampling

Plant tissue for DNA extraction was obtained from field collected silica gel dried samples (Chase and Hills, 1991) of most of Malagasy *Angraecum* s.l. and Angraecinae species. The remaining materials, in the form of sequences data, came from previous studies

(Carlsward et al., 2006; Micheneau et al., 2008a; Rakotoarivelo et al., 2013; Simo-Droissart et al., 2013). A total of 194 specimens of which 69 were newly sampled were included in our analyses, comprising 98 *Angraecum* s.l., 17 *Jumellea*, 10 *Aeranthes*, and 40 other group genera (Appendix 1). *Acampe ochracea*, *Aerides odorata*, *Phalaenopsis cornu-cervi*, *Vanda tricolor* (subtribe Aeridinae), and *Polystachya fulvilabia* (subtribe Polystachyinae) were used as outgroups based on previous phylogenetic studies (Carlsward et al., 2006; Micheneau et al., 2008a; Chase et al., 2015). Because of conservation policy in Madagascar National Parks, the collection of herbarium specimens was not allowed in protected areas. Therefore, pictures were taken to serve as vouchers and were stored at the Marie-Victorin Herbarium (MT), which eventually will be accessible via Canadensys (in progress). Elsewhere, voucher specimens were collected and will be deposited at the national herbarium of Madagascar (TAN) and MT. Overall, 32 of the 69 sampled specimens were vouchered (Appendix 1).

1.3.2. Morphological data

To investigate morphological evolution and diversification pattern, 39 characters (13 vegetative, and 26 floral) were scored for the taxa represented in our molecular sampling. Type specimens preserved in the herbaria at Kew (K) and Paris (P), where over 80% of holotypes are located, were scored for each *Angraecum* s.l. species. Missing characters were documented from literature descriptions (Perrier de la Bathie, 1941; Stewart et al., 2006; Cribb and Hermans, 2009).). For type specimens preserved in other herbaria (e.g. B, BM, MO) that we did not borrow and for other genera, characters were scored from photographs of living material and voucher specimens as well as from literature descriptions (Stewart et al., 2006; Cribb and Hermans, 2009). Character descriptions and other background information are provided in Appendix 2, while the generated matrix is presented in Appendix 3.

1.3.3. PCR amplification and DNA sequencing

Total DNA was extracted from 20–30 mg of silica-dried gel leaf material following the modified hexadecylmethylammonium bromide (2x CTAB, 2% (w/v)) extraction protocol of

Doyle and Doyle (1987); 1% polyvinylpyrrolidone (PVP) and 0.2% of β-mercaptoethanol was added to the total volume of the extraction buffer. Three plastid DNA markers were amplified: *mat*K coding gene, *rps*16 intergeneric spacer, and *trn*L intron. The amplification of the *mat*K region was performed using the barcoding primers 472F/1248R designed by Yu et al. (2011). The *rps*16 region was amplified using the primers 1F/2R designed by Oxelman et al. (1997). The *trn*L intron was amplified using the primers 49873F/50272R designed by Taberlet et al. (1991). The nuclear ribosomal internal transcribed spacer ITS2 region was amplified using the primers S2F/S3R designed by Chen et al. (2010). The PCR reactions contained, 1X PCR reaction buffer, 2.5 mM MgCl2, 0.16 µM of each primer, 0.2 mM of each dNTP, 0.4 % bovine serum albumin (BSA), 2 units of Taq DNA polymerase, 30 ng of template DNA, adjusted to a final volume of 25 µL with de-ionized water. PCR conditions are the same as described in Yu et al. (2011). PCR amplifications were performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA), and resulting PCR products were purified using exonuclease I and shrimp alkaline phosphatase (ExoSAP; Silva et al., 2001). The purified products were cycle sequenced using a BigDye® terminator sequencing kit (Life Technologies, Carlsbad, CA). Sequences were analyzed with an Applied Biosystems 3130xl genetic analyzer at the Omaha's Henry Doorly Zoo and Aquarium (NE, USA). Sequence fragments were aligned to generate a consensus sequence using Sequencher® 4.10 (Gene Codes Corporation; Ann Arbor, MI). Sequence data were also obtained from the Canadian Center for DNA Barcoding (part of *mat*K and ITS2) and from GenBank for previous studies (Carlsward et al., 2006; Micheneau et al., 2008a; Rakotoarivelo et al., 2013; Simo-Droissart et al., 2013). Following automatic alignment using SeaView v4 (Gouy et al., 2010), alignments were edited manually using BioEdit v7.1.3 (Hall, 1999). All newly generated sequences have been deposited in GenBank (Appendix 1), and the matrices in TreeBase (in progress).

1.3.4. Phylogenetic analyses

Four matrices were produced: (1) combined plastid, (2) nuclear ribosomal, (3) morphological matrix, and (4) combined plastid and morphological matrix. They were analyzed using maximum parsimony (MP) and Bayesian analyses (BA). Tree searches under parsimony were conducted in PAUP* version 4.0 Beta (Swofford, 2002). A preliminary heuristic search was performed with 1000 replicates of random addition sequence, tree bisection–reconnection (TBR) branch swapping, retaining twenty most parsimonious trees at each replicate. Starting with the trees kept in memory from this initial analysis, a second heuristic search was performed with TBR and 10000 trees were saved. A strict consensus tree was constructed for each analysis. Branch support was estimated using 5000 bootstraps replicates under a heuristic strategy with one random addition-sequence replicate TBR branch swapping. Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2003). The best nucleotide substitution model was selected with jModelTest2 (Posada and Crandall, 1998) using the Akaike information criteria (AIC) (Akaike, 1974). For all regions, the GTR+I+G model scored best and was selected. For the combined molecular and morphological matrix, data were partitioned as DNA and standard respectively. Two parallel runs of eight Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) each, and four swaps per swapping cycles for 15 million generations were undertaken. Trees were sampled every 1000 generations, and the first 25% generations were discarded as burnin. The 50% majority consensus tree with Bayesian clade credibility was built from post-burnin trees.

1.3.5. Estimation of divergence times

Divergence times were estimated using a relaxed molecular clock approach as implemented in BEAST (Drummond and Rambaut, 2007). The combined plastid matrix was used as input data in BEAUti. The GTR+G+I model was selected as substitution model. A relaxed lognormal molecular clock model was selected. The Yule model was selected as tree prior. The age for the root of the tree was set to a normal distribution with mean 35 Ma and a standard deviation of 3 (giving a 95% CI ranging from 30.07 – 39.93 Ma). Because fossil data are rare in the Orchidaceae (Iles et al., 2015.) only three having been recorded so far, none of them close to our group we used the age estimate of *Phalaenopsis* (35 Ma) found by Gustafsson et al. (2010) to calibrate the stem root of Vandeae. The prior distribution of the 'ucld.mean' parameter was set to an exponential distribution (mean=10.0, initial value=1.0). Four separate runs were performed in BEAST with 50 million generations each, sampling parameters and trees every 1000 generations. Trees were summarized with burn-in values set to the first 25% of trees sampled using TreeAnnotator and were summarized in a maximum clade credibility tree.

1.3.6. Diversification analyses

In order to assess diversification patterns, we evaluated the state-dependent diversification of morphological characters using the Binary State Speciation and Extinction (BiSSE) and the MultiState Speciation and Extinction (MuSSE) models implemented in the R package ‘diversitree’ (FitzJohn, 2012), and the speciation/extinction and phenotypic/evolution models using BAMM v.1.0 (Rabosky, 2014). Four floral characters were chosen for these analyses because of their taxonomic interest: flower colors, flower size, spur length, and labellum position (Appendix 2). Three of these characters (flower color, flower size and spur length) have been used previously to delineate sections in *Angraecum* (e.g., Perrier de la Bathie, 1941; Garay, 1973) while the labellum position was added because it appeared useful for sectional delimitation. Since these methods require ultrametric and fully bifurcating trees, we used the BEAST tree as input.

Two characters were analyzed using BiSSE: flower color and labellum position. Since we were interested in the effect the green and white flower colors may have on diversification, the taxa that did not fit in these two colors (for instance, pink or purple flowered taxa) were excluded from the analyses using the ‘drop.tip2’ function of the R package ‘phyloch’ (Heibl, 2008). Three models were tested: (1) a full model that allows all parameters ($\boldsymbol{\lambda}$**:** speciation rate, $\boldsymbol{\mu}$**:** extinction rate, $\boldsymbol{q}$**:** transition rate) to vary, (2) a constrained model allowing speciation and transition rates to vary while keeping extinction rates equal between states ($\boldsymbol{\mu}0 \sim \boldsymbol{\mu}1$), and (3) a constrained model allowing extinction and transition rates to vary while keeping speciation rates equal between states ($\boldsymbol{\lambda}0 \sim \boldsymbol{\lambda}1$). Two characters were analyzed with MuSSE: flower size and spur length. Both characters were treated as ordered. A preliminary run was performed with the unconstrained model to determine the best transition model. For flower size, which has five character states (1: tiny, 2: small, 3: medium, 4: large, 5: very large), two linear transition models were tested: (1) a constrained model where character evolution was possible in both directions between neighboring states (1 ↔ 2 ↔ 3 ↔ 4 ↔ 5), and (2) a constrained model where evolution could be either unidirectional or bidirectional between neighboring states (1 ←2 ←3 ↔ 4 → 5). For spur length, which has five character states (1: very short, 2: short, 3: medium, 4: long, 5: very long), two transition models were also tested: (1) a constrained linear model where character evolution was bidirectional and possible only among neighboring states (1 ↔ 2 ↔ 3 ↔ 4 ↔ 5), and (2) a constrained model where character evolution could be either unidirectional or bidirectional between states that are not necessarily adjacent (nonlinear) (1 ←2 ↔ 3 ↔ 4 ↔ 5, 1←3, 1←4). Having established the best fit transition model, we used it as a constraint while testing the three models of diversification: (1) an unconstrained model that allowed all parameters ($\boldsymbol{\lambda}$, $\boldsymbol{\mu}$, $\boldsymbol{q}$) to vary, (2) a constrained model allowing speciation and transition rates to vary while keeping extinction rates equal between states, and (3) a constrained model allowing extinction and transition rates to vary while keeping speciation rates equal between states. After testing the models with BiSSE and MuSSE, the posterior probabilities of the parameters were computed under a Bayesian framework after setting the priors to be exponential. The MCMC was run for 10000 generations sampling parameters every 100 generations, and the posterior distributions of parameters were summarized using the function 'profiles.plot' implemented in 'diversitree' (FitzJohn, 2012).

For the speciation/extinction model implemented in BAMM, we estimated our total sampling at 40% and we fractioned the data according to the number of species per genus being represented in our phylogeny (Appendix 4) in order to reduce the bias in estimating parameters under an assumption of incomplete sampling (Rabosky, 2014). To reduce the weight of the outgroup taxa we excluded them from the analyses using the 'drop.tip2' function of the R package 'phyloch' (Heibl, 2008). For the phenotypic/evolution model, we assessed the regime of morphological evolution of two of the four characters mentioned above, flower size and spur length (Appendix 4), since this model only treats continuous characters. For each model (speciation/extinction and phenotypic/evolution), BAMM was run for 5,000,000 generations and parameters were sampled every 1000 generations. The parameter priors were set using parameters generated from the R package 'BAMMtools' (Rabosky et al., 2014). The rate shift configurations and the rate through-time generated from 'bammdata outputs' were analyzed using the R package 'BAMMtools'.

Many criticisms have been made concerning the SSE's family (BiSSE, MuSSE, GeoSSE, etc.) error rate and limitations (Maddison and FitzJohn, 2015; Rabosky and Goldberg, 2015). It has been reported that "within-clade pseudoreplications" might result in erroneously significant results (Maddison and FitzJohn, 2015). The use of additional methods like BAMM (Rabosky et al., 2013) has been proposed to reinforce the results implemented under SSE's (Rabosky and Goldberg, 2015). Both methods use different approaches but are complementary: MuSSE looks at the effects of character states on diversification, while BAMM detects macroevolutionary rate shifts across phylogenetic trees.

1.3.7. Character state reconstruction

We examined the character evolution of four floral traits previously used in taxonomy (flower colors, flower size, spur length, and labellum position) using the Markov discrete character evolution (Pagel, 1994; Lewis, 2001) as implemented in the R package 'diversitree' (FitzJohn, 2012). Because this method requires ultrametric and fully bifurcating trees, we used the BEAST tree as input. No constraint was applied to the analyses leaving all parameters free.

1.4. Results

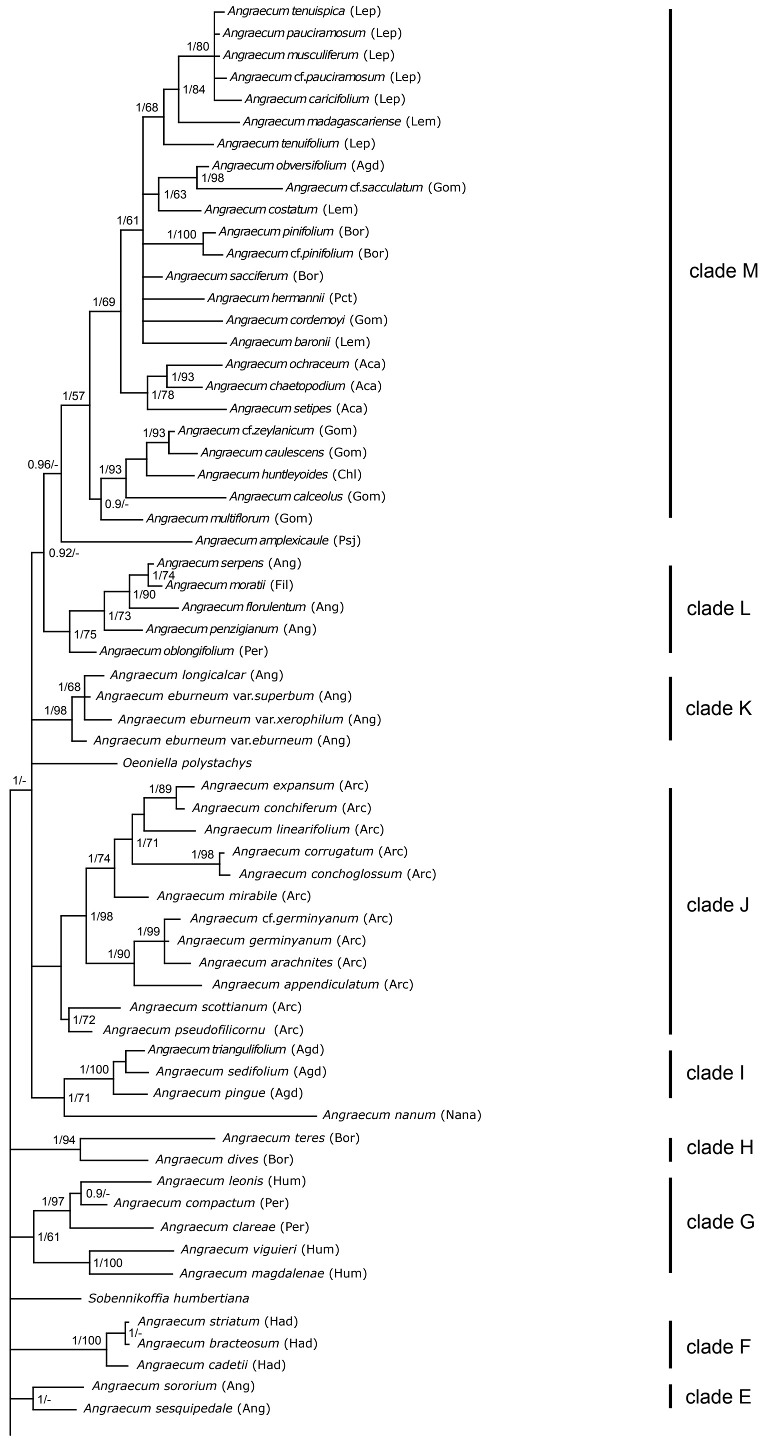
1.4.1. Phylogenetic relationships

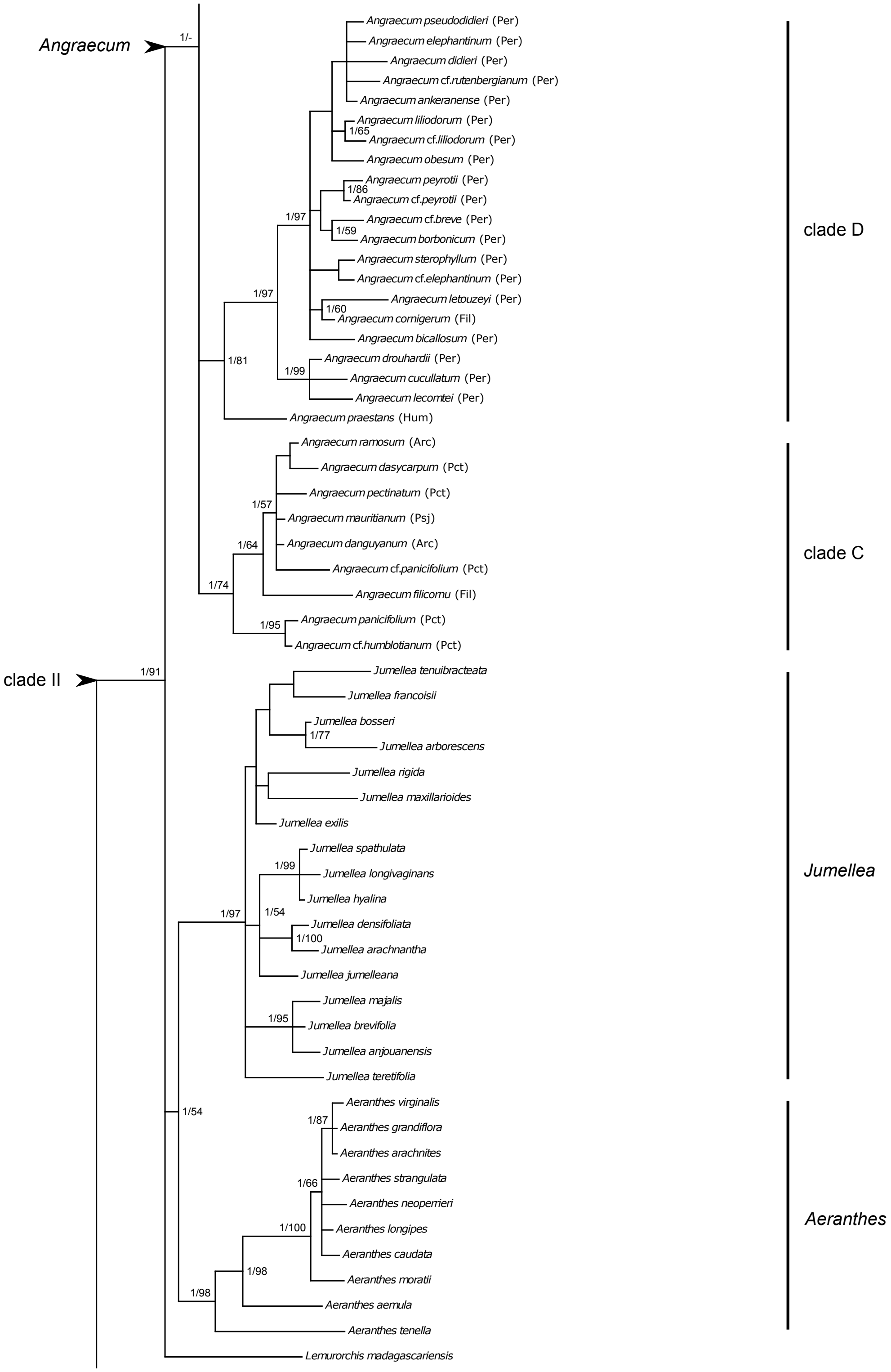
Of the 69 newly sampled specimens that we sequenced, all were fully amplified with *mat*K for a total length of 942 base pairs (bp); 66 with *rps*16 (1192 bp) except *Angraecum sterophyllum*, *A. rhynchoglossum* and *Lemurella papillosa* which failed to amplify; and 65 with *trn*L (1553 bp) except *A. pseudofilicornu*, *A. rhynchoglossum*, *Oeoniella polystachys* and *Oeonia rosea*. Only 13 of 69 samples were sequenced with ITS2 for a total length of 447 bp. When combined with GenBank sequences data, each individual matrix was composed of 190 taxa for *mat*K, 140 for *rps*16, 170 for *trn*L and 88 for ITS2. Because of the small amount of samples available with ITS2, we decided to not combine them with the plastid matrices. Therefore, the combined plastid matrix contained 3687 characters and 194 taxa. Sequences that were unavailable were treated as missing data. For the *trn*L intron, the ambiguous variable sites (360 bp) were excluded from our analyses. For the whole data set, 2361 characters were constant, 467 (13.8%) variable characters were parsimony-uninformative, and 537 (16%) characters were parsimony-informative. The combined plastid matrix produced five trees of 2202 steps with a consistency index (CI) of 0.56 and a retention index (RI) of 0.77.

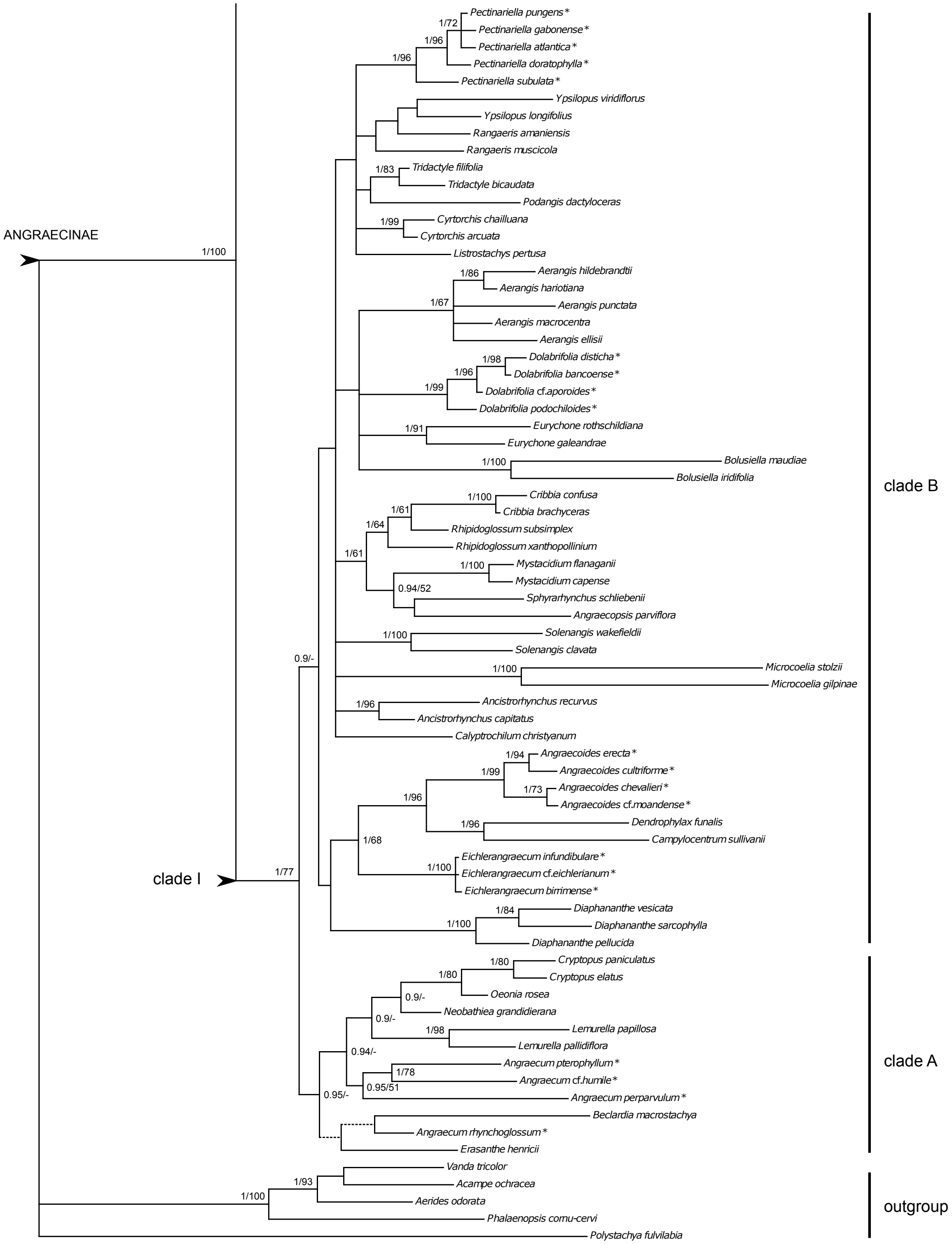
The MrBayes, BEAST, and MP analyses were congruent. The 50% majority-rule consensus tree from Bayesian analyses of the combined plastid matrix is displayed in Fig. 1.1. Our results support a monophyletic subtribe Angraecinae (PP 1.0, BP 100). Two well-supported clades were identified within Angraecinae: clade I (PP 1.0, BP 77) comprised of Malagasy, IOI, African and American genera, and clade II (PP 1.0, BP 91) with Malagasy and IOI genera. Clade II had more branch support and showed more resolution than clade I. Two main subclades are observed within clade I: a Malagasy-IOI clade A (PP 1.0), and an African-American clade B (PP 1.0). Four Malagasy *Angraecum* s.l. species (*A. perparvulum*, *A.* cf. *humile*, *A. pterophyllum*, and *A. rhynchoglossum*) are nested within clade A. Three major subclades were observed in clade II: *Aeranthes* (PP 1.0, BP 98), *Jumellea* (PP 1.0, BP 97), and *Angraecum* (PP 1.0). Here, we define *Angraecum* as a monophyletic group including all *Angraecum* species and all other taxa nested within it in clade II. Based on branch support (PP, BP) and morphological resemblance, eleven clades are observed within *Angraecum* (Fig. 1.1, clade C to M). From the base to the top of the tree are: clade C (PP 1.0, BP 74) comprised members of sections *Pectinaria*, *Pseudojumellea*, *Arachnangraecum* and *Filangis*; clade D (PP 1.0, BP 81) comprising member of sections *Perrierangraecum*, *Angraecum*, *Arachnangraecum* and *Filangis*; clade E (PP 1.0) which includes *A. sesquipedale*, and *A. sororium* of section *Angraecum*; clade F (PP 1.0, BP 100) section *Hadrangis*; clade G (PP 1.0, BP 61) with section *Humblotiangraecum* and a member of section *Perrierangraecum*; clade H (PP 1.0, BP 94) section *Boryangraecum*; clade I (PP 1.0, BP 100) section *Angraecoides*; clade J section *Arachnangraecum*; clade K (PP 1.0, BP 98) section *Angraecum* (*A. eburneum*); clade L (PP 1.0, BP 75) comprised of members of sections *Angraecum* and *Pseudojumellea*; clade M (PP 1.0, BP 57) composed of sections *Acaulia*, *Boryangraecum*, *Chlorangraecum*, *Gomphocentrum*, *Lemurangis*, and *Lepervenchea*. Clades E and J received weak support despite the fact that their positions were supported in a strict consensus tree. Two species are not included in any of the clades, *A. nanum* sister to clade I (PP 1.0, BP 72), and *A. amplexicaule* straddles between clade L and M (Fig. 1.1). Furthermore, two Malagasy genera, *Oeoniella* and *Sobennikoffia*, are nested within *Angraecum*. Even though many clades were strongly supported within *Angraecum*, resolution between clades was lacking.

The phylogeny obtained from ITS2 was lacking resolution and was slightly incongruent with the combined plastid ones (Appendix 5). Notably, the Malagasy Angraecinae genera *Aeranthes*, *Jumellea*, and *Lemurorchis* were embedded within *Angraecum*, rendering it paraphyletic. Nonetheless, all represented clades within *Angraecum* appeared to be congruent with those in the plastid data (Fig. 1.1). Our combined plastid and morphological analyses yielded a topology that is congruent with the combined plastid analyses with slight differences in branch supports (Appendix 6). Within *Angraecum*, clade M (PP 1.0, BP 75) gained support while clade D became weaker. The position of *Lemurorchis* was ambiguous, forming a polytomy with the *Aeranthes* – *Jumellea* clade in the molecular analyses while it was embedded with *Angraecum* in the combined analyses. The morphological analyses alone supported the monophyly of Angraecinae sensu Carlsward et al. (2006) with many polytomies observed within the clade (Appendix 7). According to the morphological phylogeny, most genera were monophyletic (*Aeranthes*, *Jumellea*, and most of the African genera) except *Angraecum*, where conflicts were also observed compared to the plastid tree.

Fig. 1.1. Phylogenetic relationships within subtribe Angraecinae. 50% Bayesian majority-rule consensus tree from combined plastid data (*mat*K, *rps*16 and *trn*L). Values above branches or at nodes represent posterior probability (PP) and bootstrap percentage (BP) support. Dashes represent branches that collapsed in MP strict consensus tree. Taxa with asterisk are *Angraecum* sensu Garay species. Abbreviations in brackets denote sections sensu Garay (1973): Aca = *Acaulia*, Agd = *Angraecoides*, Ang = *Angraecum*, Arc = *Arachnangraecum*, Bor = *Boryangraecum*, Chl = *Chlorangraecum*, Fil = *Filangis*, Gom = *Gomphocentrum*, Had = *Hadrangis*, Hum = *Humblotiangraecum*, Lem = *Lemurangis*, Lep = *Lepervenchea*, Pct = *Pectinaria*, Per = *Perrierangraecum*, Psj = *Pseudojumellea*.


Angraecum tenuispica (Lep)
Angraecum pauciramosum (Lep)
1/80
Angraecum musculiferum (Lep)
Angraecum cf.pauciramosum (Lep)
1/84
Angraecum caricifolium (Lep)
1/68
Angraecum madagascariense (Lem)
Angraecum tenuifolium (Lep)
Angraecum obversifolium (Agd)
1/98
Angraecum cf.sacculatum (Gom)
1/63
Angraecum costatum (Lem)
1/61
1/100
Angraecum pinifolium (Bor)
Angraecum cf.pinifolium (Bor)
Angraecum sacciferum (Bor)
Angraecum hermannii (Pct)
1/69
Angraecum cordemoyi (Gom)
Angraecum baronii (Lem)
Angraecum ochraceum (Aca)
1/93
Angraecum chaetopodium (Aca)
1/57
1/78
Angraecum setipes (Aca)
1/93
Angraecum cf.zeylanicum (Gom)
Angraecum caulescens (Gom)
0.96/-
1/93
Angraecum huntleyoides (Chl)
Angraecum calceolus (Gom)
0.9/-
Angraecum multiflorum (Gom)
Angraecum amplexicaule (Psj)
0.92/-
Angraecum serpens (Ang)
1/74
Angraecum moratii (Fil)
1/90
Angraecum florulentum (Ang)
1/73
Angraecum penzigianum (Ang)
1/75
Angraecum oblongifolium (Per)
Angraecum longicalcar (Ang)
1/68
Angraecum eburneum var.superbum (Ang)
1/98
Angraecum eburneum var.xerophilum (Ang)
Angraecum eburneum var.eburneum (Ang)
Oeoniella polystachys
1/-
1/89
Angraecum expansum (Arc)
Angraecum conchiferum (Arc)
Angraecum linearifolium (Arc)
1/71
1/98
Angraecum corrugatum (Arc)
1/74
Angraecum conchoglossum (Arc)
Angraecum mirabile (Arc)
1/98
Angraecum cf.germinyanum (Arc)
1/99
Angraecum germinyanum (Arc)
1/90
Angraecum arachnites (Arc)
Angraecum appendiculatum (Arc)
Angraecum scottianum (Arc)
1/72
Angraecum pseudofilicornu (Arc)
Angraecum triangulifolium (Agd)
1/100
Angraecum sedifolium (Agd)
1/71
Angraecum pingue (Agd)
Angraecum nanum (Nana)
1/94
Angraecum teres (Bor)
Angraecum dives (Bor)
Angraecum leonis (Hum)
0.9/-
1/97
Angraecum compactum (Per)
Angraecum clareae (Per)
1/61
Angraecum viguieri (Hum)
1/100
Angraecum magdalenae (Hum)
Sobennikoffia humbertiana
Angraecum striatum (Had)
1/-
1/100
Angraecum bracteosum (Had)
Angraecum cadetii (Had)
Angraecum sororium (Ang)
1/-
Angraecum sesquipedale (Ang)
clade M
clade L
clade K
clade J
clade I
clade H
clade G
clade F
clade E


Angraecum
1/-
Angraecum pseudodidieri (Per)
Angraecum elephantinum (Per)
Angraecum didieri (Per)
Angraecum cf.rutenbergianum (Per)
Angraecum ankeranense (Per)
Angraecum liliodorum (Per)
1/65
Angraecum cf.liliodorum (Per)
Angraecum obesum (Per)
Angraecum peyrotii (Per)
1/86
Angraecum cf.peyrotii (Per)
1/97
Angraecum cf.breve (Per)
1/59
Angraecum borbonicum (Per)
Angraecum sterophyllum (Per)
Angraecum cf.elephantinum (Per)
1/97
Angraecum letouzeyi (Per)
1/60
Angraecum cornigerum (Fil)
Angraecum bicallosum (Per)
1/81
Angraecum drouhardii (Per)
1/99
Angraecum cucullatum (Per)
Angraecum lecomtei (Per)
Angraecum praestans (Hum)
clade D
Angraecum ramosum (Arc)
Angraecum dasycarpum (Pct)
Angraecum pectinatum (Pct)
1/57
Angraecum mauritianum (Psj)
1/64
Angraecum danguyanum (Arc)
Angraecum cf.panicifolium (Pct)
1/74
Angraecum filicornu (Fil)
1/95
Angraecum panicifolium (Pct)
Angraecum cf.humblotianum (Pct)
clade C
clade II
1/91
Jumellea tenuibracteata
Jumellea francoisii
Jumellea bosseri
1/77
Jumellea arborescens
Jumellea rigida
Jumellea maxillarioides
Jumellea exilis
Jumellea spathulata
1/99
Jumellea longivaginans
Jumellea hyalina
1/97
1/54
Jumellea densifoliata
1/100
Jumellea arachnantha
Jumellea jumelleana
Jumellea majalis
1/95
Jumellea brevifolia
Jumellea anjouanensis
Jumellea teretifolia
Jumellea
Aeranthes virginalis
1/87
Aeranthes grandiflora
1/54
Aeranthes arachnites
Aeranthes strangulata
1/66
Aeranthes neoperrieri
1/100
Aeranthes longipes
Aeranthes caudata
1/98
Aeranthes moratii
1/98
Aeranthes aemula
Aeranthes tenella
Aeranthes
Lemurorchis madagascariensis


Pectinariella pungens*
1/72
Pectinariella gabonense*
1/96
Pectinariella atlantica*
1/96
Pectinariella doratophylla*
Pectinariella subulata*
Ypsilopus viridiflorus
Ypsilopus longifolius
Rangaeris amaniensis
Rangaeris muscicola
1/83
Tridactyle filifolia
Tridactyle bicaudata
Podangis dactyloceras
1/99
Cyrtorchis chailluana
Cyrtorchis arcuata
ANGRAECINAE
1/100
Listrostachys pertusa
1/86
Aerangis hildebrandtii
Aerangis hariotiana
1/67
Aerangis punctata
Aerangis macrocentra
Aerangis ellisii
1/98
Dolabrifolia disticha*
1/96
Dolabrifolia bancoense*
1/99
Dolabrifolia cf.aporoides*
Dolabrifolia podochiloides*
1/91
Eurychone rothschildiana
Eurychone galeandrae
1/100
Bolusiella maudiae
Bolusiella iridifolia
clade B
1/100
Cribbia confusa
1/61
Cribbia brachyceras
1/64
Rhipidoglossum subsimplex
1/61
Rhipidoglossum xanthopollinium
1/100
Mystacidium flanaganii
Mystacidium capense
0.94/52
Sphyrarhynchus schliebenii
Angraecopsis parviflora
1/100
Solenangis wakefieldii
Solenangis clavata
0.9/-
1/100
Microcoelia stolzii
Microcoelia gilpinae
1/96
Ancistrorhynchus recurvus
Ancistrorhynchus capitatus
Calyptrochilum christyanum
1/94
Angraecoides erecta*
1/99
Angraecoides cultriforme*
1/73
Angraecoides chevalieri*
1/96
Angraecoides cf.moandense*
1/96
Dendrophylax funalis
1/68
Campylocentrum sullivanii
Eichlerangraecum infundibulare*
1/100
Eichlerangraecum cf.eichlerianum*
clade I
1/77
Eichlerangraecum birrimense*
1/84
Diaphananthe vesicata
1/100
Diaphananthe sarcophylla
Diaphananthe pellucida
1/80
Cryptopus paniculatus
1/80
Cryptopus elatus
0.9/-
Oeonia rosea
Neobathiea grandidierana
0.9/-
1/98
Lemurella papillosa
Lemurella pallidiflora
0.94/-
1/78
Angraecum pterophyllum*
clade A
0.95/51
Angraecum cf.humile*
0.95/-
Angraecum perparvulum*
Beclardia macrostachya
Angraecum rhynchoglossum*
Erasanthe henricii
Vanda tricolor
1/93
Acampe ochracea
1/100
Aerides odorata
outgroup
Phalaenopsis cornu-cervi
Polystachya fulvilabia

1.4.2. Divergence time estimates

The maximum credibility tree of the calibrated relaxed molecular clock analysis of Angraecinae is shown in Appendix 8. Our results suggest that Angraecinae shared a most recent common ancestor (MRCA) in the late Oligocene (~ 26.1 Ma), and started to diverge in the early Miocene. Diversification started at approximately 21.1 Ma (giving a node height highest posterior density (HPD) intervals at 95% ranging from 18.2 – 33.5 Ma) for clade I and 17.18 Ma (95% HPD: 14.6 – 27.8 Ma) for clade II. In clade I, the African-American clade B diverged at approximately 19.46 Ma (95% HPD: 14.6 – 27.8 Ma), while the Malagasy-IOI clade A diverged at approximately 18.1 Ma (95% HPD: 14.6 – 27.8 Ma). Within clade II, *Angraecum*, *Aeranthes* and *Jumellea* started to diversify at around 14.56 Ma (95% HPD: 9.9 – 19.2 Ma), 10.7 Ma (95% HPD: 5.8 – 15.2 Ma), and 7.8 Ma (95% HPD: 4.9 – 11 Ma) respectively. Two waves of diversification are observed in *Angraecum*, during the Pliocene (~ 6 – 2.6 Ma) and during the Pleistocene (~ 2.6 – 0.2 Ma). *Angraecum* section *Hadrangis* which is endemic to the Mascarene Islands diverged at approximately 1.66 Ma (95% HPD: 0.4 – 3.3 Ma), and the divergence time for the two species *A. bracteosum* and *A. striatum* that are endemic to Reunion is estimated at 0.2 Ma (95% HPD: 0 – 0.7 Ma). Since this age is younger than the estimated age of the Island, approximately 2.1 Ma (Baksi and Hoffman, 2000), it is reasonably consistent with the age obtained in our analyses.

1.4.3. Species diversification

Results from BiSSE showed that the second model ($\mu 0 \sim \mu 1$) received the best AIC score for flower color and labellum position (Table 1.1). The green and white colors had equal rates of speciation (Fig. 1.2A), while the uppermost labellum showed a higher speciation rate compared to the lowermost one (Fig. 1.2B). Results from MuSSE showed that the second transition model had the best AIC score (Table 1.1); this model was used to test the diversification models. The diversification model with extinction rates equal between states

Table 1.1. Comparaison of diversificaiton model used for the BiSSE and MuSSE analyses. Bold indicates the best fit model selected to test diversification of Angraecinae. Arrow represents transition allowed between states: ↔, reversible, →, irreversible. Parameters: λ, speciation rate; μ, extinction rate; q, character transition rate. Character states: A0, green; A1, white; B0, uppermost; B1, lowermost; C1, tiny; C2, small; C3, medium; C4, large; C5, very large; D1, very short; D2, short; D3, medium; D4, long; D5, very long. Abbreviations: AIC, Akaike information criterion; M, model tested; lnLik, Log likelihood.

Character	**Model**	**lnLik**	**AIC**
A color of flower	full	-567.496	1146.992
	M1 (λ0 ~ λ1)	-567.604	1145.208
	M2 (μ0 ~ μ1)	**-567.496**	**1144.992**
	M3 (q01 ~ q10)	-569.488	1148.976
B position of labellum	full	-599.017	1210.035
	M1 (λ0 ~ λ1)	-601.055	1212.110
	M2 (μ0 ~ μ1)	**-599.017**	**1208.035**
	M3 (q01 ~ q10)	-599.018	1208.036
C size of flower (pre-run)	full	-716.052	1492.105
	M1 (1 ↔ 2 ↔ 3 ↔ 4 ↔ 5)	-723.425	1482.851
	M2 (1 ←2 ←3 ↔ 4 → 5)	**-722.499**	**1474.999**
size of flower	full	-722.499	1474.999
	M1 (λi ~ λj)	**-722.666**	**1467.332**
	M2 (μi ~ μj)	-726.511	1475.022
D spur length (pre-run)	full	-727.404	1514.808
	M1 (1 ↔ 2 ↔ 3 ↔ 4 ↔ 5)	-737.145	1510.290
	M2 (1 ←2 ↔ 3 ↔ 4 ↔ 5, 1←3, 1←4)	**-729.235**	**1496.471**
spur length	full	-729.235	1496.471
	M1 (λi ~ λj)	**-729.235**	**1488.471**
	M2 (μi ~ μj)	-735.279	1500.559

received the best score for flower size and spur length, suggesting that character states have an effect on speciation. Medium and large flowers showed higher speciation rates compared to tiny and small flowers (Fig. 1.2C). All spur length states had a similar effect on speciation rates (Fig. 1.2D).

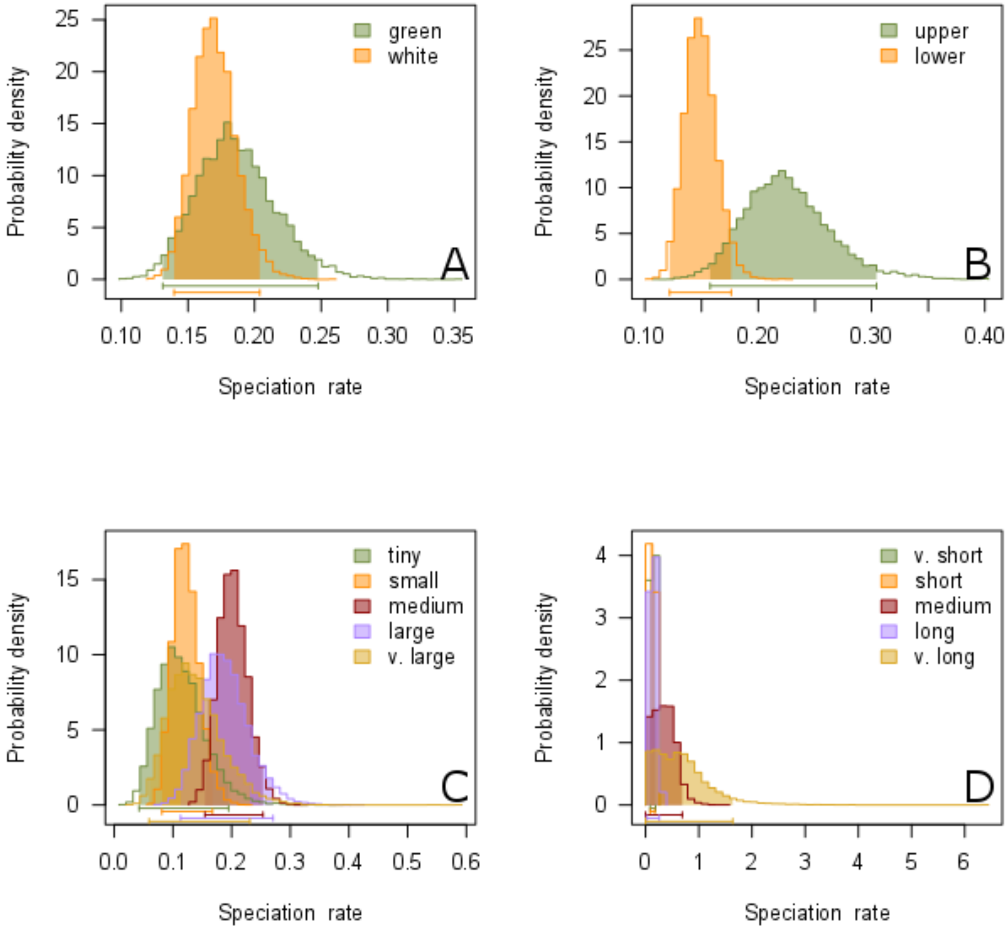


Fig. 1.2. Posterior probability distributions for the speciation rates (in Ma) of morphological characters using equal rate speciation ($\boldsymbol{\mu}0 \sim \boldsymbol{\mu}1$) with the BiSSE model and equal rate extinction (λi ~ λj) with the MuSSE model: flower color (A), labellum position (B), flower size (C), and spur length (D). Abbreviation: v, very.

The speciation/extinction model from BAMM revealed 5 distinct configuration shifts from the 95% credible set (Fig. 1.3) of which 66% of the samples in the posterior distribution showed no shift, 14% showed a single shift at the node of clade II, 6.6% had one within the *Aeranthes* clade, 5.9% had one shift at the branch of *Beclardia macrostachya*, and 3.6% of the posterior distribution had one shift at the branch of clade I. Our BAMM results showed a decline of speciation rate-through-time (RTT) for Angraecinae, starting from approximately 0.39 during the Miocene to 0.23 towards the present (Fig. 1.3).

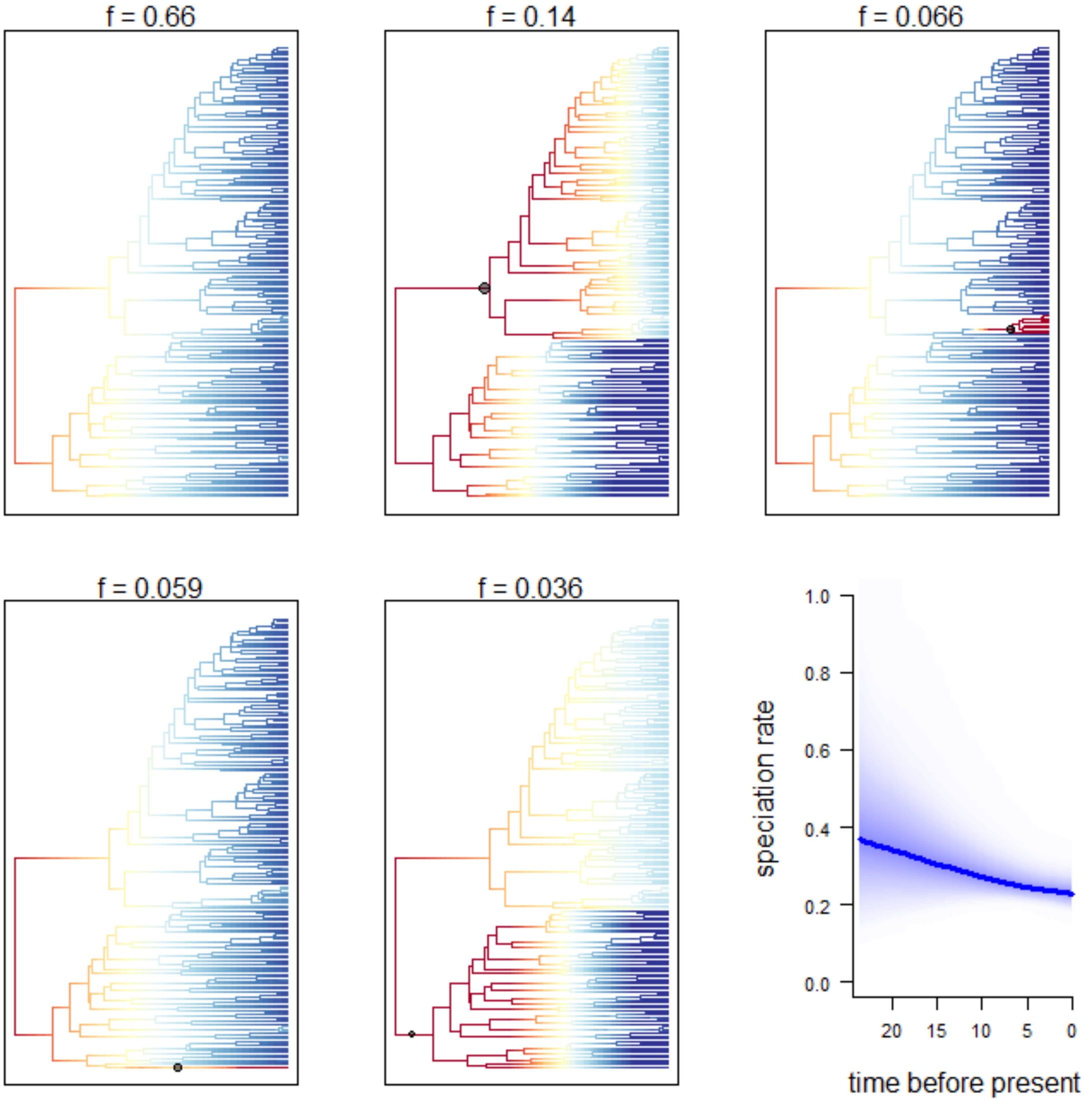


Fig. 1.3. Configuration shifts from the 95% credible set sampled by BAMM from the Angraecinae phylogeny and evolutionary rates through time. The intensity of colors on branches reflects the relative probability density of speciation rates (cool colors = slow, warm = fast). Black circles denote the position of the macroevolutionary regime shifts present in each sample. Blue curve indicates the mean speciation rate-through-time trajectory of Angraecinae in million years.

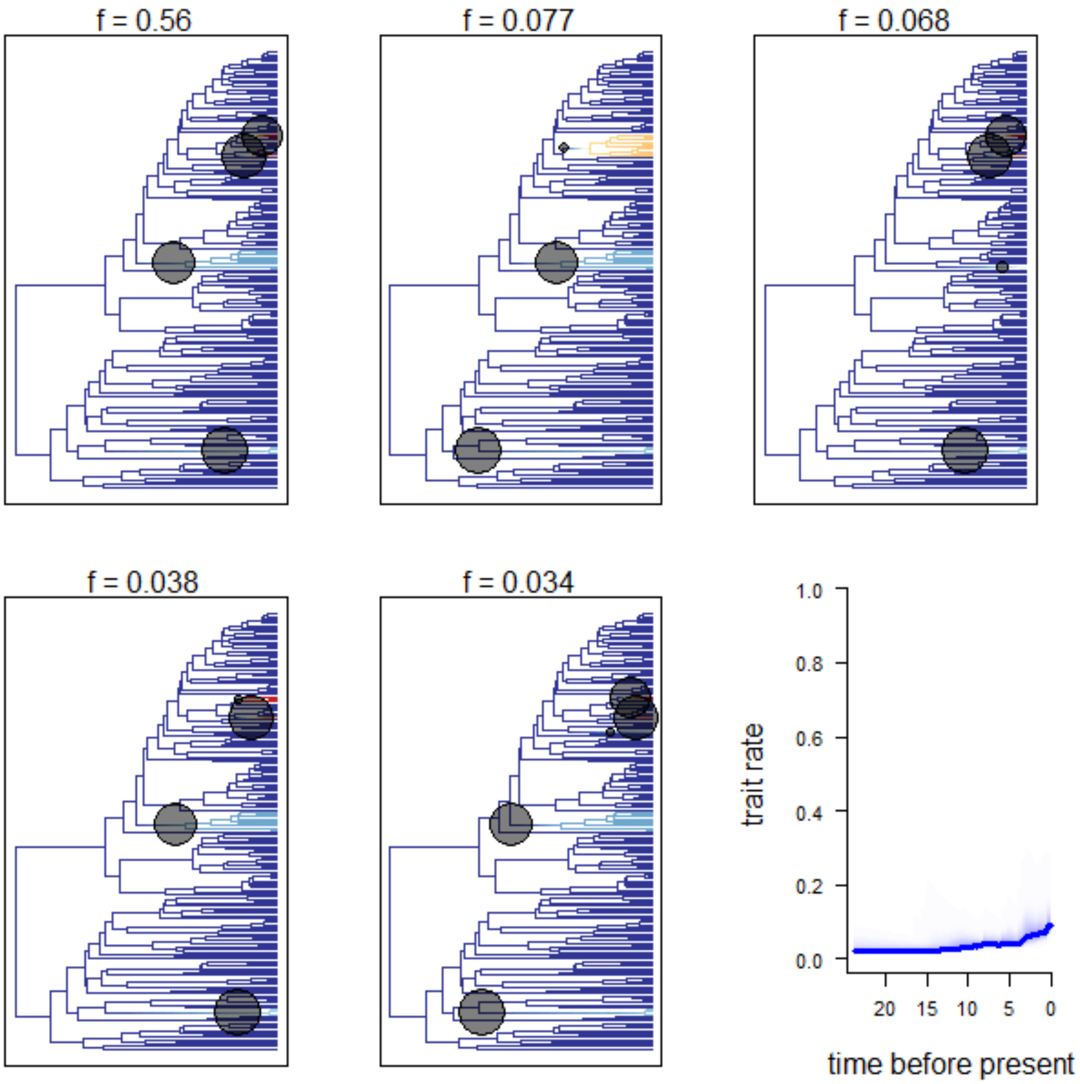


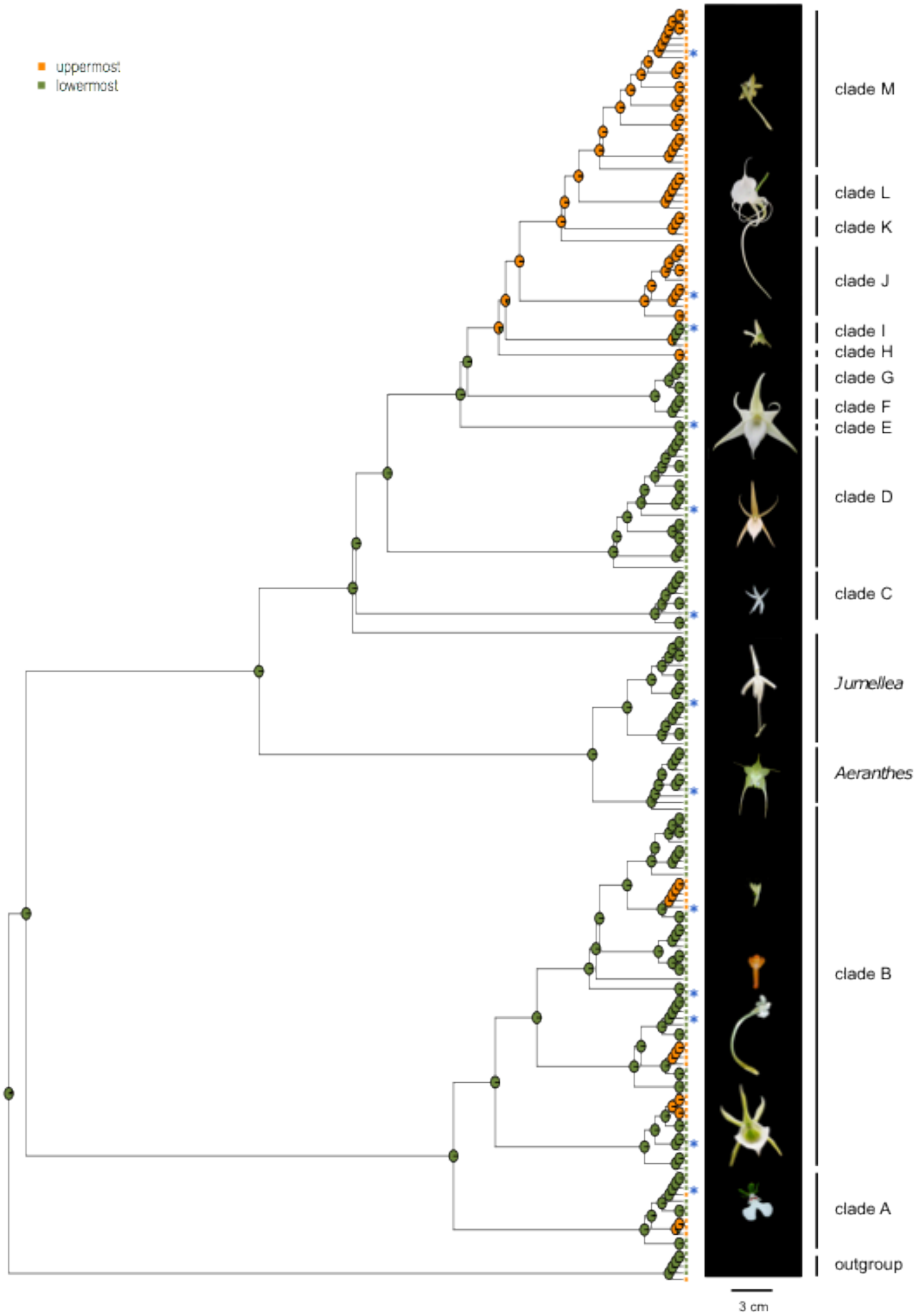
Fig. 1.4. Best configuration shifts from the 95% credible set sampled by BAMM for the evolution of spur length across the phylogeny of Angraecinae. Color intensity on branches reflects the relative probability density of the instantaneous rate of phenotypic evolution. Black circles denote the position of the macroevolutionary regime shifts present in each sample. Blue curve denotes the mean evolution rate-through-time trajectory.

The phenotypic/evolutionary model showed 32 distinct configurations on spur length. The five that received the best sample frequencies are displayed in Fig. 1.4. Four main shifts are observed, one at the branch of *Eichlerangraecum* clade, one at the branch of clade A, one at the branch of *Angraecum appendiculatum* and one shift at the branch of *Angraecum corrugatum*. The RTT phenotypic evolution showed an increased rate in spur length starting from 0.02 during the Pliocene to 0.08 in the Pleistocene and to the present (Fig. 1.4). No shift has been detected regarding flower size within Angraecinae and the RTT phenotypic evolution was constant (Appendix 9).

1.4.4. Ancestral state reconstructions

Our results showed that a labellum in the lower position is plesiomorphic in Angraecinae, while an upper labellum is apomorphic and evolved at least five times independently (Fig. 1.5). White flowers appear to be symplesiomorphic in Angraecinae, while green flowers are apomorphic and evolved several times in Angraecinae and two times independently in *Angraecum* (Appendix 10A). A medium flower appears to be the ancestral state in Angraecinae, while large and small flowers are derived (Appendix 10B). Long spur is the ancestral state and short spur is derived and arose several times independently (Appendix 10C). Our results showed that the color, the size, and the spur length are homoplastic within *Angraecum*. The taxa that showed uppermost labella appeared to be monophyletic and received very strong support in the phylogeny (*Angraecoides*, *Dolabrifolia*, *Pectinariella*, clades H to M).

Fig. 1.5. Ancestral state reconstructions of floral traits in Angraecinae implemented in 'diversitree'; colors represent character states and pie charts represent the probability of ancestral states at nodes. Asterisks (*) indicate taxa illustrated in the pictures to the right to represent the flower shape of each clade except for *Jumellea* which is represented by *Jumellea comorensis* (not sampled in the phylogeny). Photo: Andriananjamanantsoa.


🟧 uppermost
🟩 lowermost
clade M
clade L
clade K
clade J
clade I
clade H
clade G
clade F
clade E
clade D
clade C
Jumellea
Aeranthes
clade B
clade A
outgroup
3 cm

1.5. Discussion

1.5.1. Systematics of Angraecinae

The problem we encountered when amplifying the ITS region was primarily caused by endophytes: instead of amplifying orchid DNA, the primers amplified fungi. This was also reported by several authors while working with the Orchidaceae (e.g. Carlsward et al., 2006). The incongruence observed with the morphological phylogeny illustrates the existence of homoplasies in Angraecinae and the difficulty of delineating natural groups using morphology in this group. The variation in branch support between the combined plastid and combined plastid + morphological analyses are probably due to synapomorphic characters that were observed in some clades but were lacking in others, as well as strong plesiomorphic characters that are encountered in subtribe Angraecinae. The poor resolutions observed within or between clades were probably due in part to a lack of sampling or caused by low genetic variation. More regions are needed to bring more resolution to the phylogeny, especially on the relationships between clades.

Our results confirmed the monophyly of Angraecinae and the distinction of the Malagasy–IOI clades from the African–American one (Carlsward et al., 2006, Micheneau et al., 2008a). Our results confirmed also the polyphyly of *Angraecum* s.l. Indeed, apart from the African sections already transferred to new genera (Szlachetko et al., 2013) four additional Malagasy species were embedded with clade A: *Angraecum* cf. *humile*, *A. perparvulum*, *A. pterophyllum*, and *A. rhynchoglossum* belonging to sections *Lemurangis*, *Nana*, *Pectinaria*, and section *Acaulia* respectively (Garay, 1973). Micheneau et al. (2008) first reported the existence of rare *Angraecum* species from Madagascar and the Mascarenes (*A. sp*. TP84, section *Nana*) in clade A. In our treatment, this taxon was unrelated to ours, and embedded with *Lemurella* (result not shown). To keep *Angraecum* monophyletic, these species need to be removed from the genus.

1.5.2. Systematics of *Angraecum*

The paraphyly observed in the ITS2 data could be due to a lack of sampling and of low genetic variation. Carlsward et al. (2006) had detected a paralogy problem in the ITS region in the Malagasy Angraecinae and most notably in clade II and therefore they excluded this region from subsequent analyses, including the genera *Aeranthes*, *Jumellea*, *Lemurorchis*, *Oeoniella* and *Sobennikoffia*. Since most of the sequences we used to reconstruct the ITS phylogeny came from their work, except for *Angraecum* which came mostly from our samples, and given that only ITS2 was sequenced by us, we believe that it is difficult to reconciliate the data at this point. Given the potential variability of ITS sequences, it might be worth attempting to resolve the problems of paralogy and primer design in order to increase our knowledge of Angraecinae.

The plastid results also showed that *Angraecum* is paraphyletic. Two Malagasy genera, *Oeoniella* and *Sobennikoffia*, are nested within this clade, as was reported by Carlsward et al. (2006). However, *Aeranthes* and *Jumellea* form natural groups sister to *Angraecum* as also reported by Micheneau et al. (2008). Schlechter (1918) admitted the close resemblance between *Oeoniella* and *Angraecum*, but emphasized the differences in column shape and stipe length. These two genera differ from *Angraecum* in their labellum shape. If we look closer at *Oeoniella* and unfold the labellum, the flower looks similar to *Angraecum eburneum*, except that the perianth is soft, the labellum spurless, and the ovary untwisted. *Sobennikoffia*, a small genus of four species, was included within *Angraecum* until transferred to a new genus by Schlechter (1925) because of the three-lobed labellum.

Of the 16 sections of Garay (1973) represented in our phylogeny, only one is monophyletic, the Mascarene section *Hadrangis*, all other sections are paraphyletic (*Gomphocentrum*, *Lemurangis* and *Leperventchea*) or polyphyletic (*Acaulia*, *Angraecum*, *Angraecoides*, *Arachnangraecum*, *Boryangraecum*, *Filangis*, *Humblotiangraecum*, *Pectinaria*, *Perrierangraecum* and *Pseudojumellea*). Micheneau et al. (2008a) mentioned the unnaturalness of Garay's sections, and pointed out the complexity of dealing with the sections with small and greenish flowers. We decided to not consider *A. nanum* within clade I because of their morphological differences. Although these species produce green flowers, *A. nanum* which represents section *Nana*, is characterized by tiny plant with racemose inflorescences and minute flowers, while clade I is characterized by erect or pendent plants with one-flowered inflorescences and larger flowers. The position of *A. amplexicaule* is ambiguous but in the BEAST analyses it was embended within clade L (Appendix 8). Morphologically, *A. amplexicaule* has an inflorescence and flower shape similar to those of species in clade L except that the habit is more robust within clade L and the leaves are coriaceous. Descpite the weak support, we decided to include *A. pseudofilicornu* and *A. scottianum* in clade J because all species in this clade share the same inflorescence type and flower shape except that the sepals and petals are reduced and the habit crassulescent with the two species. Furthermore, in the ITS2 topology *A. scottianum* was embended within the clade (Appendix 5).

The character (type of inflorescence, flower size and color, and spur length) used by many authors (e.g., Schlechter, 1925; Perrier de la Bathie, 1941; Garay, 1973; Stewart et al., 2006; Cribb and Hermans, 2009) to delineate sections in *Angraecum* are of limited taxonomic interest and their distribution is not coherent with the phylogeny. Spur length and flower size are generally correlated (small flowers have short spur and large flowers have long ones), with the exception of *A. appendiculatum* and *A. corrugatum*. These two Mascarene species, often considered peloric forms of *A. arachnites* and *A. conchoglossum*, respectively (Garay, 1973; Hermans and Cribb, 2005; Micheneau et al., 2008a), instead lost or have changed the genes responsible for labellum and spur development (Hossain and Levy, 2014). The phenotypic plasticity observed in these two species was considered to be the result of species radiation (Micheneau et al., 2008a). But colonization of new habitats (with new selection regimes) might affect gene expression that is responsible for floral development (Theissen and Saedler, 1995; Hsu et al., 2003; Chang et al., 2010; Pan et al., 2011; Ding et al., 2013). Chang et al. (2010) showed that the size and shape of sepal/petal/labellum in *Oncidium* are regulated by the OMADS5 gene. These species are spurless, the labellum changed from suborbicular concave, typical in section *Arachnangraecum*, to linear-lanceolate, and they became self-pollinated (Pailler et al., 2013). There is no evidence here of a loss of bilateral symmetry (there is still slight zygomorphy) that would be expected in peloric flowers. Alternately, variation in spur length could be due to genotypic drift (Mallet et al., 2014; Luo et al., 2015). For instance, Stewart et al. (2006) observed that spur length in subspecies of *A. eburneum* was shorter the further away from Madagascar a subspecies was.

1.5.3. Labellum position a missing character to delineate sections in *Angraecum*

After evaluating several morphological characters (Appendix 2), we noted that the position of the labellum, uppermost or lowermost, coincided with the delimitation with clades in *Angraecum* (Fig. 1.5). The degree of resupination in an orchid flower can vary from 0° to approximately 360° depending on the inflorescence (Arditti, 2003), and might be specific to a taxon. The 180° resupination results in a lowermost labellum and a 360° resupination (double twist of pedicel or ovary) in an uppermost labellum. The position of the labellum has been used in Orchidaceae to delimit sections within genus *Bulbophyllum* (Fischer et al., 2007), and in Gesneriaceae to delimit the genus *Alloplectus* (Clark and Zimmer, 2003). The newly defined African genera (Szlachetko et al., 2013) *Angraecoides*, *Dolabrifolia*, *Pectinariella* are also characterized by an uppermost labellum. Our results showed that Clades C to G have lowermost labella, while clades H to M are composed exclusively of species with uppermost labella. The dispersion of the species of section *Angraecum* sensu Garay (Fig. 1.1: clade E vs clade K) is a concrete example demonstrating the usefulness of this character to delimit sections. The labellum position is difficult to observe on herbarium sheets and often the only way to clearly see it is on living specimens. This could explain the fact that none of the original species descriptions mentioned this character (Schlechter, 1925; Perrier de la Bathie, 1941; Garay, 1973). Given these new findings, species description should be updated.

1.5.4. Temporal framework and paleoclimate events in *Angraecum*

Our age estimate for Angraecinae and *Angraecum* is approximately 4 Ma older than that obtained by Micheneau et al. (2010); the age of two endemic species of the Reunion Island (*A. bracteosum* and *A. striatum*) is younger in our results, however. This can be explained by our calibration. We set the root of the tree to 35 Ma following Gustafsson et al. (2010), while Micheneau et al. (2010) followed Ramirez et al. (2007) and fixed it at 30.37 Ma. The main difference between the two calibrations is based on the number of fossils used in the analyses: Ramirez et al. (2007) used a single fossil, whereas Gustafsson et al. (2010) included three to calibrate their phylogeny of the Orchidaceae. We did not use secondary calibrations, while Micheneau et al. (2010) used island ages as constraints in their analysis. Since no fossil was available to directly calibrate our phylogeny, these age estimates remain approximations. Nonetheless, our calibration is consistent with the divergence time of the Vandeae estimated by Givnish et al. (2015), and the divergence time estimate for *Angraecum* matches well with the diversification age of most Malagasy Angiosperm endemic genera (Buerki et al., 2013). During the Miocene, the climate of Madagascar shifted gradually from cool dry to warm humid. Contraction of the arid forest and expansion of the current tropical forest in Madagascar have been documented as a result of the northern migration of the island towards the equator (Buerki et al., 2013). This migration was associated with the establishment of the trade winds, and later of the monsoons, which increased moisture levels throughout eastern Madagascar. Two diversification events were observed in *Angraecum*, during the Pliocene and in the Pleistocene. This could be explained by the Quaternary glaciations event, where the climate fluctuated between cold to warm (Ruddiman, 2001). The diversity increases recorded within *Aeranthes*, *Angraecum* and *Jumellea* during the Pleistocene accounts for approximately 60% of their species (Appendix 8). This coincides with the radiation bursts observed in Malagasy tree ferns during the same period (Janssen et al., 2008) which somehow reflect the presence of humid and warm climate.

1.5.5. Diversification in *Angraecum*

The BiSSE and MuSSE results revealed that flower size and labellum position appear associated with the diversification of Angraecinae and *Angraecum*. An uppermost labellum concurred with a higher speciation rate compared to a lowermost labellum. Medium and large flowers were associated with higher speciation rates when compared to minute, small or very large flowers. The overlap in the posterior probabilities observed in flower color and spur length (Fig. 1.2) could be interpreted as an equal rate of speciation between color and length categories. Floral divergence in Orchidaceae has been associated with pollinator shifts (Peter and Johnson, 2014). Fischer et al. (2007) pointed out the importance of flower orientation in plant evolution and on species diversification. Lowermost labella serve as landing platforms for pollinators (Fischer et al., 2007), while uppermost labella are associated with either autogamy or a switch to pollinating insects that prefer walking rather than flying (Rudall and Bateman, 2002). Long spurs have been shown to be associated with specific pollinators in *Angraecum* (Nilsson et al., 1987; Wasserthal, 1997). Because of this specificity, long-spurred flowers are more efficiently pollinated (Fulton and Hodges, 1999; Hodges et al., 2004; Boberg et al., 2014), though it does not necessarily result in a greater speciation rate, as appears to be the case in *Angraecum*.

The speciation/extinction analyses obtained from BAMM model could be resumed to three best configuration shifts in Angraecinae, one shift at the MRCA of clade I, one at the MRCA of clade II, and one within the *Aeranthes* clade. BAMM detected general shifts where diversification could potentially have arisen (at the MRCA of *Aeranthes*, *Angraecum*, and *Jumellea*), but could not detect evidence on specific clades associated with diversification regimes that we expected within *Angraecum* (the shift from lowermost to uppermost labella, or the high speciation rate detected by BiSSE with flower color). A lack of performance of the statistical models used in BAMM was pointed out by Rabosky and Goldberg (2015). The shift detected within *Aeranthes* could be a bias of incomplete sampling (Fig. 1.3). Within *Jumellea* we selectively sampled at least one representative of each section of the genus (Rakotoarivelo et al., 2013), while sampling was more random in *Aeranthes*. Better samples are required before any conclusion on the diversification regimes operating in this clade can be drawn.

The phenotypic/evolution analyses obtained from BAMM model showed that there was no shift associated with labellum size, while at least four shifts were detected with spur length (Fig. 1.4). This leads us to conclusion that flower size is evolutionary constant within clades in the whole subtribe Angraecinae, while the evolution of spur length is more variable in some clades than in others. Rakotoarivelo et al. (2013) pointed out the lability of spur length in *Jumellea*, and its inefficiency on delimiting sections. Since flower size is generally conserved within clades, it is difficult to ascertain the extent to which this character may influence speciation, even though this character usually is associated with pollinator type. The shifts observed in clade C and J with spur length evolution suggest that these clades are the most phenotypically diverse in the whole Angraecinae. However it does not necessary mean that there is species diversification associated with the rate of spur length evolution. If we look at clade C, there is no phylogenetic signal showing that short or long spurs led to more speciation, which excludes all hypotheses of high rate diversification associated with this character. The rate shifts observed in clade J were caused by the two Mascarene species *A. appendiculatum*, and *A. corrugatum*.

Our BAMM analyses showed a slowdown in time of the diversification rate in Angraecinae in general (Fig. 1.3). The results showed an accumulation of lineages rather than rapid radiation in *Angraecum*. To test for homogeneity in the diversification rate through time, we calculated the gamma statistic of Pybus and Harvey (2000) as implemented in the R package 'ape'. Our significantly negative result ($\gamma = -2.204816$) rejects the null hypothesis for a constant-rate, suggesting that the speciation rate was initially high but slowed through time, which is congruent with the BAMM results. This early burst in lineages could be explained by the fact that *Angraecum* species develop a crassulacean acid metabolism (CAM) which allows them to tolerate desiccation (Kluge et al., 1997). Whitman et al. (2011) reported that lithophytic Angraecinae are tolerant to limited moisture availability, and chronic bushfire did not kill the population of *Angraecum* sororium and Jumellea rigida but only reduced their expansion. But, diversification slowdown could be also the result of ecological limitation, competition, access to pollinators, species carrying capacity (Gavrilets et Vose, 2005; Phillimore et Price, 2008; Jonsson et al., 2012), or maintenance of niche similarity (Moen et Morlon, 2014). Lineage diversification is usually high right after colonization of a new niche, but slows through time as niches get occupied and ecological conditions for speciation decrease (Reddy et al., 2012). Scantlebury et al. (2013) reported that diversification slowdown in Malagasy fauna is a general pattern of adaptive radiation like that observed in amphibians and birds (Vences et al., 2009; Jonsson et al., 2012; Reddy et al., 2012). However, it has been shown that the diversification pattern of the tropical rain forest has been an accumulation of lineages through time and not sudden adaptive radiations (Couvreur et al., 2011). Our results appear to support this hypothesis.

It is intriguing that each clade in *Angraecum* is associated with specific floral traits. *Aeranthes* and *Jumellea* are species rich and occupy the same ecological niche as *Angraecum*. The three genera diverged approximately at the same time, but *Angraecum* has more species. If we look at the morphological differentiation between the three genera, *Jumellea* and *Aeranthes* have distinctive traits that characterize them as clades, while *Angraecum* has variable traits specific to each subclade. The diversity of *Angraecum* could perhaps be partly explained by its morphological evolution. Taxonomic diversity has been demonstrated to be complemented by morphological and ecological diversity (Shi and Rabosky, 2015). Pollinators could have played a significant role in this diversification process. Little is known about *Angraecum* pollinators, but we know now that it is pollinated by different kinds of pollinators (Micheneau et al., 2006; Micheneau et al., 2008c; Micheneau et al., 2009; Pailler et al., 2013) not only hawk-moths as traditionally thought (Nilsson et al., 1985; Nilsson et al., 1987; Wasserthal, 1997). Micheneau et al. (2008a) reported that the radiation of *Angraecum* in the Mascarenes was caused by a change in pollinators on these Islands. The absence of the original pollinators is considered to have resulted in auto-pollination in some Angraecinae species (Micheneau et al., 2008b; Hermans and Hermans, 2013; Pailler et al., 2013), which could be associated with a decrease or loss of rewards such as fragrance or nectar for the species (Pailler et al., 2013).

1.6. Conclusion

The present study presents the most comprehensive phylogenetic reconstruction of genus *Angraecum* to date including all the sections sensu Garay except section *Afrangraecum*. With the African sections being removed (Szlachetko et al., 2013) *Angraecum* counts currently ca. 190 species (including varieties). Our results confirmed the paraphyly or polyphyly of *Angraecum* sections, but showed the morphology to be consistent with the phylogeny. The position of the labellum, lowermost or uppermost, allowed us to delineate several clades. An updated systematic revision of the genus is required considering these findings. Our study revealed that many characters are associated with species diversification of *Angraecum*, the orientation of the labellum being one. However, our analyses failed to detect shifts that could have been caused by morphological diversification. Overall, the evolution and diversification of *Angraecum* resulted from species accumulation through time rather than rapid radiation.

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Chapitre 2: Systematics and taxonomic revision of genus *Angraecum* (Orchidaceae, Angraecinae) and two new genera in the orchids of Madagascar

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Contribution respective des auteurs:

Tahiana Andriananjamanantsoa: Conception du projet, collecte, traitement et analyse des données, interprétation et rédaction

Luc Brouillet: Supervision, conseils, recommandations, corrections et direction générale

2.1. Résumé/ Abstract

Résumé

Angraecum est le plus grand genre de la sous-tribu des Angraecinae avec ca. 195 espèces. Les études phylogénétiques moléculaires récentes appuyées par des données morphologiques ont montré qu'*Angraecum* doit être recirconscrit afin d'en préserver la monophylie. Un synopsis du genre *Angraecum* avec les 14 sections que nous reconnaissons ici est présenté, incluant cinq sections nouvelles: *Africanae*, *Oeoniella*, *Robusta*, *Sobennikoffia* et *Stellariangraecum*. Deux nouveaux genres, *Acaulia* et *Parangraecum*, sont proposés pour accommoder des espèces phylogénétiquement séparées du genre *Angraecum*.

Mots-clés: *Acaulia*, Angraecinae, *Angraecum*, Orchidaceae, *Parangraecum*, classification des sections

Abstract

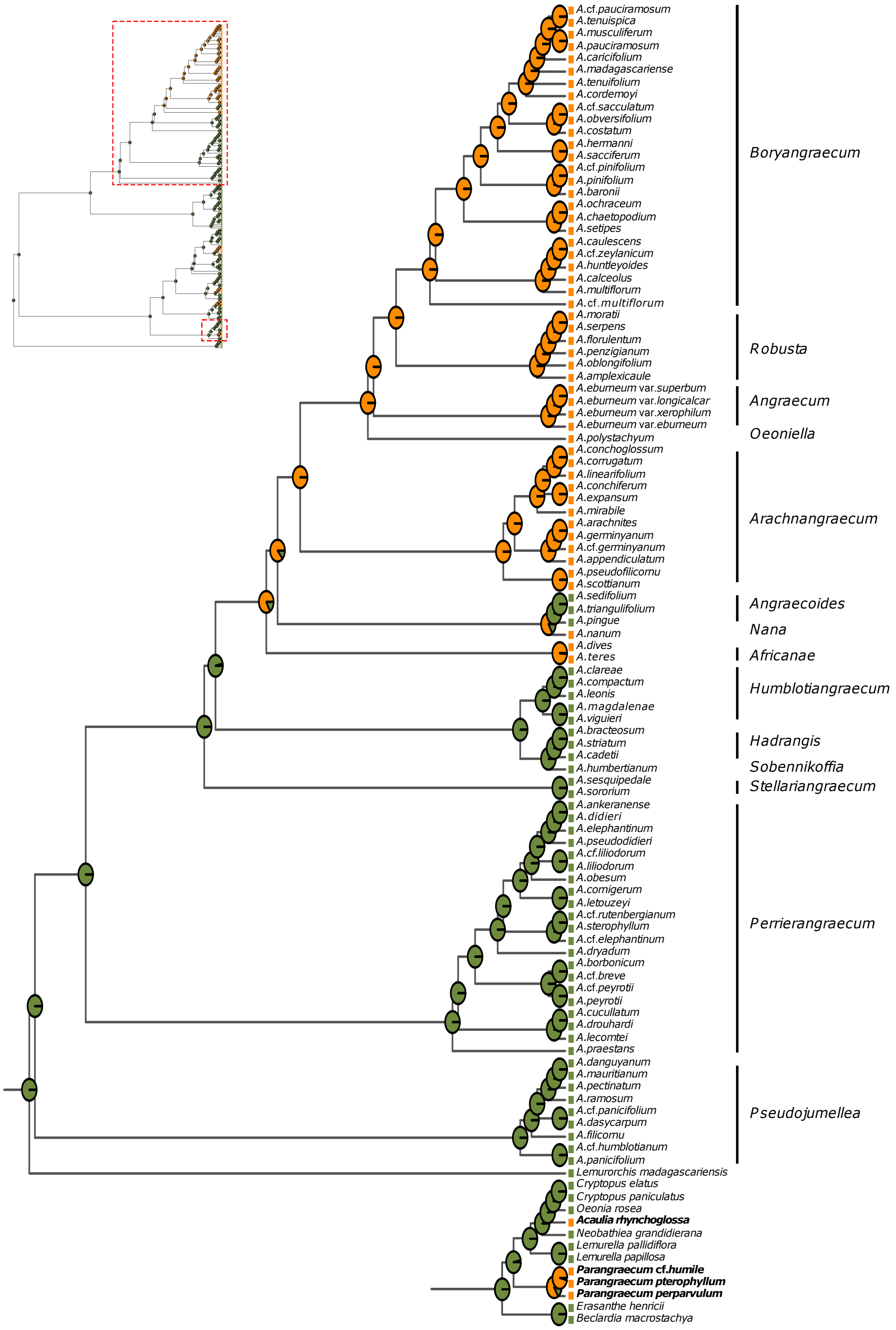
Angraecum is the largest genus of subtribe Angraecinae with ca. 195 species. Recent molecular phylogenetic studies supported by morphological data showed that *Angraecum* must be re-circumscribed to maintain monophyly. A synopsis of *Angraecum* with 14 sections recognized here is presented, including five new sections: *Africanae*, *Oeoniella*, *Robusta*, *Sobennikoffia* and *Stellariangraecum*. Two new genera, *Acaulia* and *Parangraecum*, are proposed to accommodate species phylogenetically segregated from *Angraecum*.

Keywords: *Acaulia*, Angraecinae, *Angraecum*, Orchidaceae, *Parangraecum*, sectional classification

2.2. Introduction

Angraecum is the largest genus in subtribe Angraecinae (Orchidaceae, tribe Vandeae) and includes about 195 species (chap. 1). Recent molecular and morphologic phylogenetic studies have shown that *Angraecum* is polyphyletic as traditionally circumscribed and that the sections proposed by Garay (1973) are often poly- or paraphyletic (Micheneau et al., 2008a; chap. 1). To maintain monophyly of the genus and sections, new circumscriptions are required. Traditionally, sectional classification has been based on morphological characters, especially floral traits (e.g., Garay, 1973; Stewart et al., 2006; Cribb and Hermans, 2009). These characters have been used as well to recognize informal groups within Malagasy Angraecinae like *Jumellea* (Perrier de la Bathie, 1941), but have been demonstrated to be evolutionary labile (Rakotoarivelo et al., 2012; chap. 1) and of limited use for systematic purpose. In order to render *Angraecum* monophyletic, we propose to include all species that are nested within the *Angraecum* clade within a single genus, *Angraecum*, as suggested by Andriananjamanantsoa et al. (chap. 1), and to remove elements that are found within other clades of subtribe Angraecinae. Likewise, we are assigning to sections all species grouped within a clade (Fig. 2.1). Four species were removed from *Angraecum* (*A.* cf. *humile*, *A. perparvulum*, *A. pterophyllum*, and *A. rhynchoglossum*) and accommodated into two new genera (*Acaulia* and *Parangraecum*).

Fig. 2.1. Partial phylogenetic relationships of *Angraecum* showing ancestral state reconstructions of labellum position implemented in 'diversitree' (chap. 1); complete tree presented in upper left corner, with selected partial trees in red-dashed boxes shown on the right; taxa in bold are the new genera *Acaulia* and *Parangraecum*. Colors represent labellum character states (orange, uppermost; green, lowermost) and pie charts represent the probability of ancestral states at node. Abbreviation: A, *Angraecum*.


A.cf.pauciramosum
A.tenuispica
A.musculiferum
A.pauciramosum
A.caricifolium
A.madagascariense
A.tenuifolium
A.cordemoyi
A.cf.sacculatum
A.obversifolium
A.costatum
A.hermanni
A.sacciferum
A.cf.pinifolium
A.pinifolium
A.baronii
A.ochraceum
A.chaetopodium
A.setipes
A.caulescens
A.cf.zeylanicum
A.huntleyoides
A.calceolus
A.multiflorum
A.cf.multiflorum
A.moratii
A.serpens
A.florulentum
A.penzigianum
A.oblongifolium
A.amplexicaule
A.eburneum var.superbum
A.eburneum var.longicalcar
A.eburneum var.xerophilum
A.eburneum var.eburneum
A.polystachyum
A.conchoglossum
A.corrugatum
A.linearifolium
A.conchiferum
A.expansum
A.mirabile
A.arachnites
A.germinyanum
A.cf.germinyanum
A.appendiculatum
A.pseudofilicornu
A.scottianum
A.sedifolium
A.triangulifolium
A.pingue
A.nanum
A.dives
A.teres
A.clareae
A.compactum
A.leonis
A.magdalenae
A.viguieri
A.bracteosum
A.striatum
A.cadetii
A.humbertianum
A.sesquipedale
A.sororium
A.ankeranense
A.didieri
A.elephantinum
A.pseudodidieri
A.cf.liliodorum
A.liliodorum
A.obesum
A.cornigerum
A.letouzeyi
A.cf.rutenbergianum
A.sterophyllum
A.cf.elephantinum
A.dryadum
A.borbonicum
A.cf.breve
A.cf.peyrotii
A.peyrotii
A.cucullatum
A.drouhardi
A.lecomtei
A.praestans
A.danguyanum
A.mauritianum
A.pectinatum
A.ramosum
A.cf.panicifolium
A.dasycarpum
A.filicornu
A.cf.humblotianum
A.panicifolium
Lemurorchis madagascariensis
Cryptopus elatus
Cryptopus paniculatus
Oeonia rosea
Acaulia rhynchoglossa
Neobathiea grandidierana
Lemurella pallidiflora
Lemurella papillosa
Parangraecum cf.humile
Parangraecum pterophyllum
Parangraecum perparvulum
Erasanthe henricii
Beclardia macrostachya
Boryangraecum
Robusta
Angraecum
Oeoniella
Arachnangraecum
Angraecoides
Nana
Africanae
Humblotiangraecum
Hadrangis
Sobennikoffia
Stellariangraecum
Perrierangraecum
Pseudojumellea

Fourteen clades (Fig. 2.1) are recognized here as sections of *Angraecum*, including nine sections of Garay (1973): *Angraecoides* Schltr., *Angraecum* Bory, *Arachnangraecum* Schltr., *Boryangraecum* Schltr., *Hadrangis* Schltr., *Humblotiangraecum* Schltr., *Nana* (Cordem.) Garay, *Perrierangraecum* Schltr. and *Pseudojumellea* Schltr. Five new sections are defined following our phylogenetic analysis (chap. 1): *Africanae* Andriananjamanantsoa, *Oeoniella* Andriananjamanantsoa, *Robusta* Andriananjamanantsoa, *Sobennikoffia* Andriananjamanantsoa and *Stellariangraecum* Andriananjamanantsoa. Modifications were made to published descriptions to better describe the sections. Sections *Oeoniella* and *Sobennikoffia* are new combinations for the genera *Oeoniella* and *Sobennikoffia*, fully embedded within *Angraecum* (Fig. 2.1), as confirmed by phylogenetic studies (Carlsward et al., 2006; chap. 1). Andriananjamanantsoa et al. (chap. 1) mentioned the morphological resemblance between these genera and *Angraecum*, despite the differences in the form of the labellum highlighted by Schlechter (1918, 1925). In this treatment, all species that have been sampled in the phylogenetic reconstructions are presented in bold.

2.3. Sections of *Angraecum*

2.3.1. *Angraecum* Bory, Voy. 1: 359, t. 19 (1804).

TYPE: *Angraecum eburneum* Bory

Epiphytic, lithophytic, rarely terrestrial plants; stem erect, commonly short, or elongate, rarely reduced; leaves distichous, articulate with leaf-sheaths commonly distinct, sometimes densely imbricate; inflorescence axillary, sessile or pedunculate, one-flowered or racemose, usually one per node; flowers sessile or pedicellate, alternate or secund along rachis, sepals and petals free, spreading, more or less reflexed; labellum lowermost or uppermost, sessile, entire, more or less concave, spreading, basally enveloping the column, rarely lobed in the apex; spur distinct, very short (less than 5 mm) to very long (up to 35 cm); column fleshy, very short, without a foot; clinandrium rather shallow, in front deeply bilobed with an abbreviated, tooth-like rostellum in the middle; pollinia 2, globose, sulcate, attached to a common viscidium or each pollinium may be attached more or less to a separate viscidium;

ovary round in section sometimes angular, resupinate from 180° to 360°, rarely non-resupinate.

2.3.2. *Angraecum* sect. *Africanae* Andriananjamanantsoa sect. nov.

TYPE: *Angraecum teres* Summerh.

DISTRIBUTION: Kenya, Socotra, Somalia, Tanzania

Epiphytic, medium or large plants (to 25 cm height); stems erect, short, internodes short; leaves densely imbricate, coriaceous; inflorescences racemose, many-flowered (up to 10), infrafoliar, longer or equaling leaves, one to four per stem; flowers small, greenish, sepals and petals linear-lanceolate; labellum uppermost, navicular, shorter or equaling dorsal sepal, apex attenuate; spur longer or equaling dorsal sepal, isodiametric; ovary round in section with 360° resupination.

ETYMOLOGY: The name refers to the geographical distribution of the species that are restricted to Eastern Africa (Stewart et al., 2006).

This section groups all African species placed previously in section *Boryangraecum*. Further phylogenetic work is required to confirm the placement of *A. geniculatum* G.Will. suggested here. This species from Zambia was accommodated in section *Boryangraecum* by Garay (1973). Indeed, species in section *Africanae* are very similar to those in section *Boryangraecum* Schltr. sensu Garay found in Madagascar and the western Indian Ocean Island. Geographical distribution seems to have an effect on genetic drift which is so far the most plausible explanation of the segregation of the species. The other explanation could be convergence evolution, which seems frequent in the Angraecinae (chap. 1).

Species included

Angraecum dives Rolfe, *A. geniculatum* G.Will., ***A. teres*** Summerh.

2.3.3. *Angraecum* sect. *Angraecoides* (Cordem.) Garay, Kew Bull. 28: 495–516 (1973).

LECTOTYPE: *Angraecum pingue* Frapp., designated by Garay, Kew Bull. 28: 495–516 (1973).

Mystacidium sect. *Angraecoides* Cordem. Rev. Gin. Bot. II: 413 (1899).

Angraecoides (Cordem.) Szlach., Mytnik & Grochocka, Biodiv. Res. Conserv. 29: 1–23 (2013).

DISTRIBUTION: Madagascar, Mauritius, Reunion

Epiphytic on trunk, medium or large plants (to 25 cm height); stems erect, elongate, internodes short; leaves alternate spiral, often succulent; inflorescences always one-flowered, subterminal, longer than leaves, one to four per stem; flowers small, greenish or greenish yellow, sepals and petals linear-lanceolate; labellum lowermost, concave or rounded at the base, shorter or equaling dorsal sepal, rostrate-acuminate; spur longer or equaling dorsal sepal, with a wide opening, isodiametric; ovary round in section with 180° resupination.

We restrict section *Angraecoides* to species with a lowermost labellum. The African genus *Angraecoides* (Cordem.) Szlach., Mytnik & Grochocka (former *Angraecum* sect. *Conchoglossum*, endemic to Africa) needs a new name since the type species of this genus and of section *Angraecoides*, *A. pingue*, belongs to *Angraecum* sect. *Angraecoides* according to phylogenetic data (Fig. 2.1, chap. 1) and not to the segregate entity.

Species included

Angraecum pingue Frapp. ex Cordem., ***A. sedifolium*** Schltr., ***A. triangulifolium*** Senghas.

2.3.4. *Angraecum* sect. *Angraecum* Benth., Benth. & Hook. F. Gen. Pl. 3: 583 (1883).

TYPE: *Angraecum eburneum* Bory

DISTRIBUTION: Comoros, Kenya, Madagascar, Mascarenes, Seychelles, Tanzania

Epiphytic or lithophytic, very large plants (up to 1 m height); stems short to elongate, internodes long to 5 cm when existing; leaves densely imbricate, coriaceous; inflorescences racemose, suprafoliar, longer than leaves, many-flowered (up to 10), one to five per stem; flowers very large, sepals and petals linear-lanceolate, green; labellum uppermost, orbicular concave, white, apex acuminate; spur medium to very long (up to 45 cm), green, isodiametric from the base, tapering towards the tip; ovary round in section with 360° resupination.

This section is restricted to *A. eburneum* and its varieties. It is strongly supported in morphological and molecular phylogenetic analyses (chap. 1). Previous descriptions were based on the significantly large size of the plants and flowers (Schlechter, 1925; Perrier de la Bathie, 1941; Garay, 1973; Stewart et al., 2006; Cribb et Hermans, 2009) without taking in consideration the position of the labellum (uppermost vs lowermost) or the number of flower per inflorescence (racemose vs few-flowered inflorescences). The taxonomic disposition of *A. longicalcar* (Bosser) Senghas raises question about its recognition as a distinct species versus a variety of *A. eburneum* as proposed by Bosser (1965). Further molecular phylogenetic and morphometric studies are required before making a decision as to its exact position.

Species included

A. eburneum Bory **var. *eburneum***, *A. eburneum* var. *giryamae* (Rendle) Senghas & P.J.Cribb, ***A. eburneum* var. *longicalcar*** Bosser, ***A. eburneum* var. *superbum*** (Thouars) H.Perrier, ***A. eburneum* var. *xerophilum*** H.Perrier.

2.3.5. *Angraecum* sect. *Arachnangraecum* Schltr., Fedde, Repert. Sp. Nov. Beih. 33: 309 (1925).

LECTOTYPE (in hoc loco electus): *Angraecum arachnites* Schltr.

DISTRIBUTION: Comoros, Kenya, Malawi, Madagascar, Mascarenes, Mozambique, South Africa, Tanzania, Zimbabwe

Epiphytic on trunk, large plants (to 30 cm height); stems elongate, pendent, often with long internode (up to 5 cm); leaves alternate, coriaceous; inflorescences one-flowered, subterminal, longer than leaves, one to three per stem; flowers medium to large, sepals and petals tubular, arachniform, white green sometimes ocher; labellum uppermost, rather broad, more or less suborbicular in outline, concave, white, apex acuminate; spur elongate to 13 cm, sigmoid, greenish white, sometimes ocher, slightly large at the base, tapering towards the tip; ovary round in section with 360° resupination.

The lectotype designated by Garay (1973), *A. ramosum* Thouars, was misplaced in this section and has been moved to section *Pseudojumellea* (Micheneau et al., 2008a). The type specimen is in fruit which is in fact confusing because the habit and the inflorescence look very similar to sect. *Arachnangraecum* (stems elongate, inflorescence longer than leaves, pedicellate ovary round in section). This might be the reason why Perrier de la Bathie (1941) transferred several species of sect. *Arachnangraecum* as varieties of *A. ramosum* (e.g. *A. arachnites*, *A. conchoglossum* and *A. germinyanum*) and later why Garay (1973) chose *A. ramosum* as type of the section. The major differences lie on the flower shape, where the arachniform, uppermost labellum and the sigmoid spur are typical of section *Arachnangraecum* but are not present in *A. ramosum*. Therefore, we are designating a new lectotype to represent the section as originally described by Schlechter (1925), with a modification to the original description following recent morphological and phylogenetic evidence (chapter 1).

Species included

Angraecum ampullaceum Bosser, ***A. appendiculatum*** Frapp. ex Cordem., ***A. arachnites*** Schltr., ***A. conchiferum*** Lindl., ***A. conchoglossum*** Schltr., ***A. corrugatum*** (Cordem.) Micheneau, ***A. expansum*** Thouars, ***A. germinyanum*** Hook.f., ***A. linearifolium*** Garay, ***A. mirabile*** Schltr., ***A. pseudofilicornu*** H.Perrier, ***A. scottianum*** Rchb.f., *A. teretifolium* Ridl.

2.3.6. *Angraecum* sect. *Boryangraecum* Schltr., Fedde, Repert. Sp. Nov., Beih. 33: 308 (1925).

TYPE: *Angraecum pumilio* Schltr.

Angraecum sect. *Acaulia* Garay, Kew Bull. 28: 495–516 (1973). Type: *Angraecum rhynchoglossum* Schltr.

Angraecum sect. *Chlorangraecum* Schltr. Fedde, Repert. Sp. Nov., Beih. 33: 310 (1925). Lectotype: *Angraecum chloranthum* Schltr., designated by Garay, Kew Bull. 28: 495–516 (1973) [syn. of *Angraecum huntleyoides* Schltr., Bot. Jahrb. Syst. 38: 160 (1906)].

Angraecum sect. *Gomphocentrum* (Benth.) Garay, Kew Bull. 28: 495–516 (1973).

Mystacidium sect. *Gomphocentrum* Benth., J. Linn. Soc. Bot. 18: 337 (1881). Type: *Angraecum caulescens* Thouars

Angraecum sect. *Lemurangis* Garay, Kew Bull. 28: 495–516 (1973). Type: *Angraecum madagascariense* (Finet) Schltr. (= *Macroplectrum madagascariense* Finet).

Angraecum sect. *Lepervenchea* (Cordem.) Schltr., Beih. Bot. Centralbl. 36(2): 157 (1918).

Lepervenchea Cordem., Rev. Gin. Bot. II: 415 (1899). Type: *Angraecum tenuifolium* Frapp. ex Cordem.

DISTRIBUTION: Comoros, Madagascar, Mauritius, Mozambique, Reunion, Seychelles

Epiphytic on trunk, medium to large plants (up to 30 cm); stems very short to elongate, erect or pendent, internodes often long (to 5 cm) sometimes short or absent; leaves imbricate, thin; inflorescences one-flowered or racemose (up to 10 flowers), infra or suprafoliar, short sometimes longer than leaves, one to more than five per stem, sometimes branched; flowers small, yellowish green, sub-diaphanous, sepals and petals linear-lanceolate; labellum uppermost, navicular, shorter or longer than dorsal sepal, apex acute or acuminate; spur shorter or longer than dorsal sepal, often globular at the tip; ovary round in section with 360° resupination.

We modified the original description to better describe the section wich assembles here species previously placed in sections *Acaulia*, *Angraecoides*, *Boryangraecum*, *Chlorangraecum*, *Gomphocentrum*, *Lemurangis* and *Lepervenchea* by Garay (1973). Schlechter (1925) hesitated to separate sect. *Hildebrandtiangraecum* (sect. *Gomphocentrum* sensu Garay) and *Micrangraecum* (sect. *Nana* sensu Garay) from *Boryangraecum* because of their morphological resemblance (mostly floral). Phylogenetic reconstructions and morphological data supported *Nana* as a separate section, as circumscribed by Garay (1973), which is phylogenetically distant from sect. *Boryangraecum* but closely related to sect. *Africanae* and *Angraecoides* (chapter 1). However, further molecular analyses, including a more complete sampling, will be required to confirm the composition of section *Boryangraecum*.

Species included

A. acutipetalum Schltr. var. *acutipetalum*, *A. acutipetalum* var. *analabeensis* H.Perrier ex Hermans, *A. acutipetalum* var. *ankeranae* H.Perrier ex Hermans, *A. alleizettei* Schltr., *A. andasibeense* H.Perrier, *A. andringitranum* Schltr., ***A. appendiculoides*** Schltr., *A. aviceps* Schltr., ***A. baronii*** (Finet) Schltr., *A. brachyrhopalon* Schltr., ***A. calceolus*** Thouars, ***A. caricifolium*** H.Perrier, ***A. caulescen*****s** Thouars, ***A. chaetopodum*** Schltr., *A. chermezonii* H.Perrier, *A. cilaosianum* (Cordem.) Schltr., ***A. cordemoyi*** Schltr., *A. cornucopiae* H.Perrier, *A. corynoceras* Schltr., *A. costatum* Frapp. ex Cordem., *A. crassifolium* Schltr., *A. curvicaule* Schltr., *A. curvipes* Schltr., *A. dauphinense* (Rolfe) Schltr., *A. decaryanum* H.Perrier, *A. dupontii* Pailler, *A. falcifolium* Bosser, *A. ferkoanum* Schltr., *A. flavidum* Bosser, *A. floribundum* Bosser, ***A. hermannii*** Schltr., ***A. huntleyoides*** Schltr., *A. inapertum* Thouars, ***A. madagascariense*** (Finet) Schltr., ***A. multiflorum*** Thouars, *A. muscicolum* H.Perrier, ***A. musculiferum*** H.Perrier, *A. myrianthum* Schltr., ***A. obversifolium*** Frapp. ex Cordem., ***A. ochraceum*** (Ridl.) Schltr., *A. oeonioides* Bosser, *A. onivense* H.Perrier, *A. parvulum* Ayres ex S.Moore, *A. patens* Frapp. ex Cordem., ***A. pauciramosum*** Schltr., *A. pergracile* Schltr., *A. perhumile* H.Perrier, ***A. pinifolium*** Bosser, *A. pseudopetiolatum* Frapp. ex Cordem., *A. pumilio* Schltr., *A. rhizanthium* H.Perrier, *A. rhizomaniacum* Schltr., *A. rostratum* Ridl., ***A. sacciferum*** Lindl., ***A. sacculatum*** Schltr., *A. salazianum* Schltr., *A. scalariforme* H.Perrier, ***A. setipes*** Schltr., *A. sinuatiflorum* H.Perrier, *A. tamarindicolum* Schltr., ***A. tenuifolium*** Frapp. ex Cordem., *A. tenuipes* Summerh., ***A. tenuispica*** Schltr., *A. undulatum* (Cordem.) Schltr., *A. verecundum* Schltr., *A. vesiculatum* Schltr., *A. vesiculiferum* Schltr., *A. viridiflorum* Cordem., *A. xylopus* Rchb.f., ***A. zaratananae*** Schltr., ***A. zeylanicum*** Lindl.

2.3.7. *Angraecum* sect. *Hadrangis* Schltr., Beih. Bot. Centralbl. 36(2): 158 (1918).

TYPE: *Angraecum striatum* Thouars

DISTRIBUTION: Mauritius, Reunion

Epiphytic on canopy or trunk, large plants (to 35 cm height); stems erect, short or elongate, internodes short; leaves dense, coriaceous; inflorescences racemose, few to several-flowered (up to 10), suprafoliar, short or equaling leaves, one to four per stem; flowers medium, white, sepals and petals lanceolate-ovate; labellum lowermost, ovate, deeply concave, shorter or equaling dorsal sepal, white overlaid green at the moth, apex acute; spur shorter than dorsal sepal, conic or saccate, with a wide opening, green; ovary round in section with 180° resupination.

This is the only section which both was recognized as a natural group from its original description (Schlechter, 1918) and supported in morphological and molecular phylogenetic studies (chap. 1).

Species included

Angraecum bracteosum Balf. & S.Moore, ***A. cadetii*** Bosser, *A. jeannineanum* Fournel & Micheneau, ***A. striatum*** Thouars.

2.3.8. *Angraecum* sect. *Humblotiangraecum* Schltr., Fedde, Repert. Sp. Nov., Beih. 33: 310 (1925).

TYPE: *Angraecum leonis* (Rchb. f.) André [*Aeranthes leonis* Rchb. f.]

DISTRIBUTION: Madagascar, Comoros

Epiphytic on canopy or trunk, medium to large plants (to 35 cm height); stems erect, short or barely developed, internodes short to elongated (to 5 cm); leaves thin to thick, coriaceous or fleshy; inflorescences racemose, with up to four flowers, suprafoliar, longer than leaves, one to four per stem; flowers very large, white, sepals and petals linear-lanceolate to lanceolate-ovate; labellum lowermost, broadly ovate, deeply concave, longer or equaling dorsal sepal, sometimes white overlaid green at the moth, apex acute; spur long (to 15 cm), with a wide often infundibuliform opening, tapering from the median to tip, greenish white towards the tip; ovary triquetrous or round in section with 180° resupination.

Some species previously placed in section *Perrierangraecum* (e.g. *A. clareae* and *A. compactum*) fit better in this section. Their position is supported by phylogenetic evidence (chap. 1). The triquetrous ovary, most often observed in sect. *Perrierangraecum*, and the plant habit, similar to some species from this section, might be reasons why these species were misplaced. The spur shape of *A. praestans* is remarkably similar to that of *A. viguieri* and this is probably one reason why Garay (1973) included this species in section *Perrierangraecum*.

Species included

Angraecum aloifolium Hermans & P.J.Cribb, ***A. clareae*** Hermans, la Croix & P.J.Cribb, ***A. compactum*** Schltr., *A. dollii* Senghas, ***A. leonis*** (Rchb.f.) André, ***A. magdalenae*** Schltr. & H.Perrier var. *magdalenae*, *A. magdalenae* var. *latiilabellum* Bosser, ***A. viguieri*** Schltr.

2.3.9. *Angraecum* sect. *Nana* (Cordem.) Garay, Kew Bull. 28: 495–516 (1973).

Mystacidium sect. *Nana* Cordem., Rev. Gin. Bot. II: 414 (1899).

LECTOTYPE: *Angraecum nanum* Frapp., designated by Garay, Kew Bull. 28: 495–516 (1973).

Angraecum sect. *Micrangraecum* Schltr., Fedde, Repert. Sp. Nov., Beih. 33: 308 (1925). Type: *Angraecum pusillum* Lindl.

DISTRIBUTION: South Africa, Ethiopia, Madagascar, Mauritius, Kenya, Reunion, Tanzania, Zambia, Zimbabwe

Epiphytic, tiny plants (to 5 cm heigth); stems short, internodes reduced; leaves thin, graminiform; inflorescences often one-sided racemose, infrafoliar, longer than leaves, one to three per stem; flowers minute, greenish, sepals and petals lanceolate-ovate; labellum uppermost, navicular, apex acute; spur shorter than dorsal sepal, isodiametric; ovary round in section with 360° resupination.

We modified the original description and restricted the section to species with racemose inflorescences of minute, greenish flowers. Garay (1973) included all dwarf plants that bear minute flowers in this section, even though their vegetative and floral traits differed. All dwarf plants with thick leaves and small flowers (*A.* cf. *humile*, *A. perparvulum*, *A. pterophyllum*) are now placed in genus *Parangraecum*. Further phylogenetic and morphological studies, including a wider sampling of species, are required to validate the section because we only sampled *A. nanum* in the analyses. We suspect that some of the species in this section could be varieties or synonyms because the morphological differences are few (tiny plants, grass-like, inflorescence and flowers very similar).

Species included

Angraecum chamaeanthus Schltr., *A. decipiens* Summerh., *A. microcharis* Schltr., *A. minus* Summerh., *A. minutum* Frapp. ex Cordem., ***A. nanum*** Frapp. ex Cordem., *A. pusillum* Lindl.

2.3.10. *Angraecum* sect. *Oeoniella* (Schltr.) Andriananjamanantsoa comb. nov.

Oeoniella Schltr. Beih. Bot. Centralbl. 36(2): 176 (1918).

TYPE: *Angraecum polystachyum* (Thouars) A. Rich. [*Oeoniella polystachys* (Thouars) Schltr.]

DISTRIBUTION: Comoros, Madagascar, Mascarenes, Seychelles

Epiphytic on trunk or lithophytic undergrowth, medium plants (to 15 cm height); stems elongate, erect or climbing, internodes long (to 5 cm); leaves subimbricate, coriaceous; inflorescences racemose, suprafoliar, longer than leaves, many-flowered (up to 10), one to four per stem; flowers medium sized, sepals and petals linear-lanceolate, green; labellum uppermost, orbicular, rotund at the base, white, apex acuminate; spurs very short, green, isodiametric; ovary round in section, non-resupinate.

We believe that *Oeoniella aphrodite* could be a variety of *Oeoniella polystachyus* as they merely differ in size (Baker, 1877), which could be interpreted also as phenotypic variation: *O. aphrodite* appears larger than *O. polystachys*. Further investigations involving molecular and morphological work (including morphometry) are required before making any decision.

Species included and transfered from *Oeoniella* to *Angraecum*

Angraecum aphrodite (Balf. & S. Moore) Andriananjamanantsoa comb. nov.

Basionym: *Listrostachys aphrodite* Balf. & S. Moore, J.G. Baker, Fl. Mauritius: 354 (1877).

Synonym: *Oeoniella aphrodite* (Balf.f. & S.Moore) Schltr., Beih. Bot. Centralbl. 36(2): 177 (1918).

Angraecum polystachyum (Thouars) A. Rich., Mém. Mus. Hist. Nat. 4: 66 (1818).

Basionym: *Epidendrum polystachys* Thouars, Hist. Orchid.: t. 82 (1822).

Synonym: *Oeoniella polystachys* (Thouars) Schltr., Beih. Bot. Centralbl. 36(2): 177 (1918).

2.3.11. *Angraecum* sect. *Perrierangraecum* Schltr., Fedde, Repert. Sp. Nov., Beih. 33: 309 (1925).

LECTOTYPE (in hoc loco electus): *Angraecum rutenbergianum* Kraenzl.

DISTRIBUTION: South Africa, Comoros, Madagascar, Malawi, Reunion, Zimbabwe

Epiphytic on canopy or trunk, medium to large plants (to 35 cm height); stems erect, short to elongate, internodes very short rarely long (to 5 cm); leaves fleshy, usually thick, fan shape; inflorescence usually one-flower, sometimes racemose with up to four flowers, suprafoliar, very short, one or two per stem; flowers medium to large, white, sepals and petals linear-lanceolate to lanceolate-ovate; labellum lowermost, broadly ovate, equal to dorsal sepal, slightly concave, apex acute; spur short to elongate (to 15 cm), isodiametric at base, tapering to tip, greenish white towards the tip; ovary round in section often triquetrous with 180° resupination.

We designated a new type for the section since the one proposed by Garay (1973), *Angraecum triquetrum*, is a synonym of *Jumellea triquetra* (Thouars) Schltr. In the original description, Schlechter (1925) kept this species in section *Perrierangraecum* even though he already transfered it to *Jumellea*. The presence of *Angraecum praestans* in this section is difficult to accept without phylogenetic support (chap. 1) because the similarities of this species to species of sect. *Humblotiangraecum* are intriguing (the labellum and spur shapes in *A. praestans* and *A. viguieri* appear very similar). Indeed, the infudibular form of the spur is unusual in section *Perrierangraecum*. This form appears to represent an example of convergent evolution within *Angraecum*. We also added *A. cornigerum* (section *Filangis*) to this section because its morphological characters fit well here, a fact that is strongly supported by the phylogeny.

Species included

Angraecum ambrense H.Perrier, ***A. ankeranense*** H.Perrier, *A. bicallosum* H.Perrier, ***A. borbonicum*** Bosser, ***A. breve*** Schltr., *A. chimanimaniense* G.Will., *A. clavigerum* Ridl., *A. compressicaule* H.Perrier, *A. coriaceum* (Thunb. ex Sw.) Schltr., ***A. cornigerum*** Cordem., *A. coutrixii* Bosser, *A. crassum* Thouars, ***A. cucullatum*** Thouars, *A. curnowianum* (Rchb.f.) T.Durand & Schinz, *A. curvicalcar* Schltr., ***A. didieri*** (Baill. ex Finet) Schltr., ***A. drouhardii*** H.Perrier, ***A. dryadum*** Schltr., ***A. elephantinum*** Schltr., *A. elliotii* Rolfe, *A. equitans* Schltr., *A. humbertii* H.Perrier, *A. imerinense* Schltr., *A. kranzlinianum* H.Perrier, ***A. lecomtei*** H.Perrier, ***A. letouzeyi*** Bosser, ***A. liliodorum*** Frapp. ex Cordem., *A. litorale* Schltr., *A. longicaule* Humbert, *A. mahavavense* H.Perrier, *A. meirax* (Rchb.f.) H.Perrier, ***A. obesum*** H.Perrier, ***A. palmicolum*** Bosser, ***A. peyrotii*** Bosser, ***A. praestans*** Schltr., ***A. pseudodidieri*** H.Perrier, *A. rigidifolium* H.Perrier, ***A. rutenbergianum*** Kraenzl., *A. sambiranoense* Schltr., *A. stella-africae* P.J.Cribb, ***A. sterrophyllum*** Schltr., *A. trichoplectron* (Rchb.f.) Schltr., *A. urschianum* Toill.-Gen. & Bosser.

2.3.12. *Angraecum* sect. *Pseudojumellea* Schltr., Beih. Bot. Centralbl. 36(2): 157 (1918).

Angraecum sect. *Filangis* Garay, Kew Bull. 28: 495–516 (1973). Type: *Angraecum filicornu* Thou.

Angraecum sect. *Pectinaria* (Benth.) Schltr., Beih. Bot. Centralbl. 36(2): 157 (1918). Type: *Angraecum pectinatum* Thou.

Mystacidium sect. *Pectinaria* Benth., Benth. & Hook. f., Gen. P1. 3: 585 (1883).

LECTOTYPE: *Angraecum mauritianum* (Poir.) Frapp. [*Orchis mauritiana* Poir.], designated by Garay, Kew Bull. 28: 495–516 (1973).

DISTRIBUTION: Madagascar, Mauritius, Reunion

Epiphytic on trunk or undergrowth lithophytic, medium to large plants (to 1 m height); stems erect, climbing or pendent, internodes usually long (to 5 cm); leaves thin or coriaceous, stems and leaves usually spotted black; inflorescences one-flower, subterminal, short sometimes longer than leaves, one to five per stem; flowers small to medium sized, white, sepals and petals linear-lanceolate to elliptic; labellum lowermost, linear-lanceolate to elliptic, concave, longer or equaling dorsal sepal, apex acute; spur short to elongate (to 12 cm), isodiametric at base, tapering to tip, white sometimes greenish white towards the tip; ovary round in section with 180° resupination.

We modified the original description to better match the phylogenetic composition of the section. Two sections of Garay (1973) are subsumed within it: *Pectinaria* and *Pseudojumellea*. As originally proposed by Schlechter (1918), we moved *A. filicornu* (the type of section *Filangis*) to section *Pseudojumellea*, since this position is strongly supported by the phylogeny (chap. 1).

Species included

Angraecum danguyanum H.Perrier, *A. darainense* P.J. Cribb & Nusb., ***A. dasycarpum*** Schltr., ***A. filicornu*** Thouars, ***A. humblotianum*** Schltr., *A. implicatum* Thouars, ***A.***

mauritianum (Poir.) Frapp., *A. melanostictum* Schltr., ***A. panicifolium*** H.Perrier, ***A. pectinatum*** Thouars, *A. platycornu* Hermans, P.J. Cribb & Bosser, ***A. ramosum*** Thouars.

2.3.13. *Angraecum* sect. *Robusta* Andriananjamanantsoa sect. nov.

TYPE: *Angraecum oblongifolium* Toill.-Gen. & Bosser

DISTRIBUTION: Madagascar, Comoros

Epiphytic on trunk, medium to very large plants (up to 1 m height); stems elongate, pendent, robust, often with long internode (up to 5 cm); leaves alternate, often coriaceous; inflorescences one to three-flowered, subterminal, shorter than leaves, one to five per stem; flowers medium, white, sepals and petals lanceolate-ovate; labellum uppermost, usually smaller than dorsal sepal, elliptic to lanceolate, concave, apex acuminate; spur short to elongate (to 12 cm), sigmoid, slightly enlarged at the base, tapering towards the tip, green; ovary round in section with 360° resupination.

ETYMOLOGY: The name refers to the structure of the stem, which is very robust and woody-like.

This section looks very similar to section *Arachnangraecum*, except that the flowers are smaller and the margin of sepals and sepals always entire. The species in this section were previously placed in section *Angraecum* (*A. dendrobiopsis*, *A. penzigianum*, *A. serpens*) because of their racemose inflorescence, and *Pseudojumellea* (*A. oblongifolium*) because of their medium-sized flowers and one-flowered inflorescence. We also added *A. amplexicaule* and *A. moratii* previously placed in section *Filangis* to this section because of morphological characters and phylogenetic evidence (Fig. 2.1).

Species included

Angraecum amplexicaule Toill.-Gen. & Bosser, *A. dendrobiopsis* Schltr., ***A. florulentum*** Rchb.f., ***A. moratii*** Bosser, ***A. oblongifolium*** Toill.-Gen. & Bosser, ***A. penzigianum*** Schltr., ***A. serpens*** (H.Perrier) Bosser.

2.3.14. *Angraecum* sect. *Sobennikoffia* (Schltr.) Andriananjamanantsoa comb. nov.

Sobennikoffia Schltr., Repert. Spec. Nov. Regni Veg. Beih. 33: 361 (1925).

TYPE: *Sobennikoffia robusta* (Schltr.) Schltr. [*Oeonia robusta* Schltr.]

DISTRIBUTION: Western Madagascar

Epiphytic on canopy or trunk, large plants (to 40 cm height); stems erect, short or elongate, internodes short; leaves dense, coriaceous; inflorescences racemose, many-flowered (up to 10), suprafoliar, longer than leaves, one to four per stem; flowers large, white, sepals and petals lanceolate-ovate; labellum lowermost, ovate, deeply concave, longer or equaling dorsal sepal, white overlaid green at the moth, apex trilobed; spur longer or equaling dorsal sepal, infundibuliform with a wide opening, green; ovary round in section with 180° resupination.

In the phylogeny (Fig. 2.1; chap. 1), sect. *Sobennikoffia* is sister to sections *Hadrangis* and *Humblotiangraecum*. Morphologically, they share several traits: large flowers, lowermost labellum, broadly ovate and deeply concave, often white overlaid green at the moth, and spurs widely open at the base. This section is widespread from north western to south western Madagascar.

Species included and transferred from *Sobennikoffia* to *Angraecum*

Angraecum fournierianum Kraenzl, Gard. Chron. 3(15): 808 (1894).

Synonym: *Sobennikoffia fournieriana* (Kraenzl.) Schltr., Repert. Spec. Nov. Regni Veg. Beih. 33: 362 (1925).

Angraecum humbertianum (H.Perrier) Andriananjamanantsoa comb. nov.

Basionym: *Sobennikoffia humbertiana* H.Perrier, Notul. Syst. Paris, 7: 134 (1938).

Angraecum poissonianum (H.Perrier) Andriananjamanantsoa comb. nov.

Basionym: *Sobennikoffia poissoniana* H.Perrier, Notul. Syst. Paris, 14: 164 (1951).

Angraecum robustum (Schltr.) Schltr., Beih. Bot. Centralbl. 33(2): 437 (1915).

Basionym: *Oeonia robusta* Schltr., Ann. Inst. Bot.-Géol. Colon. Marseille, 3(1): 184, t. 13 (1913).

Synonym: *Sobennikoffia robusta* (Schltr.) Schltr., Repert. Spec. Nov. Regni Veg. Beih. 33: 362.

2.3.15. *Angraecum* sect. *Stellariangraecum* Andriananjamanantsoa sect. nov.

TYPE: *Angraecum sororium* Schltr.

DISTRIBUTION: Madagascar

Epiphytic or lithophytic, very large plants (to 50 cm height); stems erect, internodes very short; leaves densely imbricate, coriaceous; inflorescences usually one or two-flowered, sometimes racemose with up to five flowers, suprafoliar, short or equaling leaves, one to four per stem; flowers very large, white or cream, sepals and petals linear-lanceolate to lanceolate-ovate; labellum lowermost, broadly ovate, slightly concave, longer or equaling dorsal sepal, apex acute; spur very long (up to 35 cm), isodiametric from the base, tapering towards the tip, pale green; ovary round in section with 180° resupination.

ETYMOLOGY: The name comes from a local appellation, ‘Comet Orchid’, which designates *Angraecum* species (*A. sesquipedale* and *A. sororium*) that have large, white flowers with a long spur.

Two species previously placed in sect. *Angraecum*, *A. sesquipedale* and *A. sororium*, are accommodated in this section according to both morphology and molecular evidence (Fig. 2.1). The lowermost position of the labellum and the untwisted ovary are the major characters that separate species in this section from section *Angraecum* (its previous section). A third species, *Angraecum protensum* Schltr., which was not included in previous phylogenetic studies, is referable to this section. This species has a very similar habit to *A. sororium* and inhabits limestone inselbergs in the Middle West central highland of Madagascar.

Species included

Angraecum protensum Schltr., ***A. sesquipedale*** Thouars **var. *sesquipedale***, *A. sesquipedale* var. *angustifolium* Bosser & Morat, *A. sesquisectangulum* Kraenzl., ***A. sororium*** Schltr.

2.3.16. Incertae sidis

Two species poorly know from herbaria specimen should be removed from *Angraecum*:

Angraecum gracile Thouars, Hist. Orchid.: t. 77 (1822). Garay (1973) and Stewart et al. (2006) consider this endemic species to Mauritius a synonymy of *Chamaeangis gracilis* (Thouars) Schltr., Beih. Bot. Centralbl. 36(2): 108 (1918).

Angraecum palmiforme Thouars, Hist. Orchid.: 68. (1822). Garay (1973) putted this species in section *Angraecum*, while Stewart et al. (2006) believe this endemic species to Reunion is probably extinct.

2.3.17. Species excluded from *Angraecum*

The following species were misidentified and are relegated to synonymy:

Angraecum metallicum Sander = *Aerangis stylosa* (Rolfe) Schltr., Beih. Bot. Centralbl. 33(2): 427 (1915).

Angraecum ramulicolum H.Perrier = *Aerangis pallidiflora* H.Perrier, Notul. Syst. (Paris) 7: 36 (1938).

Angraecum triquetrum Thouars = *Jumellea triquetra* (Thouars) Schltr., Beih. Bot. Centralbl. 33(2): 430 (1915).

Taking into account all previous phylogenetic studies (Carlsward et al., 2006; Micheneau et al., 2008a; Szlachetko et al., 2013; chap. 1), all African sections are excluded from *Angraecum*. Nonetheless, further studies with a more complete sampling are needed before attributing unplaced species to genus, especially within the former section *Afrangraecum* sensu Garay (1973) which includes ten species restricted to continental Africa.

In fact, only one species of this section (*A. multinominatum*) has been sampled in a phylogenetic reconstruction (Szlachetko et al., 2013) that appeared to be related to African Angraecinae genera rather than *Angraecum* sensu stricto, but it has not been attributed to any genera yet.

2.4. New genera of Malagasy orchids

2.4.1. *Acaulia* (Garay) Andriananjamanantsoa comb. nov.

TYPE: *Acaulia rhynchoglossa* (Schltr.) Andriananjamanantsoa [=*Angraecum rhynchoglossum* Schltr.]

Angraecum sect. *Acaulia* Garay, Kew Bull. 28: 495–516 (1973). Type: *Angraecum rhynchoglossum* Schltr.

DISTRIBUTION: Central highland and eastern Madagascar

Epiphytic plants, small, stemless; leaves 2-6, distinctly lanceolate-ovate, constricted at the base, 23 x 5 mm; inflorescences 16 mm long, 1-flowered (a second aborted when present), equaling or exceeding leaves; flowers small, pale green, petals and sepals linear-lanceolate 0.6 x 7.5 mm, dorsal sepal slightly longer; labellum uppermost 6 x 3 mm, base expanded and very rounded, apex acuminate; spur funnel-shaped and tapering below, often expanding a little at the apex, at least twice longer than labellum, ca. 25 mm long; ovary round in section with 360° resupination.

HABITAT: At low altitudes, usually on small trees or twigs (1 to 2 m from the ground), frequent in secondary forests.

CONSERVATION STATUS: Data deficient but possibly threatened because of its limited range.

Plants of this monotypic genus have a habit very similar to *Lemurella* Schltr., but the leaves are narrower, the stem reduced and the inflorescence one-flowered (Fig. 2.2). It differs from other species that were included in *Angraecum* section *Acaulia* by Garay (1973) in its very acuminate labellum and linear sepals and petals. These other species are now part of section *Boryangraecum*.

Fig. 2.2. *Acaulia rhynchoglossa* (Photo: Andriananjamanantsoa)

SPECIMEN EXAMINED: Madagascar, Antananarivo Prov., Vohitrilongo mountain, Perrier 14973 (holotype, https://science.mnhn.fr/institution/mnhn/collection/p/item/p00098421)

New species combination

Acaulia rhynchoglossa (Schltr.) Andriananjamanantsoa comb. nov.

Angraecum rhynchoglossum Schltr., Repert. Spec. Nov. Regni Veg. Beih. 33: 339 (1925).

2.4.2. *Parangraecum* Andriananjamanantsoa gen. nov.

TYPE: *Parangraecum pterophyllum* (H.Perrier) Andriananjamanantsoa [= *Angraecum pterophyllum* H.Perrier]

DISTRIBUTION: Eastern Africa (Ethiopia, Kenya, Rwanda, Swaziland, Tanzania, Zimbabwe), Madagascar (Central highland and Eastern), Reunion

Leaves elliptic or linear, semi-terete or slightly triquetrous, inflorescences racemose, flowers almost actinomorphic, labellum similar to petals but with raised edges, gynostemium pale green, spur globular.

Epiphytic plants, small and erect; stems usually elongate (up to 5 cm long), but very short or even inexistent in some species; leaves 2-10, inflorescences 1--5-flowered, flowers mostly one-sided, borne from lower leaf axils; flowers white; sepals ovate (3 x 2 mm), apex attenuate; petals similar to sepals, smaller (2.5 x 1.8 mm); labellum uppermost, ovate-concave (2.5 x 1.8 mm), white overlain with green at base; spur shorter than sepal (2 x 1.2 mm); ovary glabrous, 2 mm long.

HABITAT: Humid and evergreen forests, on trunk or lower branches.

CONSERVATION STATUS: Not evaluated but probably of little concern because it is abundant in the field (personal observation).

ETYMOLOGY: For the morphology (vegetative and floral) that is similar to that of *Angraecum*.

The leaves are fleshy and the apex acute. The inflorescence is usually short and the flowers dense (Fig. 2.3). Three *Angraecum* species (*A. humile*, *A. perparvulum*, and *A. pterophyllum*), which form a monophyletic group, are included in this genus. Two other species (*A. rubellum* and *A. tenellum*), which were not included in phylogenetic studies, appear better accommodated in this genus; this needs to be validated with molecular data.

Fig. 2.3. *Parangraecum* cf. *humile* (Photo: Andriananjamanantsoa)

SPECIMEN EXAMINED: Madagascar, Toamasina Prov., Mantadia, Onive basin, Perrier 18647 (holotype, https://science.mnhn.fr/institution/mnhn/collection/p/item/p00334625).

New species combination

Parangraecum humile (Summerh.) Andriananjamanantsoa comb. nov.

Basionym: *Angraecum humile* Summerh., Kew Bull. 13: 269 (1958).

Parangraecum perparvulum (H.Perrier) Andriananjamanantsoa comb. nov.

Basionym: *Angraecum perparvulum* H.Perrier, Notul. Syst. (Paris) 7: 123 (1938).

Parangraecum pterophyllum H.Perrier Andriananjamanantsoa comb. nov.

Basionym: *Angraecum pterophyllum* H.Perrier, Notul. Syst. (Paris) 7: 106 (1938).

Parangraecum tenellum (Ridl.) Andriananjamanantsoa comb. nov.

Basionym: *Mystacidium tenellum* Ridl., J. Linn. Soc., Bot.21: 489 (1885).

Angraecum tenellum (Ridl.) Schltr., Beih. Bot. Centralbl. 33(2): 438 (1915).

Angraecum bemarivoense Schltr., Repert. Spec. Nov. Regni Veg. Beih. 33: 332 (1925).

Parangraecum rubellum (Bosser) Andriananjamanantsoa comb. nov.

Basionym: *Angraecum rubellum* Bosser, Adansonia, n.s. 10: 22 (1988).

2.5. Acknowledgements

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Chapitre 3: Biogeography of *Angraecum* (Orchidaceae, Angraecinae), out of Madagascar case-dispersal

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Contribution respective des auteurs:

Tahiana Andriananjamanantsoa: Conception du projet, collecte, traitement et analyse des données, interprétation et rédaction

Luc Brouillet: Supervision, conception du projet, conseils, recommandations, corrections et direction générale

3.1. Résumé/Abstract

Résumé

Objectifs: Cette étude vise à étudier la répartition des espèces d'*Angraecum* dans le monde, un groupe d'orchidées très diversifié à Madagascar et dans la région occidentale de l'Océan Indien, et une partie de l'Afrique tropicale. Nous avons évalué l'origine biogéographique de la sous-tribu des Angraecinae, et évalué l'effet de changement de stratification sur la diversification au sein du genre *Angraecum* à Madagascar. Nous voulons aussi analyser la diversification des espèces d'*Angraecum* après un changement de la strate occupée dans l'habitat.

Localisation: Madagascar, région occidentale de l'Océan Indien, Afrique

Méthodes: Nous avons évalué différents modèles de transition de la stratification écologique qui pourrait affecter la diversification d'*Angraecum* en utilisant les modèles multistate speciation and extinction. Ensuite, à partir de la phylogénie moléculaire datée des Angraecinae (chap. 1), nous avons reconstruit les aires ancestrales de la sous-tribu des Angraecinae en utilisant le programme BioGeoBEARS.

Résultats: Le meilleur modèle de transition suggère que le passage des épiphytes de canopée vers des lithophytes sous canopée ou sur inselberg, ou vers des épiphytes de tronc est le changement d'habitat (strate) le plus probable ayant pu conduire à la diversification d'*Angraecum*. L'analyse biogéographique montre que Madagascar est le lieu d'origine de la sous-tribu des Angraecinae et du genre *Angraecum* à partir d'un ancêtre du sud-est asiatique. Les lignées ancestrales d'*Angraecum* proviendraient du Nord-Est de Madagascar et sont apparues au Miocène. Chez *Angraecum*, la migration à l'intérieur de Madagascar a commencé vers la fin du Miocène, alors que la migration hors de Madagascar a commencé plus tard, généralement au Pléistocène.

Conclusions: Notre étude a montré un nouveau cas de dispersion à partir de Madagascar pour la sous-tribu des Angraecinae et le genre *Angraecum*. Chez *Angraecum*, les évenements de dispersion ont eu lieu au cours du Pliocène-Pléistocène et ont débuté à partir du Nord-Est de Madagascar. Les cyclones jouent un rôle important dans la dispersion à longue distance des orchidées dans la région occidentale de l'Océan Indien. La diversification d'*Angraecum* dans les îles Comores et les Mascareignes reflète l'importance des nouveaux habitats et la similarité des niches écologiques dans l'expansion des espèces.

Mots-clés: Angraecinae, *Angraecum*, histoire biogéographique, diversification, niche, nord est de Madagascar, Orchidaceae, taux de transition

Abstract

Aim: This study investigates the worldwide distribution of *Angraecum*, a highly diversified orchid group restricted to Madagascar, the western Indian Ocean, and parts of tropical Africa. We assessed the biogeographical origins of the Angraecinae and of *Angraecum*. Our goals were also to analyze the diversification of *Angraecum* species following changes in the strata occupied within the forest.

Location: Africa, Madagascar, western Indian Ocean region

Methods: Using a dated molecular phylogeny of subtribe Angraecinae (chapter 1), we performed an ancestral area reconstruction of subtribe Angraecinae as implemented in BioGeoBEARS. We also tested different transition models related to forest habitat stratification, which may have affected the diversification of *Angraecum*, using the multistate speciation and extinction models.

Results: The best model suggested that transitions from canopy epiphytes to lithophytes (both undergrowth and inselberg), and to trunk epiphytes were the most probable shifts in stratification that might have led to the diversification in *Angraecum*. Historical biogeographic analysis showed that Madagascar was the place of origin of Angraecinae as well as *Angraecum*. Ancestral lineages of *Angraecum* came probably from the northern tropical lowland forest of Madagascar and arose during the Miocene. Within the genus, expansion within Madagascar started in the late Miocene, while dispersal out of Madagascar occurred mostly during the Pleistocene.

Main conclusion: Our study highlighted a new case of dispersal out of Madagascar in Angraecinae and *Angraecum*. In *Angraecum*, dispersal events took place during the Pliocene-

Pleistocene, and they started from the northeast Madagascar. Cyclones appeared to have played an important role in orchid seed dispersal in the western Indian Ocean region. The diversification of *Angraecum* in the Comoros and Mascarenes Islands reflects the importance of new habitats and niche similarities in range expansion.

Keywords: Angraecinae, *Angraecum*, historical biogeography, diversification, niche, north-east Madagascar, Orchidaceae, transition rates

3.2. Introduction

The Western Indian Ocean Region (WIOR) offers valuable insights for historical biogeography studies because of its biodiversity richness (Goodman and Benstead, 2005; Buerki et al., 2013), and due on one hand to islands dating from the Cenozoic (Madagascar and Seychelles), which may have experienced vicariance, and on the other to recently formed islands (Comoros and Mascarene), which could have been subject to dispersal events. Island biogeographical theory predicts that the largest proportion of lineages within an island region comes from the nearest island and/or mainland source (MacArthur and Wilson, 1967; Warren et al., 2010). Based on geological history, only dispersal can explain the presence of many oceanic island biotas (de Queiroz, 2005). Thus, Madagascar has been a major source of colonizing lineages for surrounding islands (Raxworthy et al., 2002; Weyeneth et al., 2008; Krüger et al., 2012; Buerki et al., 2013), especially for Comoros and the Mascarenes (Buerki et al., 2013). Many studies have revealed that Malagasy biotas originated from Africa (e.g. Yoder et al., 1996; Yoder et al., 2006; Bacon et al., 2015). However, the majority of lineages in Madagascar have post-Gondwanan origins (Vences, 2004; Yoder and Nowak, 2006), suggesting that dispersal played a more important role in their origins than vicariance (Warren et al., 2010). At the same time, it is now widely accepted that present-day levels of diversity and endemism in Madagascar may be due to a combination of divergence by vicariance and in situ diversification by post Cenozoic dispersers (Vences et al., 2001; Yoder and Nowak, 2006; Warren et al., 2010; Kuntner and Agnarsson, 2011; Strijk et al. 2012). Recent studies supported an Asian origin and affinities to many lineages within Madagascar (e.g. Battistini and Richard-Vindard, 1972; Stoddart, 1984; Cheke and Hume, 2008; Buerki et al., 2013), opening up a new perspective on historical biogeography of the island and the WIOR.

Rapid radiation is often highlighted as a primary cause of the diversification Madagascar biotas (Janssen et al., 2008; Townsend et al., 2009; Vences et al., 2009; Anthony et al., 2010; Jonsson et al., 2012; Christidis et al., 2014), as well as the Mascarene lineages (Thébaud et al., 2009). This mechanism is usually considered a result of ecological opportunity and/or founder effect (Thébaud et al., 2009; Matzke, 2013a; Alsos et al., 2015). The main way to validate wether species diversity is caused by adaptive radiation or multiple dispersal events is through the use of species rich taxa in biogeographical analyses. Genus *Angraecum* is of particular interest because of its high diversification (chap. 1) and of its widespread geographical distribution in the Western Indian Ocean Region ranging from Africa to Sri Lanka. Diversification studies suggested that the great number of species within *Angraecum* resulted from a steady accumulation of lineages through-time (chap. 1) and not from a rapid radiation as suggested for many Malagasy taxa (e.g. Townsend et al., 2009; Vences et al., 2009). The significant number of species present in Madagascar and the Indian Ocean Islands (IOI: Comoros, Mascarenes, and Seychelles), especially the Comoros and Mascarenes, raised the hypothesis of a possible origin of *Angraecum* in Madagascar. The relatively young age of subtribe Angraecinae (Ramirez et al., 2007; Gustafsson et al., 2010, Iles et al., 2015), as well as *Angraecum* (Jacquemyn et al., 2005; chap. 1) eliminates a priori a post-Gondwanan vicariance hypothesis. Using Mascarene Angraecoid data, Micheneau et al. (2008a) suggested a Malagasy origin for Angraecinae. This study was conducted with a limited sample of taxa (mostly taxa from the Mascarenes). The absence of resolved generic and species relationships limits the ability to evaluate historical and geographical hypotheses. With a fully reconstructed phylogeny of *Angraecum* (chap. 1), it is now possible to evaluate the historical biogeographic pattern of the group.

The elevational gradient is often pointed out as the main cause of diversification in orchids (Jacquemyn et al., 2005; Acharya et al., 2011) and epiphytism was a key innovation (apomorphic state in Orchidaceae) responsible for a species burst in the family. *Angraecum*, with over 95% of its species epiphytic, took advantage of this innovation, being the most diverse genus in subtribe Angraecinae. The genus has mainly diversified in Madagascar and the adjacent islands (Comoros and Mascarenes). Species diversification is often associated with historical biogeography and ecological niche opportunities (Schluter, 2000; Whittaker et al., 2008; Strijk et al., 2013). In fact, the origin of diversification of *Angraecum* in Madagascar was reported to be associated with paleoclimate events, essentially the establishment of the monsoon in the northern part of the island during the Miocene (Buerki et al., 2013), and the contraction and expansion of the tropical rainforest during the post-Pleistocene global cooling (Ruddiman, 2001). In *Angraecum*, we noted four types of habitat distribution according to the stratum (forest layer): in the canopy, on trunks, on undergrowth rocks, and on inselbergs (granite or limestone). Considering each layer as an ecological niche, the shift of stratification could be viewed as the colonization of a new niche. The ability of orchid seeds to occupy a habitat is determined by many factors such as niche opportunity, mycorrhizal source availability, or climatic conditions (Dressler, 1981–1993; Arditti and Ghami, 2000; Jersakkova and Malinova, 2007). Furthermore, adapted pollinators are required to maintain the population's reproduction whatever the niche occupied. Understanding the mechanisms that help to colonize different strata within the forest will be helpful in understanding the distribution patterns of *Angraecum* in tropical rain forests.

Although the biogeography of subtribe Angraecinae has been studied previously (Micheneau et al., 2008), no study has included temporal events which are important to estimate the underlying causes of the distribution. Thus, many questions remain unanswered such as how, when and from where did the migration occur. Furthermore, the high number of *Angreacum* species encountered in Madagascar and surrounding islands (Mascarenes and Comoros) raises questions about the importance of ecological niches and its possible limitation on species distribution. We suspected that stratification shifts in the forest might play a significant role in colonizing new habitats. Using a dated and more thoroughly sampled phylogeny of Angraecinae (chap. 1), we are inferring the historical biogeography of *Angraecum* and its close relatives. Our goals were to: (1) evaluate the effect of forest stratification changes on the diversification of *Angraecum*, (2) evaluate the biogeographical origin of Angraecinae and its probable source, (3) assess biogeographic patterns within *Angraecum*, and (4) understand the mechanism of dispersal responsible for the distribution of the lineages.

3.3. Material and methods

3.3.1. Phylogenetic inference and divergence time estimates

Phylogenetic inference and divergence time estimates used in this analysis, as well as subsequent taxonomic changes, are described more fully in chap. 1 and 2. A total of 194 taxa were included in phylogenetic analyses, comprising 96 *Angraecum* species, 93 Angraecinae, and five outgroups (*Acampe ochracea*, *Aerides odorata*, *Phalaenopsis cornu-cervi*, *Polystachya fulvilabia*, and *Vanda tricolor*). Three plastid regions were combined (*mat*K coding gene, *rps*16 intergenic spacer, and *trn*L intron) for a total of 3725 characters. DNA sequences were aligned using SeaView v4 (Gouy et al., 2010), and alignments were visually inspected and manually edited, if necessary using BioEdit v7.1.3 (Hall, 1999).

Divergence times were estimated using a relaxed molecular clock approach as implemented in BEAST (Drummond and Rambaut, 2007) with GTR+G+I model selected as the substitution model. A relaxed lognormal molecular clock model was set. The Yule speciation process with a random starting tree was selected as tree prior. The age of the tree root was set to a normal distribution with a mean of – 35 Ma and a standard deviation of 3 (giving a 95% CI ranging from 30.07 – 39.93 Ma) using the age estimate for Vandeae determined by Gustafsson et al. (2010). The prior distribution of the 'ucld.mean' parameter was set to an exponential distribution (mean=10.0, initial value=1.0). Four separate runs were performed in BEAST with 50 million generations each, sampling parameters every 1000 generations. Trees were summarized with burn-in values set to the first 25% of trees sampled using TreeAnnotator and were summarized in a maximum clade credibility tree (MCCT).

3.3.2. Diversification analyses

To assess the diversification patterns of plant habitats in *Angraecum*, we evaluated the state-dependent diversification of this character using the multistate speciation and extinction (MuSSE) models implemented in the R package 'diversitree' (FitzJohn, 2012). This habitat character has four potential states (E: canopy epiphyte, I: inselberg lithophyte, L: undergrowth lithophyte, T: trunk epiphyte). Character state distribution was provided in the character matrix of Andriananjamanantsoa et al. (in chap. 1). Since the MuSSE method requires ultrametric and fully bifurcating trees, we used the MCC tree generated from BEAST as input. All taxa that do not belong to *Angraecum* were excluded from the analyses using the ‘drop.tip2’ function of the R package ‘phyloch’ (Heibl, 2008).

Six models were tested, one full model allowing all parameters to vary (***λ***: speciation rate, ***μ***: extinction rate, and ***q:*** transition rate), and five constrained models that allow ***λ*** and ***μ*** to vary while constraining the ***q*** parameter. In the second model, only transitions between E and L (E↔L), from E to I (E→I), and from E to T (E→T) were allowed. In the third model, five transitions were allowed (E↔L, E↔T, and E→I). The fourth model allowed five transitions (E↔L, E→T, E→I, and L→T), the fifth model seven (E↔L, E↔T, L↔T, and E→I), and the sixth, eight (E↔L, E↔T, L↔T, T→I, and L→I). After selecting the best fit model (Table 3.1), we set up the prior to be exponential and computed the posterior probabilities of the parameters under a Bayesian framework. The MCMC was run for 10000 generations sampling parameters every 100 generations, and posterior probability distributions were summarized using the function ‘profiles.plot’ implemented in ‘diversitree’ (FitzJohn, 2012).

Table 3.1. Comparison of models tested in MuSSE; bold characters within the table highlight the best fit model selected for analyses. Character states: E, canopy epiphyte; I, inselberg lithophyte; L, undergrowth lithophyte; T, trunk epiphyte; ↔, reversible transition; →, irreversible transition. Abbreviations: AIC, Akaike information criterion; Df, degrees of freedom; lnLik, log likelihood; M, model.

Model	**transitions allowed**	**Df**	**lnLik**	**AIC**
M1	All (full model)	20	-300.25	640.50
M2	E↔L, E→I, E→T	12	-321.50	666.99
M3	E↔L, E↔T, E→I	13	-311.11	648.22
M4	E↔L, E→T, E→I, L→T	13	-309.65	645.30
M5	**E↔L, E↔T, L↔T, E→I**	**15**	**-301.22**	**632.45**
M6	E↔L, E↔T, L↔T, T→I, L→I	16	-307.11	646.22

3.3.3. Biogeographical history analyses

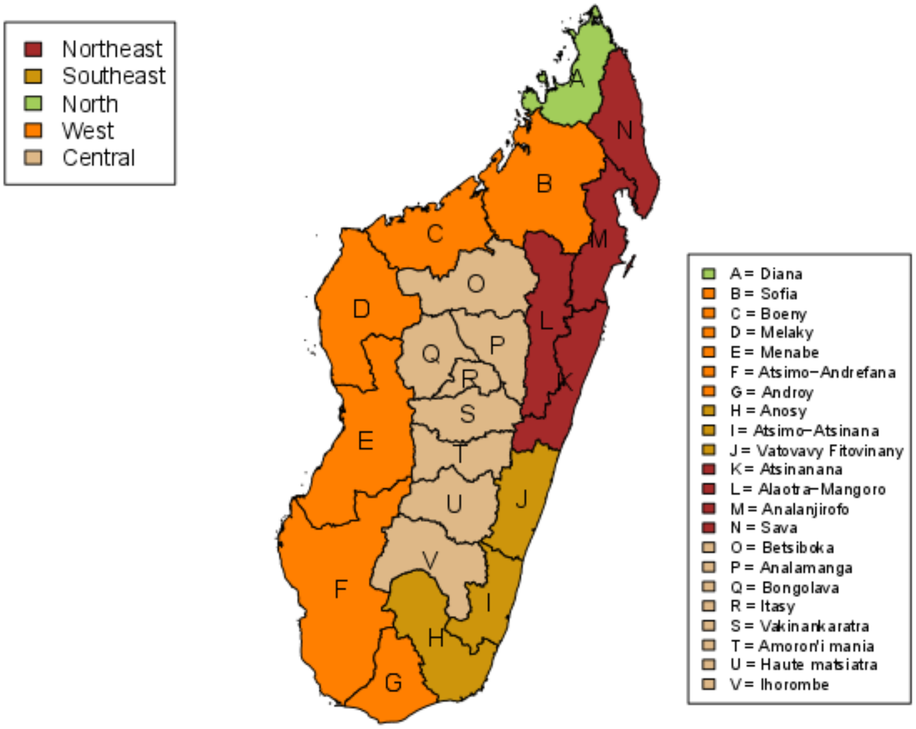
To determine the biogeographical origins of the Angraecinae, ancestral ranges were inferred using the biogeography with Bayesian and likelihood evolutionary analyses using R scripts implemented in the R package 'BioGeoBEARS' (Matzke, 2013b). The method implements different models such as the dispersal–extinction–cladogenesis (DEC) (Ree and Smith 2008) or the dispersal–vicariance (DIVA) analysis (Ronquist, 1997) with added parameters (Matzke, 2013a, 2013b). One of its particularities is that it considers founder-event speciation, described as parameter ***j***. Founder-event speciation implies that dispersal to an area outside the ancestral range results in a new species; this is thought to be especially significant in island systems (Moya et al., 1995; Matzke, 2013a; Matzke, 2014). We conducted the analyses using the tree generated from BEAST as input. The following regions were used for the overall analysis: Md, Madagascar; Ms, Mascarenes; Com, Comoros; eA, east Africa; Se, Seychelles and Sri Lanka; As, Southeast Asia; wA, west Africa; and Am, South America. Three models were tested, one unconstrained and two with constraints (M0 and M1). Because of the geographical distance between areas and their geological history, we applied differential dispersal rate parameters between areas in the constrained models (Table 3.2). Since the emergence of archipelagos in the western Indian Ocean occurred from the late Miocene to the Pleistocene (Baksi and Hoffman, 2000; Weyeneth et al. 2008), we designated three time slices related to this temporal framework: 2 Ma as maximum age for the Mascarenes, 7 Ma for the appearance of Mayotte (Comoros), and 15 Ma for the maximal age of the Comoros.

In order to gain a finer comprehension of the biogeographic history of *Angraecum*, a separate analysis was run in which a greater focus was placed on biogeographic subdivisions within Madagascar and the IOI. We divided Madagascar into five areas according to species distribution taken from Cribb and Hermans (2009), supplemented by our own field data and specimen labels. These areas are congruent with the vegetation zones of Humbert (1955), except that the mountain zone was not treated as a separate area. The west and south were grouped as the west, while the northeast and southeast were treated as separate areas in order to increase the resolution of biogeographic analyses (Fig. 3.1). Geographical units outside the IOI were grouped into a single area because of software limitation in treating more than nine areas. The following regions were retained for biogeographical analyses: within Madagascar:

Table 3.2. Time slices and constraints used in ancestral area reconstructions of subtribe Angraecinae implemented in BioGeoBEARS; age of Mascarenes (2 Ma), Mayotte (7 Ma), and Comoros (15 Ma). Areas within Madagascar: MdN, north; MdNe, northeast; MdSe, southeast; MdC, center; MdW, west. Areas outside Madagascar: Ms, Mascarenes; Cm, Comoros; Se, Seychelles/Sri Lanka; AA, Africa/America/Asia. Abbreviations: M, model constraint; Ma, million years.

Areas	**MdN**	**MdNe**	**MdSe**	**MdC**	**MdW**	**Ms**	**Cm**	**Se**	**AA**	**time slices**	**cons-traint**
MdN	1	0.5	0.4	0.3	0.2	0.01	0.3	0.01	0.01		
MdNe	0.5	1	0.5	0.5	0.1	0.01	0.3	0.3	0.1		
MdSe	0.4	0.5	1	0.5	0.1	0.01	0.01	0.01	0.01		
MdC	0.3	0.5	0.5	1	0.5	0.01	0.01	0.3	0.01		
MdW	0.2	0.1	0.1	0.5	1	0.01	0.1	0.01	0.01	**2 Ma**	**M0, M1**
Ms	0.01	0.01	0.01	0.01	0.01	1	0.01	0.01	0.01		
Cm	0.3	0.3	0.01	0.01	0.1	0.01	1	0.01	0.01		
Se	0.01	0.3	0.01	0.3	0.01	0.01	0.01	1	0.01		
AA	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0.01	1		
MdN	1	0.5	0.4	0.3	0.2	0.01	0.3	0	0.01		
MdNe	0.5	1	0.5	0.5	0.1	0.01	0.3	0	0.1		
MdSe	0.4	0.5	1	0.5	0.1	0.01	0.01	0	0.01		
MdC	0.3	0.5	0.5	1	0.5	0.01	0.01	0	0.01		
MdW	0.2	0.1	0.1	0.5	1	0.01	0.1	0	0.01	**7 Ma**	**M1**
Ms	0.01	0.01	0.01	0.01	0.01	1	0.01	0	0.01		
Cm	0.3	0.3	0.01	0.01	0.1	0.01	1	0	0.01		
Se	0	0	0	0	0	0	0	0	0		
AA	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0	1		
MdN	1	0.5	0.4	0.3	0.2	0.01	0	0	0.01		
MdNe	0.5	1	0.5	0.5	0.1	0.01	0	0	0.1		
MdSe	0.4	0.5	1	0.5	0.1	0.01	0	0	0.01		
MdC	0.3	0.5	0.5	1	0.5	0.01	0	0	0.01		
MdW	0.2	0.1	0.1	0.5	1	0.01	0	0	0.01	**15 Ma**	**M1**
Ms	0.01	0.01	0.01	0.01	0.01	1	0	0	0.01		
Cm	0	0	0	0	0	0	0	0	0		
Se	0	0	0	0	0	0	0	0	0		
AA	0.01	0.1	0.01	0.01	0.01	0.01	0	0	1		

MdN, north (Sambirano); MdNe, northeast; MdSe, southeast; MdC, center; E, MdW; outside Madagascar: AA, Africa, America and Asia; Cm, Comoros; Ms, Mascarenes; Se, Seychelles and Sri Lanka.



<u>Fig. 3.1.</u> Biogeographic areas inside Madagascar used in ancestral area reconstructions of subtribe Angraecinae implemented in BioGeoBEARS, colors indicate the different areas and letters denote the 22 administrative regions of Madagascar.

3.4. Results

3.4.1. Divergence time estimates

Phylogenetic relationships and time-calibrated trees used here were presented in chap. 1. The details of the highest posterior density (HPD) for major clades are shown in Table 3.3. The analysis suggested a most recent common ancestor in the late Oligocene (~ 26.1 Ma) for

Angraecinae, and early linage diversification around 14.56 Ma (95% HPD: 9.9 – 19.2 Ma) for *Angraecum*. Most of the Angraecinae clades diverged from the early Miocene through the Pleistocene. Two waves of diversification were observed in *Angraecum*, during the Pliocene (~ 6 – 2.6 Ma) and during the Pleistocene (~ 2.6 – 0.2 Ma).

Table 3.3. Ages of crown nodes of relevant clades presented in the phylogeny of Angraecinae inferred from BEAST (from chap. 1; Appendix 8). Comparison between the mean time to most recent common ancestor (MRCA) estimates and the node height highest posterior density (HPD) intervals at 95%.

Angraecinae clades	**MRCA (node)**	**95% HPD (node)**
Outgroup	27.6	[18.2 – 34.8]
Angraecinae	26.1	[18.1 – 33.5]
Clade I	21	[14.6 – 27.8]
Clade II	17.1	[11.7 – 23.3]
Clade A	18	[12.1 – 24.5]
Clade B	19.4	[13.7 – 25.9]
Aeranthes and *Jumellea*	14.9	[9.2 – 19.9]
Aeranthes	10.3	[5.8 – 15.1]
Jumellea	7	[4.7 – 11]
Angraecum	15.5	[10.4 - 20,6]
sect. *Pseudojumellea*	8.7	[5 – 12.6]
sect. *Perrierangraecum*	9.7	[5.9 – 13.9]
sect. *Stellariangraecum*	5.7	[1.4 – 10.4]
sect. *Humblotiangraecum*	8.5	[5 – 11.9]
sect. *Hadrangis*	1.6	[0.3 – 3.2]
sect. *Africanae*	6.1	[2.7 – 9.7]
sect. *Angraecoides*	2.8	[1 – 4.9]
sect. *Angraecum*	2	[0.5 – 3.6]
sect. *Arachnangraecum*	9.2	[6.1 – 12]
sect. *Robusta*	6.1	[3.1 – 8.7]
sect. *Boryangraecum*	6.3	[3.8 – 8.8]

3.4.2. Habitat diversification

Results from MuSSE showed that model 5 received the best AIC score (Table 3.1), suggesting that the shifts from canopy epiphytes to undergrowth lithophytes or conversely, from canopy epiphytes to trunk epiphytes or conversely, from undergrowth lithophytes to trunk epiphytes or conversely, and from canopy epiphytes to inselberg lithophytes are the most probable habitat transitions among those tested here leading to diversification within *Angraecum* (Fig. 3.2A). Canopy epiphytes had the highest diversification rate (2.14e-01), followed by trunk lithophytes (2.12e-01) and undergrowth epiphytes (1.99e-01) that had similar rates (Table 3.4). The inselberg lithophytes diversification rate was negative (- 6.90e-01).

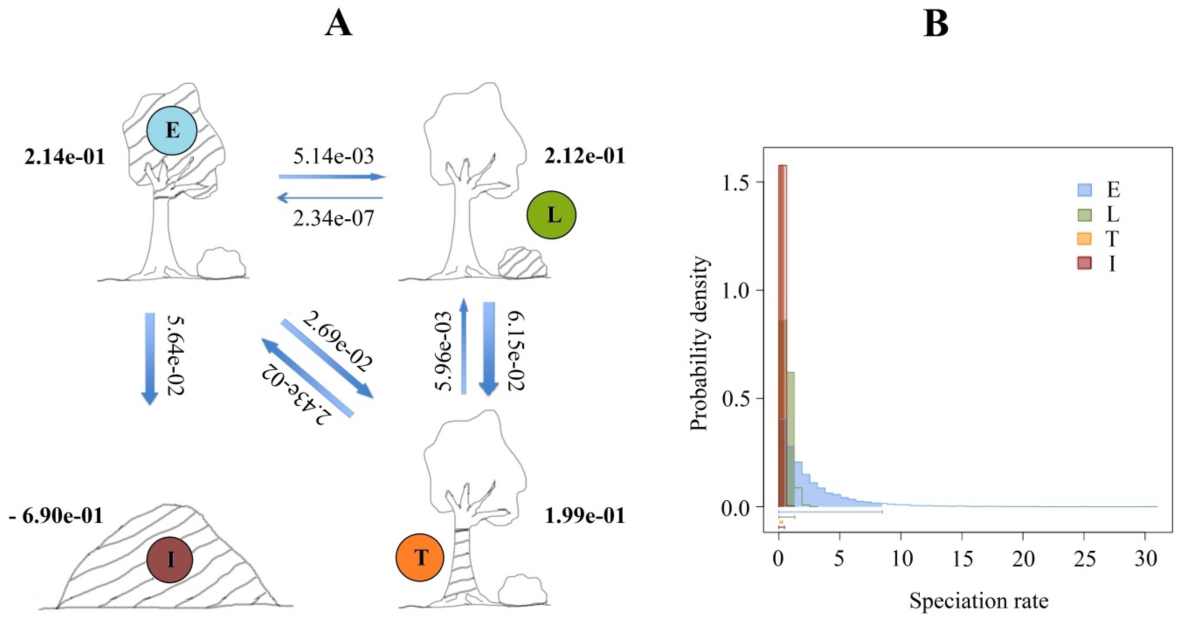


__Fig. 3.2.__ (**A**) Forest strata transition model selected in MuSSE. Arrows indicate direction of transitions; values next to arrows are transition rates; values in bold are diversification rates. E, canopy epiphytes; L, undergrowth lithophytes; T, trunk epiphytes; I, inselberg lithophytes. (**B**) Speciation rate of each character state.

Table 3.4. Diversification rates of the forest strata character states obtained from the best transition model (5) selected in MuSSE (Table 3.1).

Character states	**Code**	**speciation (*λ*)**	**extinction (*μ*)**	**diversification**
canopy epiphytes	E	2,14e-01	8,95e-08	2,14e-01
undergrowth lithophytes	L	1,99e-01	8,78e-08	1,99e-01
trunk epiphytes	T	2,12e-01	3,55e-08	2,12e-01
inselberg lithophytes	I	7,92e-08	6,90e-01	- 6,90e-01

3.4.3. Ancestral range reconstruction

A comparison of the different models used in the program BioGeoBEARS is presented in Table 3.5. Inclusion of founder-event speciation (DEC+J) in the ancestral area modelling resulted in a better AIC compared to the DEC model. The use of Mascarene and the Comoros Islands' ages as constraint in DECM1+J appears a logical optimization (species cannot reach the islands before they emerged), but did not yield a significantly better AIC when compared to both the DEC+J and DECM0+J models. A graphic representation of the biogeographical history under DECM1+J model is presented in Figures 3.3. Figure 3.3A illustrates the biogeographic history of tribe Vandeae using eight geographical areas. It suggested a Southeast Asian origin for the tribe, with a westward migration to the WIOR leading to a Malagasy origin of subtribe Angraecinae. Our analyses using finer subdivisions within Madagascar (Fig. 3.3B) yielded congruent results with a more detailed optimization for Angraecinae in Madagascar. It suggested a northeast Malagasy ancestral origin dated to the late Oligocene (~ 26.1 Ma) for subtribe Angraecinae, which started to diverge in the early Miocene (~ 21.1 Ma) to engender two major clades: clade I (African-Malagasy lineages) and clade II (Malagasy lineages). Clade I showed a northeast Malagasy origin and diverged to engender a Malagasy clade A at approximately 18.1 Ma, and an African clade B at approximately 19.5 Ma. Clade B showed E and W Africa as possible ancestral areas. The Malagasy clade II appears to have a northeast Malagasy origin and started to diversify to engender *Angraecum* at 14.6 Ma, *Aeranthes* at 10.7 Ma, and *Jumellea* at 7.8 Ma (Fig. 3.3B).

Table 3.5. Comparison of biogeographic models tested in BioGeoBEARS, bold represent selected model used for interpretation and discussions. Abbreviations: AIC, Akaike information criterion; AICwt, AIC weight; alt, alternative (model using parameter ***j***); null, strandard model; LnL, log likelihood; J, parameter ***j*** including founder effect; M: constrained model with differential dispersal rate and time slices; pval, *p*-values.

alt	null	LnLalt	LnLnul	pval	AIC1	AIC2	AICwt1	AICwt2
DEC+J	DEC	-707.4	-721.9	7.2e-08	1421	1448	1.00	1.4e-06
DECM0+J	DECM0	-586.3	-604.9	1.0e-09	1179	1214	1.00	2.2e-08
DECM1+J	DECM1	**-645.4**	-649.6	**0.0036**	**1297**	1303	**0.96**	0.038

In *Angraecum*, section *Pseudojumellea* appears to be the first to diverge within *Angraecum*. It diversified from the late Miocene through early Pleistocene. Ancestral area reconstruction suggested a northeast Madagascar origin for the section, with an expansion across Madagascar and the IOI during the Pliocene and Pleistocene. Section *Stellariangraecum* had a northeast origin and migrated west towards the central highland. The large section *Perrierangraecum* originated from northeast Madagascar and migrated to the center and later to the North. Section *Humblotiangraecum*, its sister section *Hadrangis* and section *Sobennikoffia* originated from the central highland. All sections with an uppermost labellum (*Africanae*, *Angraecum*, *Arachnangraecum*, *Boryangraecum*, *Nana*, and *Oeoniella*) originated from the northeast and colonized large ranges from Eastern Africa to WIOR and Sri Lanka.

Fig. 3.3. Ancestral area reconstructions of subtribe Angraecinae from DECM1+J analysis implemented in BioGeoBEARS: **A,** reconstruction using eight geographic areas (more detailed outside Madagascar); **B**, reconstruction using nine geographic areas (more detailed within Madagascar); dash lines indicate time slices. Areas within Madagascar: MdC, center; MdN, north; MdNe, northeast; MdSe, southeast; MdW, west. Areas outside Madagascar: AA, Africa/America/Asia; Am, South America; As, Southeast Asia; eA, east Africa; wA, west Africa; Cm, Comoros; Ms, Mascarenes; Se, Seychelles/Sri Lanka.

Fig. 3.3A. part 1

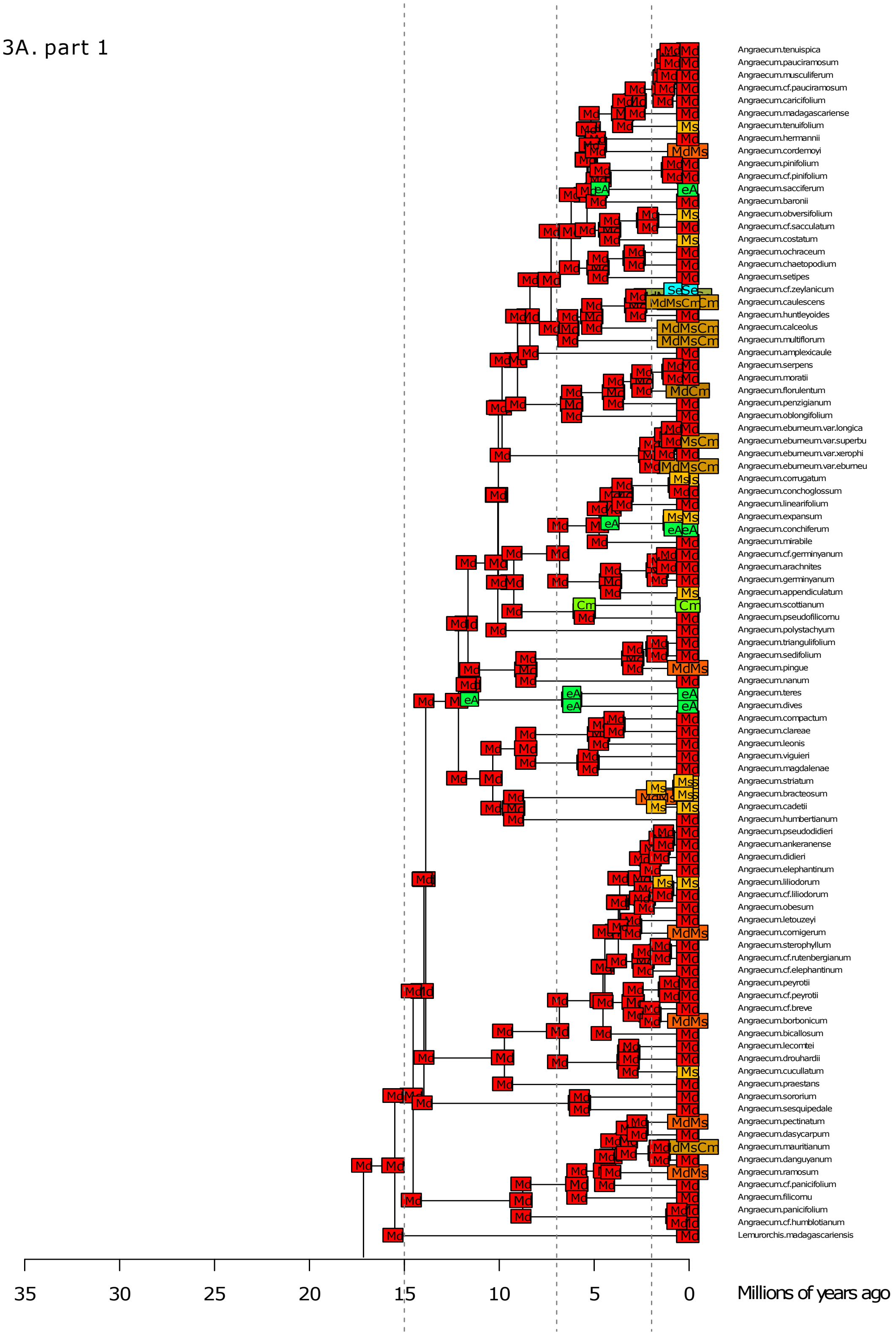


Fig. 3.3A. part 2

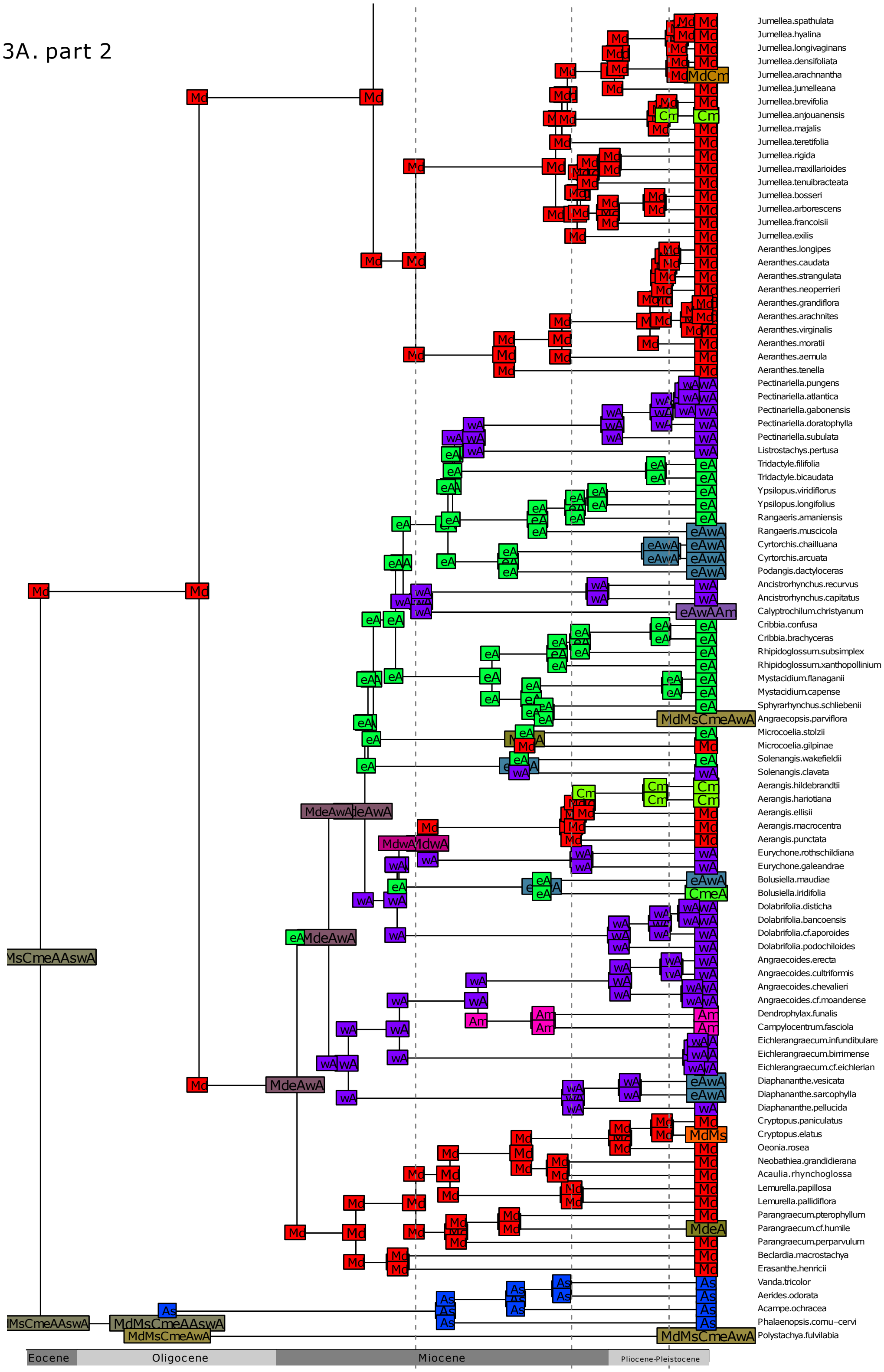


Fig. 3.3B. part 1

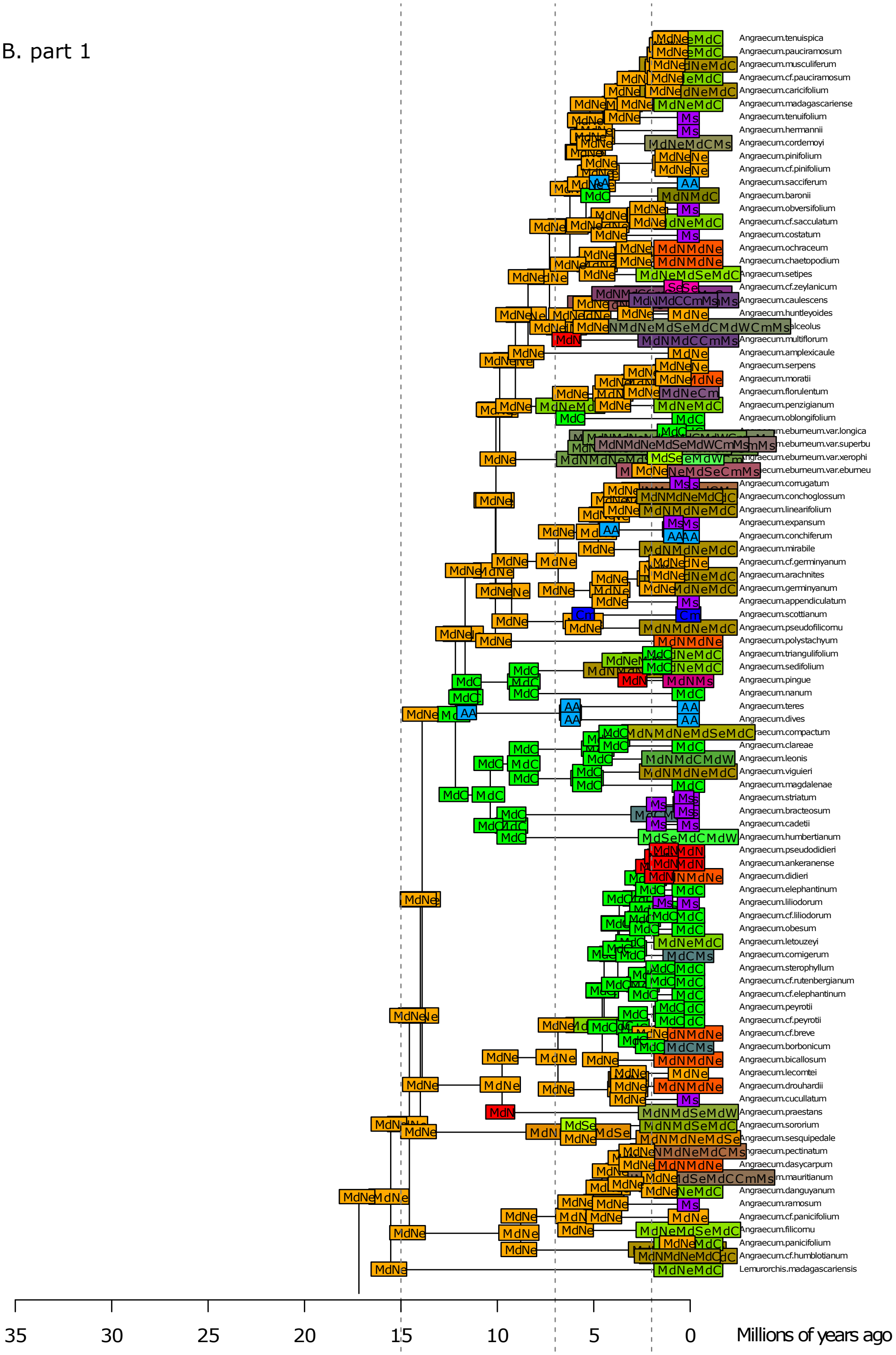
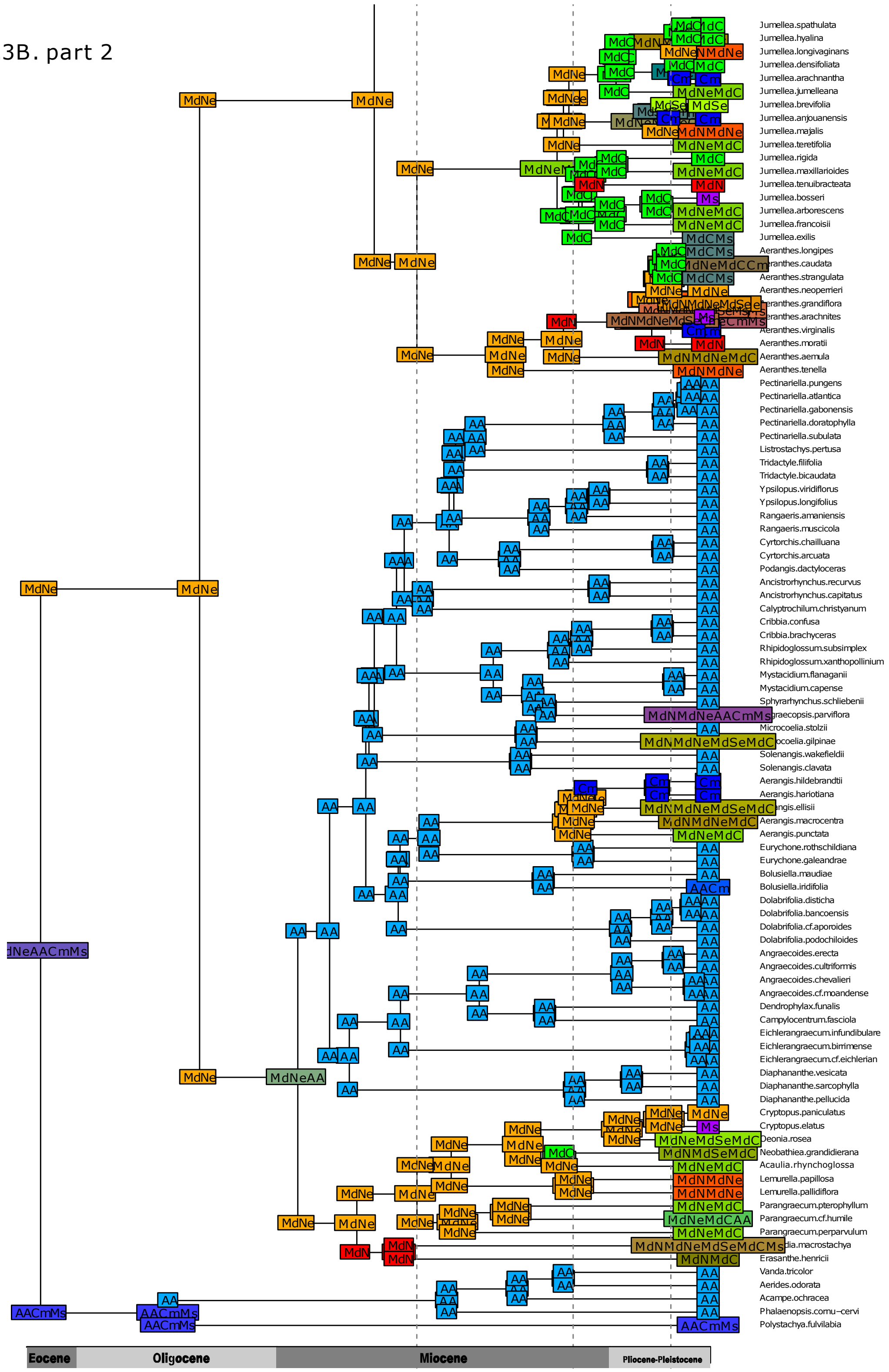


Fig. 3.3B. part 2



3.5. Discussion

3.5.1. Stratification changes and orchid diversification

Colonization of new habitats is known to drive species diversification in response to ecological opportunities (Reddy et al., 2012). The evolution of epiphytism, often associated with CAM photosynthesis, has been shown to be a key innovation leading to species diversification in the Orchidaceae (Givnish et al., 2015). Habitat change also has been demonstrated to be at the origin of diversification in subfamily Epidendroideae (Freudenstein and Chase, 2015), to which subtribe Angraecinae belongs. *Angraecum* is symplesiomorphically epiphytic, with over 95% of the species occupying that particular habitat. The shift from canopy epiphytes to undergrowth lithophytes and from canopy epiphytes to trunk epiphytes resulted in similar diversification rates in *Angraecum* (Fig. 3.2). Such shifts in the stratum occupied within the forest could be construed as changes in habitat and thus new habitat colonization events. The negative diversification rate for inselberg lithophytes could be the result of slow extinction (Donoghue and Sanderson, 2015) or an absence of speciation. These types of habitats are usually isolated and the species occupying them are highly specialized (e.g. *A. eburneum* var. *longicalcar*, *A. praestans*, *A. sororium*).

The colonization of new forest strata may expose the plants to new pollinator types, providing new evolutionary opportunities. It is possible that changes in strata may be associated with pollinator shifts. We have noticed during our field surveys that all species with green flowers (e.g. sections *Boryangraecum*) occupy exclusively the understory layer (trunk epiphytes), indicating a possible shift in pollinator. This could limit pollinator competition with species from other strata. To our knowledge, this is the first study to report the effect of changes in the strata occupied on orchid diversification and our results show that all strata appear to have contributed similarly to the diversification of *Angraecum*. Further studies are required (especially in Madagascar) to determine the pollinators of species adapted to canopy, trunk, or rock substrates.

3.5.2. Malagasy origin of subtribe Angraecinae

Our results suggest a Malagasy origin for subtribe Angraecinae in the early Miocene, with a probable source from sotheast Asia (Fig. 3.4). Using the ancestral area of Bremer (1992) to study the historical biogeography of Mascarene angraecoid orchids, Micheneau et al. (2008a) showed that Madagascar was potentially the ancestral area of the Angraecinae. Two major clades are observed in the Angraecinae, an African-Malagasy clade I with a Malagasy origin (Fig. 3.3A) that diverged in the late Miocene (~ 21 Ma), giving rise to mostly small Malagasy (clade A) and African (clade B) genera, and a Malagasy clade II that diverged later in the mid-Miocene (~ 17 Ma) and diversified into three large genera, *Angraecum* (~ 14 Ma), and *Aeranthes* (~ 10 Ma) sister to *Jumellea* (~ 7 Ma). Micheneau et al. (2008a) suggested an African origin for clade I, while our results support a Malagasy origin (Appendix 10). The Malagasy clade A has a northeast origin (Fig. 3.3B) and colonized the rest of the Island, except the western part. Northeast here refers to the northern half of the lowland tropical forests (eastern forests 0 – 800 m) of Humbert (1955). Two independent migrations, originating from northeast Madagascar are observed, one to east Africa during the Pliocene (~ 6.1 Ma) and one to the Mascarenes during the Pleistocene (~ 3.1 Ma). Several cases of out of Madagascar dispersal events have been proposed recently (e.g. Raxworthy et al., 2002; Stankiewicz et al., 2006; Krüger et al., 2012; Buerki et al., 2013), supporting our findings. Within the African Angraecinae clade B, no specific area has been found as a possible site of origin within Africa (east or west), putatively due to the limited sampling in our analysis. An increased sampling may help resolve the distribution history of Angraecinae within continental Africa. Nonetheless, a dispersal event that originated from western Africa during the late Miocene gave rise to the American Angraecinae (*Campylocentrum* and *Dendrophylax*) group. Furthermore, at least four secondary dispersals (essentially in *Aerangis*, *Angraecopsis*, *Bolusiella*, and *Microcoelia*) from continental Africa back to Madagascar and to the WIOR were observed during the Pliocene and Pleistocene (Fig. 3.3A; Fig. 3.4).

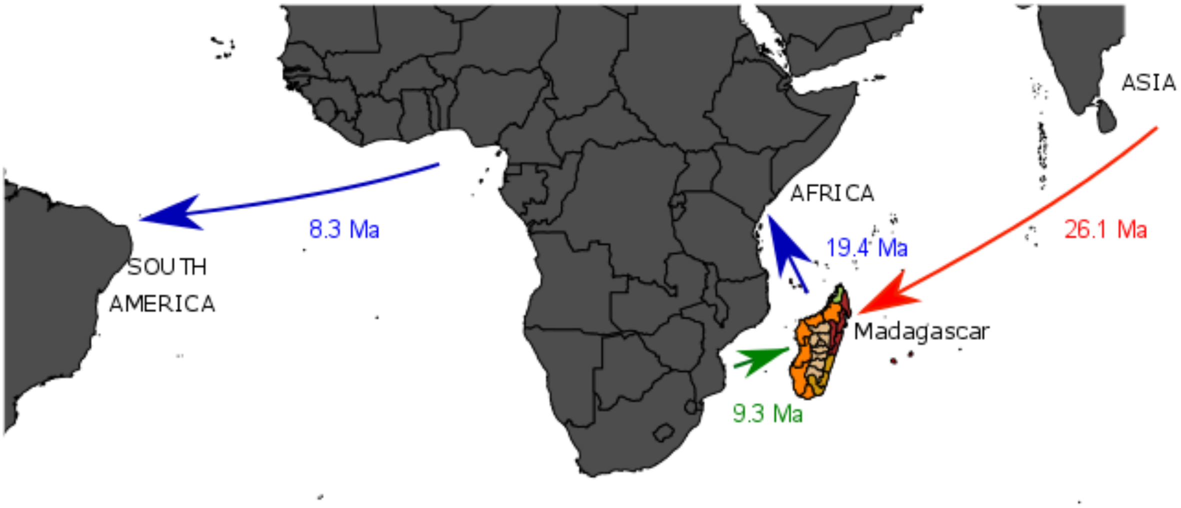


Fig. 3.4. Movements of dispersal in subtribe Angraecinae, arrows indicate the direction of the migration and numbers designate the age estimate of the events in million years. Red arrow indicates the early migration of subtribe Angraecinae from Southeast Asia towards Madagascar and Africa; blue arrows indicate the migration path of Angraecinae from Madagascar towards Africa then South America; green arrow denotes secondary migrations from Africa to Madagascar.

3.5.3. Origin of *Angraecum* and its dispersal within Madagascar

Our results suggest a Malagasy origin of *Angraecum* in the mid-Miocene. Two other Malagasy Angraecinae genera, *Aeranthes* and *Jumellea*, diversified as well during this period (Fig. 3.3B). The Miocene was characterized by the northern migration of Madagascar towards the equator, the establishment of the monsoon in the WIOR (Buerki et al., 2013), and the expansion of the Malagasy tropical forest, particularly its northern part (Yoder and Nowak, 2006; Buerki et al., 2013), which corresponds to our northeast region. These events were highlighted as the main diversification factors in *Angraecum* (chap. 1). The colonization of *Angraecum* within Madagascar started during the mid-Miocene and continued into the Pleistocene. The genus started to expand from northeast Madagascar (the northern half of the lowland humid tropical forest) and migrated to the central highland (highland tropical forest adjacent to the lowland forest of the northeast) during the late Miocene-early Pliocene. As in *Angraecum*, the northern tropical rain forest of Madagascar has been considered to be the point of origin of the expansion of many recent Malagasy taxa (Raxworthy and Nussbaum, 1995; Vences et al., 2009; Anthony et al., 2010; Rakotoarinivo et al., 2013). Two migrations to the West (*A. humbertianum* and *A. praestans*) originated from the center and the northeast, respectively, and occurred independently during the late Miocene (Fig. 3.3B). A great number of dispersal events within Madagascar happened independently during the Pliocene-Pleistocene, most of them originating from the northeast or the center. A similar pattern of dispersal is also observed in *Aeranthes* and *Jumellea* (Fig. 3.3B), though our data are incomplete there.

3.5.4. Out of Madagascar dispersal of *Angraecum*

Dispersal out of Madagascar happened during the late Miocene-early Pliocene to east Africa, during the early Pliocene to the Comoros, and during the Pleistocene to the Mascarenes, Seychelles and Sri Lanka (Fig. 3.5). In most cases, dispersal originated from the northeast or the center of Madagascar, and involved species that are still present on the island. A single speciation event in east Africa, leading to sect. *Africanae* (*A. divers* and *A. teres*), originated from central Madagascar during the late Miocene (Fig. 3.3B). At least two dispersal events (*A. conchiferum*, *A. sacciferum*) took place independently from northeast Madagascar to east Africa during the Pliocene. Additionally, two further east African species not included in our analyses probably originated from the center (*A. stella-africae*, a member of sect. *Perrierangraecum* related to *A. rutenbergianum*) and the northeast of Madagascar (*A. eburneum* var. *giryamae*, section *Angraecum*). The absence of *Angraecum* sensu stricto lineages in west Africa could be explained by the existence of a natural barrier between the two regions. Couvreur et al. (2008) reported that the Guineo-Congolian and east African regions are geographically isolated by a ca. 1000 km-wide north-south arid corridor, creating an effective barrier to dispersal for rainforest taxa. The isolation of the east African rain forests happened essentially during the Oligocene-Early Miocene (Couvreur et al., 2008) before the dispersion of *Angraecum*. Because uplift of the two archipelagos occurred in the late Miocene for the Comoros (Weyeneth et al. 2008) and in the Pliocene for the Mascarenes (Baksi and Hoffman, 2000), colonization of these islands could not have happened before these dates, which were used as time slice constraints in our analyses (Table 3.2). Dispersal to the Comoros happened several times starting in the early Pliocene and the majority during the Pleistocene (Fig. 3.3B). Most of the lineages found there originated from northeast Madagascar, and only a few endemic species are recorded (Govaerts et al., 2015). The single dispersal event observed to the Seychelles archipelago and Sri Lanka (*A.* cf. *zeylanicum*) may be a secondary migration from the Comoros that was reached first in the Pleistocene (Fig. 3.3B). Our results show that dispersal events from Madagascar to the Mascarenes occurred several times independently (Fig. 3.3B). The lineages came essentially from the northeast or the central highland of Madagascar during the Pleistocene. One dispersal event originating from the central highland of Madagascar during the Pleistocene, gave rise to the small radiation of the endemic section *Hadrangis*, characterized by the adoption of unusual pollination modes in *Angraecum* (Micheneau, 2005; Micheneau et al., 2009).

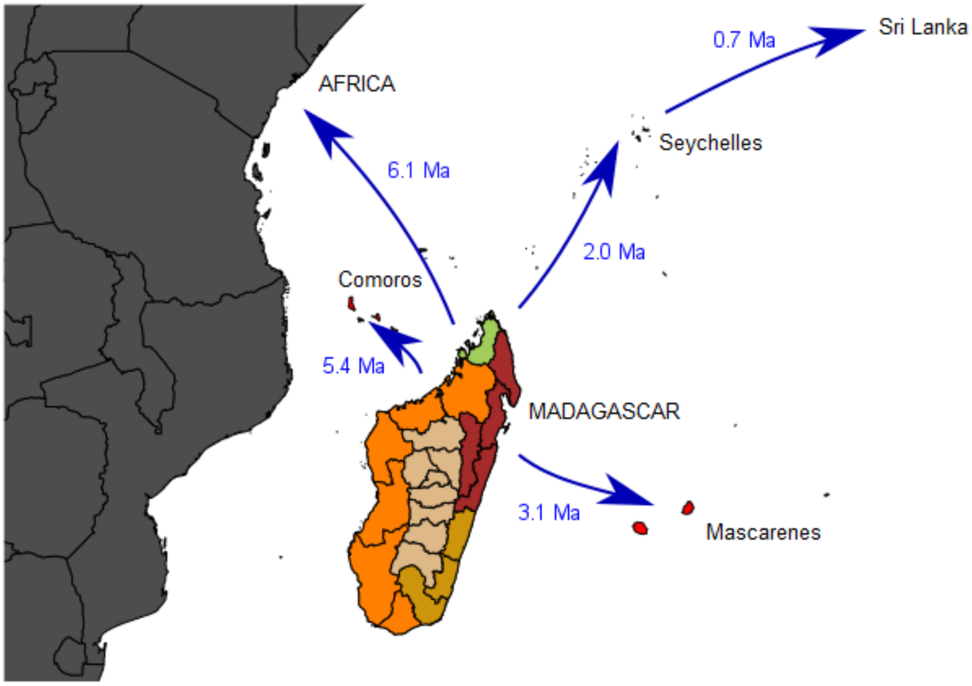


Fig. 3.5. Out of Madagascar dispersal of *Angraecum*, arrows indicate the direction of the migration and numbers designate the age estimate of the events in million years.

3.5.5. Dispersal mechanisms and colonization in *Angraecum*

The young age of the Angraecinae, approximately 26 Ma (chap. 1), cannot support hypotheses of vicariance, which would require a Cretaceous age in Madagascar, suggesting that long-distance dispersal was the main mechanism to explain area establishment in the group. In *Angraecum*, migration within or out of Madagascar could be explained by two major phenomena, the frequent passage of cyclones and prevailing winds in the region. Cyclones are accidental disturbances affecting each year the territory of Madagascar and the western Indian Ocean region, especially in summer (December to April). The average number of cyclones passing through Madagascar is estimated at 10 per season (Stankiewicz et al., 2006). Their origin lies in the clash of two drafts on the opposite front of the trade winds and the monsoon (Joly, 1941). These clashes occur only during the summer because in winter monsoon follows the same direction as the trade winds (Joly, 1941). During the cyclonic season, the wind simultaneously sweeps out several areas, shuffling the air in the entire region on its way. Cyclones are formed generally in the Indian Ocean, rarely in the Mozambique Channel. They go westward by the Mascarene Islands, pass the east coast of Madagascar, and continue northward to the Comoros islands or southward to end up in the Mozambique Channel. A return to the Indian Ocean is often observed. However, the storms rarely go eastwards over the Mozambique Channel, and none of them goes over Africa and returns to Madagascar (Stankiewicz et al., 2006). From the Early Miocene (~ 23 Ma), ocean currents and prevailing winds flowed from the Indian Ocean towards Africa, which was not the case during the Eocene (Rabinowitz and Stephen, 2006; Ali and Huber, 2010; Weyeneth et al., 2011).

Oceanic islands are known for their species diversity and high endemism (Goldberg et al., 2014, Goodman et al., 2015, Lewis et al., 2015), and isolation is considered to be the main reason (Weigelt and Kreft, 2013; Mallet et al., 2014). The two Indian Ocean archipelagos appear to be an expansion area for *Angraecum*, with approximately 45 species and 15 species recorded (Govaerts et al., 2015) from the Mascarenes and the Comoros, respectively. The uplift of these volcanic islands opened up new niches for colonization (Buerki et al. 2013), potentially driving diversification. Dispersal probability is expected to decrease with distance so that gene flow is generally lower between geographically distant populations causing a pattern of isolation-by-distance (Mallet et al., 2014). This could explain the diversification and high endemicity of the *Angraecum* species found in the Mascarenes (with one endemic section, *Hadrangis*) compared to other areas, essentially Africa and Comoros, which are half as far to Madagascar and harbour less species diversity. The differentiation of the new endemic lineages in the Mascarenes, essentially section *Hadrangis*, could be viewed as a founder-event speciation specific to this island. Jacquemyn et al. (2005) demonstrated the significant role of elevational gradients on the diversification of orchids in Reunion. Acharya et al. (2011) came to the same conclusion when studying Himalayan orchids. This could be explained by ecological similarities between Madagascar and Mascarene regions (Buerki et al. 2013). However, it has been reported that the Mascarenes have a greater affinity to the Malagasy biodiversity than do the Comoros (Buerki et al. 2013), suggesting that cyclones may have been more effective than prevailing winds in *Angraecum* dispersal.

3.6. Conclusion

The study of the historical biogeography of Angraecinae provides valuable insights into migration and colonization events in the Western Indian Ocean Region and Africa. Our results suggest an out of Madagascar dispersal for the subtribe during the Pliocene-Pleistocene. The origin of *Angraecum* is in Madagascar, more precisely the northeast of the island where the lowland wet tropical forest occurrs. Local colonization from the northeast to the central highland happened first during the late Miocene, followed by an expansion to the north and the southeast during the Pliocene-Pleistocene. Long-distance dispersal from the northeast or the central highland of Madagascar came later during the Pliocene-Pleistocene into two opposite directions: eastward to the Mascarenes and westward to the Comoros and Africa. The existence of natural barrier between the east and west tropical African forest may explain why *Angraecum* is restricted to east Africa, Comoros, Madagascar, Mascarene, Seychelles and Sri Lanka. Two main dispersal factors are suggested: cyclonic events and prevailing winds (trade wind and monsoon). Our results suggest that cyclones are likely more effective in orchid dispersal than prevailing winds. Nonetheless, the colonization and diversification of *Angraecum* in Archipelagos (Comoros and Mascarenes) reflects the importance of new habitat and niche similarities in species expansion. Changes of habitat (stratification) had no significant effect on diversification patterns in *Angraecum*. However, this change could be correlated with a pollinator shift, which requires further investigation to determine the mechanism undergone between strata.

3.7. Acknowledgements

We are very thankful to S. Joly (Montreal Botanical Garden), F.J. Lapointe, E. Gagnon (University of Montreal) and S. Renaut (Quebec Centre for Biodiversity Science) for their comments, assistance and expertise. This study was supported by Omaha's Henry Doorly Zoo and Aquarium, and the Madagascar Biodiversity Partnership.

Conclusion

Notre étude du genre *Angraecum* à Madagascar a permis d'évaluer plusieurs hypothèses, notamment la monophylie du genre *Angraecum* sensu stricto et l'exclusion des sections africaines sensu Garay. Toutefois, nous avons reconnu une nouvelle section endémique de l'Afrique de l'est (sect. *Africanae*). Nous avons aussi démontré que les caractères morphologiques sont impliqués dans la diversification d'*Angraecum*. Par contre, la diversification observée dans le genre *Angraecum* à Madagascar est le résultat d'une accumulation progressive des lignées et non pas d'une radiation adaptative rapide comme nous l'avions prédit. Finalement, nous avons pu montrer que l'ancêtre d'*Angraecum* s. s. est malgache et que la dispersion s'est effectuée de Madagascar vers d'autres régions.

Délimitation du genre *Angraecum* sensu stricto

Notre projet a permis d'accroître l'échantillonnage du genre *Angraecum* dans l'étude la plus exhaustive du groupe jamais réalisée, incluant près de 70% des espèces malgaches, dont la majorité jamais présentées dans des analyses phylogénétiques auparavant. Cet échantillonnage significatif a permis dans un premier temps de résoudre les problèmes liés à la systématique du groupe, entre autre le statut d'*Angraecum* comme genre monophylétique. Ceci a permis d'exclure définitivement la plupart des sections africaines qui étaient considérées appartenir au genre *Angraecum* sensu lato par bon nombre d'auteurs. Deuxièmement, la résolution des clades à l'intérieur d'*Angraecum* sensu stricto, maintenant monophylétique, a permis de réviser les sections proposées par Garay (1973). Cette révision fut rendue possible grâce à la concordance de nos données moléculaires avec les données morphologiques. La plus grande contribution de notre projet dans l'étude de la systématique du groupe a été la découverte d'un nouveau caractère, la position du labellum, supérieure ou inférieure selon les espèces, qui semble bien délimiter chaque section. Dans cette nouvelle classification, nous reconnaissons 14 sections, dont cinq sont nouvellement décrites: *Africanae*, *Oeoniella*, *Robusta*, *Sobennikoffia* et *Stellariangraecum*.

Notre étude a montré l’importance des convergences évolutives au sein de la sous-tribu des Angraecinae, qui jusqu’à maintenant étaient difficiles à observer avec seulement les données morphologiques, ce qui avait rendu difficile l’étude systématique du groupe. La reconnaissance de deux nouveaux genres malgaches, *Acaulia* et *Parangraecum*, témoignent de l’existence de caractères homoplasiques dans la sous-tribu. Par ailleurs, malgré la résolution de la phylogénie du genre *Angraecum* et la quantité de données morphologiques que nous avons généré, nous étions toujours incapables de définir le genre *Angraecum* sensu stricto correctement. Ceci est surtout dû aux caractères plésiomorphes qui réunissaient différents genres au sein des Angraecinae. D’autres études morphologiques beaucoup plus élargies, incluant plus d’espèces d'Angraecinae, seraient nécessaires afin de délimiter correctement le genre *Angraecum* et probablement d’autres genres appartenant à la sous-tribu.

Origine et diversité d’*Angraecum* à Madagascar

Notre étude sur la diversification des Angraecinae appuie l’hypothèse sur la diversification des plantes vasculaires des régions tropicales humides (Couvreur et al., 2011), qui résulterait d’une accumulation d'espèces à travers le temps plutôt que d’une radiation adaptative rapide. Notre étude confirme aussi une hypothèse concernant l’origine de la diversification des orchidées selon laquelle plusieurs caractères et états de caractères seraient responsables de la diversification des espèces (Freudenstein et Chase, 2015.) et non pas un seul trait ou encore moins un seul trait innovateur. Ceci renforce l’importance des données morphologiques dans les analyses macroévolutives. L’analyse intégrale de tous nos caractères morphologiques montre que beaucoup de caractères ont contribué à la diversification d’*Angraecum*, soit 22 des 39 caractères évalués (Annexe 11). Cette diversification serait en partie liée à l’expansion de la forêt tropicale humide qui a commencé dans la partie Nord Est de Madagascar au moment de l’établissement de la mousson pendant le Miocène. Cette expansion aurait été suivie d'une migration des espèces à l’intérieur de Madagascar, puis plus tard vers l’extérieur. La colonisation à l’intérieure de l’île s’est produite entre le Miocène et le début du Pliocène. Une migration vers les régions des hautes terres (forêts de montagne adjacentes à celles du Nord-Est) s'est produite vers la fin du Miocène, suivie plus tard de migrations indépendantes vers les autres régions de l'île vers le Pliocène-Pléistocène.

Notre étude suggère que Madagascar serait l'origine de la sous-tribu des Angraecinae, ainsi que du genre *Angraecum*. Ceci confirme bien notre hypothèse de départ. En effet, la présence des espèces d'*Angraecum* hors de Madagascar est le résultat d'événements de dispersion à longue distance entraînés soit par les vents dominants (alizée et mousson), qui soufflent dans la région ouest de l'Océan Indien, soit par les cyclones qui sévissent chaque année dans cette région. Le modèle de dispersion suit généralement le mouvement et la direction des vents: elle est de direction Est-Ouest pour les vents dominants, et de direction Est-Ouest ou inversement dans le cas des cyclones. L'expansion des espèces dans les îles voisines, entre autres les Comores et les Mascareignes, plus qu'ailleurs, pourrait être liée à l'âge récent de ces îles et aussi à l'affinité de leur composition floristique avec Madagascar.

Perspectives futures

Certes, notre étude aura permis de reconstruire la phylogénie d'*Angraecum* avec une meilleure résolution au moins au niveau des nœuds (les principaux clades ou sections), mais elle manque encore de résolution au niveau des espèces. Nous suggérons l'utilisation de marqueurs moléculaires autre que chloroplastiques pour avoir plus de support, d'une part, mais aussi pour valider la topologie que nous avons obtenue. A cause des problèmes liés aux champignons endophytes, nous avons eu de la difficulté à amplifier les régions nucléaires, entre autres ITS, pour beaucoup de nos échantillons, raison pour laquelle nous n'avons pas présenté ces résultats. De nouvelles recherches d'amorces plus spécifiques à notre groupe pourraient résoudre le problème en partie. Cependant, Carlsward et al. (2006) avaient rapporté que les séquences paralogues et orthologues sont fréquentes chez les Angraecinae malgaches (e.g. *Aeranthes*, *Angraecum*, *Jumellea*, etc.) pour la région ITS, ce qui nécessiterait beaucoup plus d'attention lorsqu'on fait les reconstructions phylogénétiques.

Étant donnée la diversification du genre *Angraecum* à Madagascar et dans les îles adjacentes, une étude sur les pollinisateurs pourrait fournir des informations importantes sur les mécanismes de spéciation, d'adaptation et/ou de colonisation de niche. En effet, plusieurs auteurs avancent que l'autopollinisation observée chez certaines espèces d'*Angraecum* qui se trouvent en dehors de Madagascar (Hermans et Hermans, 2013; Paillet et al., 2013) serait due à l'absence de pollinisateurs spécialisés. Cette absence aurait conduit à une nouvelle adaptation de la plante en vue de maintenir sa reproduction. Nous pensons que le changement de strates écologiques chez *Angraecum* pourrait être lié à un changement de pollinisateurs. Ceci concerne principalement les espèces à fleurs vertes qui se sont beaucoup diversifiées à Madagascar et dans les Mascareignes (ces espèces constituent plus de la moitié des espèces d'*Angraecum* dans l'île de la Réunion).

Afin de mieux définir le genre *Angraecum*, il serait recommandé d'examiner plus de caractères pour renforcer l'analyse des caractères morphologiques et anatomiques des Angraecinae. Des analyses morphométriques pourraient aussi être utile, notamment la mesure de la taille du gynostème ou de la longueur des stipes. Les travaux de Dressler (1996), Freudenstein et Rasmussen (2005), ou autres, sur les caractères morphologiques et anatomiques des orchidées doivent être appuyées pour une meilleure compréhension de l'histoire évolutive de la famille des Orchidaceae.

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Annexe 1. Voucher and sources of DNA data

Taxa, geographical origin, voucher information, and NCBI or BOLD accession numbers (*mat*K, *rps*16, *trn*L, ITS) for samples used in the study; sequences that are from different individuals are in italics with voucher information in brackets. Dashes (-) denote unavailable sequences, "unvouchered" are sequences from living collections used in previous studies (Carlsward et al., 2006; Micheneau et al., 2008a), asterisks (*) indicates newly generated sequences, and dagger (†) those with vouchers. Unvouchered specimens have been identified by H.N. Andriananjamanantsoa and J. Andriatiana (TAN). Abbreviations: Com, Comoros; Md, Madagascar; Reu, Reunion.

Acampe ochracea (Lindl.) Hochr., Asia, *Carlsward 206* (SEL), DQ091314, –, DQ091438, DQ091707; **Aerides odorata** Lour., Asia, *Chase 15081* (K), KF557954, KF558031, KF558231, –; **Aerangis ellisii** (B.S.Williams) Schltr., Md, *Chase 15080* (K), KF557992, KF558043, KF558204, *DQ091602* (Carlsward 401, FLAS); **Aerangis hariotiana** (Kraenzl.) P.J.Cribb & Carlsward, Com, *Carlsward 227* (FLAS), DQ091343, –, DQ091467, DQ091606; **Aerangis hildebrandtii** (Rchb.f.) P.J.Cribb & Carlsward, Com, Kew 2616 (K), DQ091344, –, DQ091468, DQ091608; **Aerangis macrocentra** (Schltr.) Schltr., Md, Hermans 779 (K), KF557923, –, KF558085, *DQ091601* (Kew 779, K); **Aerangis punctata** J.Stewart, Md, unvouchered (REU), KF557936, KF558040, KF558209, DQ091605; **Aeranthes aemula** Schltr., Md, *Andriananjamanantsoa & al. 00203471* (MT), KT826826*, KT826895*, KT826964*, –; **Aeranthes arachnites** (Thouars) Lindl., Md, *Fournel 126* (REU), KF557945, KF558036, KF558232, *DQ091759* (Carlsward 198, FLAS); **Aeranthes caudate** Rolfe, Md, *Chase 17918* (K), KF557929, KF558090, KF558206, –; **Aeranthes grandiflora** Lindl., Md, unvouchered (K), KF557958, KF558050, KF558158, *DQ091760* (Carlsward 238, FLAS); **Aeranthes longipes** Schltr., Md, *DuPuy 17* (K), KF557965, KF558094, KF558208, –; **Aeranthes moratii** Bosser, Md, *Chase 14643* (K), KF558024, KF558060, KF558225, –; **Aeranthes neoperrieri** Toill.Gen., Md, unvouchered (K), KF557953, KF558059, KF558199, –; **Aeranthes strangulata** Frapp. ex Cordem., Reu, *Pailler 100* (REU), KF558005, KF558097, KF558183, –; **Aeranthes tenella** Bosser, Reu, *Pailler 137* (REU), KF557997, KF558117, KF558162, –; **Aeranthes virginalis** D.L.Roberts, Com, *Chase 17901* (REU), KF557955, KF558071, KF558166, –; **Ancistrorhynchus capitatus** (Lindl.) Summerh., Africa, *Carlsward 276* (FLAS), DQ091351, –, DQ091475, DQ091643; **Ancistrorhynchus recurvus** Finet, Africa, unvouchered, DQ091354, –, DQ091478, DQ091646; **Angraecoides chevalieri** (Summerh.) Szlach., Africa, *Carlsward 208* (FLAS), AF506363, –, AF506339, AF506320; ***Angraecoides cultriformis*** (Summerh.) Szlach., Africa, *Carlsward 298* (FLAS) AF506364, –, AF506340, AF506321; ***Angraecoides erecta*** (Summerh.) Szlach., Africa, *Bytebier 801* (EA), DQ091323, –, DQ091447, DQ091566; ***Angraecoides*** **cf.** ***moandense*** De Wild., Africa, unvouchered (K), KF557944, KF558114, KF558214, –; ***Angraecopsis parviflora*** (Thouars) Schltr., Africa, *Hermans 4363* (Personal), KF557967, KF558046, KF558150, –; ***Angraecum amplexicaule*** Toill.Gen. & Bosser, Md, *Andriananjamanantsoa & al. 00203468* (MT), KT826791*, KT826861*, KT826929*, –; ***Angraecum ankeranense*** H.Perrier, Md, *Andriananjamanantsoa & al. 00203430* (MT), KT826802*, KT826872*, KT826940*, –; ***Angraecum appendiculatum*** Frapp. ex Cordem., Reu, *Pailler 107* (REU), KF557933, KF558130, KF558173, *DQ091752* (Kew 4232, K); ***Angraecum arachnites*** Schltr., Md, *Hermans 4241* (K), KF557990, KF558064, KF558250, –; ***Angraecum baronii***† (Finet) Schltr., Md, *Andriananjamanantsoa & al. 00203336* (MT), KT826787*, KT826857*, KT826925*, –; ***Angraecum borbonicum*** Bosser, Md, *Andriananjamanantsoa & al. 00203441* (MT), KT826808*, KT826878*, KT826946*, –; ***Angraecum bracteosum*** Balf.f. & S.Moore, Reu, *Micheneau 7* (REU), KF557998, KF558045, KF558266, –; ***Angraecum cadetii*** Bosser, Reu, *Pailler 157* (REU), KF557986, KF558122, KF558222; ***Angraecum calceolus*** Thouars, Md, *Andriananjamanantsoa & al. 00203470* (MT), KT826789*, KT826859*, KT826927*, –; ***Angraecum caricifolium***† H.Perrier, Md, *Andriananjamanantsoa & al. 00203338* (MT), KT826776*, KT826846*, KT826914*, –; ***Angraecum caulescens*** Thouars, Md, *Pailler 97* (REU), KF557988, –, KF558172, –; ***Angraecum*** **cf.** ***breve***† Schltr., Md, *Andriananjamanantsoa & al. 00203464* (MT), KT826803*, KT826873*, KT826941*, –; ***Angraecum*** **cf.** ***peyrotii*** Bosser, Md, *Andriananjamanantsoa & al. 00203426* (MT), KT826806*, KT826876*, KT826944*, –; ***Angraecum*** **cf.** ***elephantinum***† Schltr., Md, *Andriananjamanantsoa & al.* 00203323 (MT), KT826811*, KT826881*, KT826949*, –; ***Angraecum*** **cf.** ***germinyanum*** Hook.f., *Hermans 5540* (K), KF557973, KF558093, KF558203, –; ***Angraecum*** **cf.** ***humile***† Summerh., Md, *Andriananjamanantsoa & al. 00203453* (MT), KT826762*, KT826833*, KT826902*, –; ***Angraecum*** **cf.** ***pauciramosum*** Schltr., Md, *Andriananjamanantsoa & al. 00203436* (MT), KT826777*, KT826847*, KT826915*, –; ***Angraecum*** **cf.** ***humblotianum*** Schltr., Md, *Simo-Droissart & al. 2151* (BRLU), KF672272, KF672255, KF662345, –; ***Angraecum*** **cf.** ***liliodorum*** Frapp. ex Cordem., Md, *Andriananjamanantsoa & al. 00203472* (MT), KT826801*, KT826871*, KT826939*, –; ***Angraecum*** **cf.** ***panicifolium*** H.Perrier, Md, *Andriananjamanantsoa & al. 00203421* (MT), KT826816*, KT826885*, KT826954*, –; ***Angraecum*** **cf.** ***pinifolium***† Bosser, Md, *Andriananjamanantsoa & al. 00203456* (MT), KT826784*, KT826854*, KT826922*, –; ***Angraecum*** **cf.** ***rutenbergianum*** Kraenzl., Md, unvouchered (K), KF557938, KF558076, KF558218, *DQ091743* (Carlsward 300, FLAS);

Angraecum* cf. *sacculatum*†** Schltr., Md, *Andrianjamanantsoa & al. 00203387* (MT), KT826790*, KT826860*, KT826928*, –; ***Angraecum* cf. *zeylanicum Lindl., Sey, unvouchered (K), KF557956, KF558137, KF558230, –; ***Angraecum chaetopodium*** Schltr., Md, *Andrianjamanantsoa & al. 00203462* (MT), KT826786*, KT826856*, KT826924*, ANGMD356-11; ***Angraecum clareae*** Hermans, Md, *Hermans 3788* (K), KF557917, KF558049, KF558256, –; ***Angraecum compactum*** Schltr., Md, *Andrianjamanantsoa & al. 00203420* (MT), KT826796*, KT826866*, KT826934*, –; ***Angraecum conchiferum*** Lindl., Africa, *Bytebier 616* (EA), DQ091414, –, DQ091539, DQ091748; ***Angraecum conchoglossum*** Schltr., Md, *Micheneau 5* (REU), KF558027, –, KF558242, –; ***Angraecum cordemoyi*†** Schltr., Md, *Andrianjamanantsoa & al. 00203360* (MT), KT826782*, KT826852*, KT826920*, –; ***Angraecum cornigerum*†** Cordem., Md, *Andrianjamanantsoa & al. 00203340* (MT), KT826809*, KT826879*, KT826947*, –; ***Angraecum corrugatum*** (Cordem.) Micheneau, Reu, *Pailler 106* (REU), KF558003, KF558106, KF558194, *DQ091745* (Carlsward 391, FLAS); ***Angraecum costatum*** Frapp. ex Cordem., Reu, *Pailler 174* (REU), KF557942, KF558111, KF558180, –; ***Angraecum cucullatum*** Thouars, Reu, *Pailler 108* (REU), KF557927, KF558051, KF558245, –; ***Angraecum danguyanum*†** H.Perrier, Md, *Andrianjamanantsoa & al. 00203467* (MT), KT826818*, KT826887*, KT826956*, ANGMD305-11; ***Angraecum dasycarpum*†** Schltr., Md, *Andrianjamanantsoa & al. 00203450* (MT), KT826817*, KT826886*, KT826955*, –; ***Angraecum didieri*** (Baill. ex Finet) Schltr., Md, unvouchered (K), KF558029, KF558030, KF558213, –; ***Angraecum dives*** Rolfe, Africa, *Marimoto 42* (EA), DQ091422, –, DQ091547, DQ091756; ***Angraecum drouhardii*†** H.Perrier, Md, *Andrianjamanantsoa & al. 00203424* (MT), KT826799*, KT826869*, KT826937*, ANGMD044-11; ***Angraecum dryadum*†** Schltr., Md, *Andrianjamanantsoa & al. 00203341* (MT), KT826810*, KT826880*, KT826948*, –; ***Angraecum eburneum* var. *eburneum*** Bory, Reu, unvouchered (K), KF558000, KF558128, KF558261, *DQ091742* (Carlsward 182, FLAS); ***Angraecum eburneum* var. *superbum*†** (Thouars) H.Perrier, Md, *Andrianjamanantsoa & al. 00203463* (MT), KT826769*, KT826839*, KT826907*, *DQ091738* (Carlsward 282, FLAS); ***Angraecum eburneum* var. *xerophilum*** H.Perrier, Md, unvouchered (K), KF557935, KF558092, KF558253, –; ***Angraecum elephantinum*** Schltr., Md, *Carlsward 251* (FLAS), DQ091424, –, DQ091549, DQ091751; ***Angraecum expansum*** Thouars, Reu, *Micheneau 2* (REU), KF557974, KF558087, KF558151, –; ***Angraecum filicornu*†** Thouars, Md, *Andrianjamanantsoa & al. 00203460* (MT), KT826823*, KT826892*, KT826961*, ANGMD348-11; ***Angraecum florulentum*** Rchb.f., Com, unvouchered (K), KF557934, KF558088, KF558248, *DQ091741* (Carlsward 321, FLAS); ***Angraecum germinyanum*** Hook.f., Md, *Andrianjamanantsoa & al. 00203469* (MT), KT826765*, KT826835*, KT826904*, ANGMD254-11; ***Angraecum hermannii***

(Cordem.) Schltr., Md, unvouchered (REU), KF557970, KF558072, KF558159, –; ***Angraecum huntleyoides*** Schltr., Md, *Hermans 4248* (K), KF557961, KF558143, KF558191, –; ***Angraecum lecomtei*** H.Perrier, Md, *Andriananjamanantsoa & al. 00203440* (MT), KT826800*, KT826870*, KT826938*, ANGMD177-11; ***Angraecum leonis*** (Rchb.f.) Andre, Md, unvouchered (REU), KF557999, KF558116, KF558187, –; ***Angraecum letouzeyi*** Bosser, Md, *Andriananjamanantsoa & al. 00203429* (MT), KT826804*, KT826874*, KT826942*, –; ***Angraecum liliodorum*** Frapp. ex Cordem., Reu, unvouchered (REU), KF557982, KF558100, KF558153, –; ***Angraecum linearifolium*** Garay, Md, *Andriananjamanantsoa & al. 00203439* (MT), KT826767*, KT826837*, KT826906*, *KF672215* (Simo-Droissart & al. 2152, BRLU); ***Angraecum longicalcar*** (Bosser) Senghas, Md, unvouchered (K), KF558013, KF558052, KF558210, DQ091739; ***Angraecum madagascariense*** (Finet) Schltr., Md, *Andriananjamanantsoa & al. 00203448* (MT), KT826775*, KT826844*, KT826912*, –; ***Angraecum magdalenae*** Schltr. & H.Perrier, Md, unvouchered (K), KF557995, KF558123, KF558219, –; ***Angraecum mauritianum***† (Poir.) Frapp., Md, *Andriananjamanantsoa & al. 00203422* (MT), KT826815*, KT826884*, KT826953*, ANGMD039-11; ***Angraecum mirabile*** Schltr., Md, *Andriananjamanantsoa & al. 00203465* (MT), KT826766*, KT826836*, KT826905*, ANGMD374-11; ***Angraecum moratii*** Bosser, Md, *Andriananjamanantsoa & al. 00203447* (MT), KT826770*, KT826840*, KT826908*, –; ***Angraecum multiflorum*** Thouars, Reu, *Pailler 154* (REU), KF557948, KF558053, KF558240, –; ***Angraecum musculiferum*** H.Perrier, Md, *Andriananjamanantsoa & al. 00203432* (MT), KT826780*, KT826850*, KT826918*, –; ***Angraecum obesum*** H.Perrier, Md, *Hermans 2407* (K), KF558011, KF558054, KF558249, –; ***Angraecum oblongifolium*** Toill.Gen. & Bosser, Md, *Andriananjamanantsoa & al. 00203446* (MT), KT826771*, KT826841*, KT826909*, –; ***Angraecum obversifolium*** Frapp. ex Cordem., Reu, *Pailler 8* (REU), KF557962, KF558125, KF558259; ***Angraecum ochraceum*** (Ridl.) Schltr., Md, *Andriananjamanantsoa & al. 00203461* (MT), KT826785*, KT826855*, KT826923*, ANGMD354-11; ***Angraecum panicifolium*** H.Perrier, Md, *Andriananjamanantsoa & al. 00203444* (MT), KT826821*, KT826890*, KT826959*, ANGMD219-11; ***Angraecum pauciramosum*** Schltr., Md, *Andriananjamanantsoa & al. 00203445* (MT), KT826781*, KT826851*, KT826919*, –; ***Angraecum pectinatum***† Thouars, Md, *Andriananjamanantsoa & al. 00203473* (MT), KT826819*, KT826888*, KT826957*, *KF672211* (Simo-Droissart & al. 1684, BRLU); ***Angraecum penzigianum*** Schltr., Md, *Andriananjamanantsoa & al. 00203466* (MT), KT826772*, KT826842*, KT826910*, –; ***Angraecum perparvulum***† H.Perrier, Md, *Andriananjamanantsoa & al. 00203326* (MT), KT826764*, KT826834*, KT826903*, –; ***Angraecum peyrotii*** Bosser, Md, *Andriananjamanantsoa & al. 00203427* (MT), KT826813*, KT826882*, KT826951*, –; ***Angraecum pingue*** Frapp. ex Cordem., Md, *Andriananjamanantsoa & al. 00203451* (MT), KT826792*, KT826862*, KT826930*, –; **Angraecum pinifolium** Bosser, Md, *Andriananjamanantsoa & al. 00203425* (MT), KT826783*, KT826853*, KT826921*, –; **Angraecum praestans** Schltr., Md, *Andriananjamanantsoa & al. 00203419* (MT), KT826814*, KT826883*, KT826952*, ANGMD074-11; **Angraecum pseudodidieri** H.Perrier, Md, unvouchered (K), KF558002, KF558032, KF558200, –; **Angraecum pseudofilicornu**† H.Perrier, Md, *Andriananjamanantsoa & al. 00203435* (MT), KT826768*, KT826838*, –, –; **Angraecum pterophyllum**† H.Perrier, Md, *Andriananjamanantsoa & al. 00203443* (MT), KT826761*, KT826832*, KT826901*, ANGMD218-11; **Angraecum ramosum** Thouars, Reu, *Pailler 73* (REU), –, KF558037, KF558170, –; **Angraecum rhynchoglossum**† Schltr., Md, *Andriananjamanantsoa & al. 00203457* (MT), KT826763*, –, –, –; **Angraecum sacciferum** Lindl., Africa, *Bytebier 2226* (NBG), KF558028, KF558101, KF558181, –; **Angraecum scottianum** Rchb.f., Com, unvouchered (K), KF558017, KF558118, KF558163, *AB217521* (TBG102594); **Angraecum sedifolium**† Schltr., Md, *Andriananjamanantsoa & al. 00203396* (MT), KT826793*, KT826863*, KT826931*, –; **Angraecum serpens** (H.Perrier) Bosser, Md, *Andriananjamanantsoa & al. 00203438* (MT), KT826773*, KT826843*, KT826911*, –; **Angraecum sesquipedale** Thouars, Md, *Andriananjamanantsoa & al. 00203454* (MT), KT826824*, KT826893*, KT826962*, –; **Angraecum setipes**† Schltr., Md, *Andriananjamanantsoa & al. 00203358* (MT), KT826788*, KT826858*, KT826926*, ANGMD358-11; **Angraecum sororium**† Schltr., Md, *Andriananjamanantsoa & al. 00203400* (MT), KT826825*, KT826894*, KT826963*, –; **Angraecum sterophyllum**† Schltr., Md, *Andriananjamanantsoa & al. 00203398* (MT), KT826812*, –, KT826950*, –; **Angraecum striatum** Thouars, Reu, *Micheneau 4* (REU), KF557991, KF558058, KF558214, –; **Angraecum tenuifolium** Frapp. ex Cordem., Reu, *Pailler 116* (REU), KF557968, KF558035, KF558254, –; **Angraecum tenuispica** Schltr., Md, unvouchered (K), KF557950, KF558099, KF558189, –; **Angraecum teres** Summerh., Africa, *Bytebier 673* (EA), KF557969, KF558038, KF558198, DQ091755; **Angraecum triangulifolium** Senghas, Md, *Andriananjamanantsoa & al. 00203442* (MT), KT826794*, KT826864*, KT826932*, –; **Angraecum viguieri** Schltr., Md, *Andriananjamanantsoa & al. 00203423* (MT), KT826795*, KT826865*, KT826933*, –; **Angraecum nanum**† Frapp. ex Cordem., Md, *Andriananjamanantsoa & al. 00203325* (MT), KT826797*, KT826868*, KT826936*, –; **Beclardia macrostachya**† (Thouars) A.Rich., Md, *Andriananjamanantsoa & al. 00203322* (MT), KT826827*, KT826896*, –, –; **Bolusiella iridifolia** (Rolfe) Schltr., Africa, *Bytebier 1113* (EA), DQ091356, –, DQ091481, DQ091665; **Bolusiella maudiae** (Bolus) Schltr., Africa, *Bytebier 485* (EA), DQ091355, –, DQ091480, DQ091664; **Calyptrochilum christyanum** (Rchb.f.) Summerh., Africa, *Carlsward 194* (SEL), DQ091325, –, DQ091449, DQ091668; **Campylocentrum fasciola**

(Lindl.) Cogn., America, *Carlsward 301* (FLAS), DQ091321, –, DQ091445, DQ091564; ***Cribbia brachyceras*** (Summerh.) Senghas, Africa, *Bytebier 361* (EA), DQ091365, –, DQ091490, DQ091577; ***Cribbia confusa*** P.J.Cribb, Africa, *Kew 3936* (K), DQ091366, –, DQ091491, DQ091578; ***Cryptopus elatus*** (Thouars) Lindl., Reu, *Micheneau 6* (REU), KF558019, KF558063, KF558221, *DQ091585* (Carlsward 403, FLAS); ***Cryptopus paniculatus*** H.Perrier, Md, *Hermans 5392* (K), KF557963, KF558113, KF558264, *DQ091588* (Kew 5392, K); ***Cyrtorchis arcuata*** (Lindl.) Schltr., Africa, *Bytebier 676* (EA), DQ091380, –, DQ091505, DQ091624; ***Cyrtorchis chailluana*** (Hook.f.) Schltr., Africa, *Carlsward 156* (SEL), DQ091381, –, DQ091506, DQ091625; ***Dendrophylax funalis*** (Sw.) Benth. Ex Rolfe, America, *Carlsward 302* (FLAS), KF557981, KF558067, KF558238, *AF506310* (Whitten 1935, FLAS); ***Diaphananthe pellucida*** (Lindl.) Schltr., Africa, *Carlsward 241* (FLAS), DQ091377, –, DQ091502, DQ091620; ***Diaphananthe sarcophylla*** (Schltr. ex Prain) P.J.Cribb & Carlsward, Africa, *Bytebier 339* (EA), DQ091378, –, DQ091503, DQ091621; ***Diaphananthe vesicata*** (Lindl.) P.J.Cribb & Carlsward, Africa, *Chase 14646* (K), KF557976, KF558083, KF558205, *DQ091623* (Kew 399, K); ***Dolabrifolia bancoensis*** (Burg) Szlach. & Romowicz, Africa, *Simo-Droissart & al. 54* (BRLU), KF672280, KF672257, KF662335, –; ***Dolabrifolia* cf. *aporoides*** (Summerh.) Szlach., Africa, *Simo-Droissart & al. 15* (BRLU), KF672266, KF672230, KF662332, –; ***Dolabrifolia disticha*** (Lindl.) Szlach., Africa, *Simo-Droissart & al. 6* (BRLU), KF672265, KF672231, KF662348, –; ***Dolabrifolia podochiloides*** (Schltr.) Szlach., Africa, *Simo-Droissart & al. 12* (BRLU), KF672281, KF672238, KF662330, –; ***Eichlerangraecum birrimense*** (Rolfe) Szlach., Africa, *Smith 531* (K), KF557930, KF558098, KF558196, –; ***Eichlerangraecum* cf. *eichlerianum*** (Kraenzl.) Szlach., Africa, unvouchered (K), KF557978, KF558069, KF558263, *AF506322* (Carlsward 284, FLAS); ***Eichlerangraecum infundibulare*** (Lindl.) Szlach., Africa, unvouchered (K), KF557977, KF558077, KF558243, –; ***Erasanthe henricii*** (Schltr.) P.J.Cribb, Hermans & D.L.Roberts, Africa, unvouchered (K), –, KF558102, KF558175, –; ***Eurychone galeandrae*** (Rchb.f.) Schltr., Africa, *Carlsward 293* (FLAS), DQ091349, –, DQ091473, DQ091614; ***Eurychone rothschildiana*** (O'Brien) Schltr., Africa, *Carlsward 407* (FLAS), DQ091350, –, DQ091474, DQ091615; ***Jumellea anjouanensis*** (Finet) H.Perrier, Com, *Bryonnaud 62* (REU), –, KF558120, KF558152, –; ***Jumellea arachnantha*** (Rchb.f.) Schltr., Md, *Rakotoarivelo & al. 042* (REU), JQ905335, JQ905457, JQ905518, JQ905396; ***Jumellea arborescens*** H.Perrier, Md, unvouchered (K), KF557949, KF558095, KF558262, –; ***Jumellea bosseri*** Pailler, Reu, *Pailler 270* (REU), JQ905340, JQ905462, JQ905523; ***Jumellea brevifolia*** H.Perrier, Md, *Rakotoarivelo & al. 300* (TAN), JQ905342, JQ905464, JQ905525, JQ905401; ***Jumellea densifoliata*** Senghas, Md, *Hermans 2809* (K), KF557966, KF558041, –, *JQ905405* (Rakotoarivelo & al. 109, TAN); ***Jumellea exilis*** (Cordem.)

Schltr., Reu, *Pailler 25* (REU), KF558014, KF558048, KF558169, –; ***Jumellea francoisii*** Schltr., Md, *Rakotoarivelo & al. 230* (TAN), JQ905350, JQ905472, JQ905533, JQ905411; ***Jumellea hyalina*** H.Perrier, Md, *Rakotoarivelo & al. 006* (TAN), JQ905354, JQ905476, JQ905537, JQ905415; ***Jumellea jumelleana*** (Schltr.) Summerh., Md, *Rakotoarivelo & al. 099* (TAN), JQ905356, JQ905478, JQ905539, JQ905417; ***Jumellea longivaginans*** H.Perrier, Md, *Rakotoarivelo & al. 328* (TAN), JQ905362, JQ905484, JQ905545, JQ905423; ***Jumellea majalis*** (Schltr.) Schltr., Md, *Rakotoarivelo & al. 307* (TAN), JQ905366, JQ905488, JQ905549, JQ905427; ***Jumellea maxillarioides*** (Ridl.) Schltr., Md, unvouchered (K), KF558004, KF558134, KF558161, –; ***Jumellea rigida*** Schltr., Md, *Rakotoarivelo & al. 220* (TAN), JQ905376, JQ905498, JQ905559, JQ905437; ***Jumellea spathulata*** (Ridl.) Schltr., Md, *Rakotoarivelo & al. 209* (TAN), JQ905380, JQ905502, JQ905563, JQ905441; ***Jumellea tenuibracteata*** (H.Perrier ex Hermans) F.P.Rakotoar. & Pailler, Md, *Rakotoarivelo & al. 321* (TAN), JQ905360, JQ905482, JQ905543, –; ***Jumellea teretifolia*** Schltr., Md, *Rakotoarivelo & al. 160* (TAN), JQ905386, JQ905508, JQ905568, JQ905447; ***Lemurella pallidiflora*** Bosser, Md, *Andriananjamanantsoa & al. 00203431* (MT), KT826828*, KT826897*, KT826965*, –; ***Lemurella papillosa*** Bosser, Md, *Andriananjamanantsoa & al. 00203434* (MT), KT826829*, KT826898*, KT826966*, –; ***Lemurorchis madagascariensis*** Kraenzl., Md, *Kew 5383* (K), DQ091431, –, DQ091556, DQ091747; ***Listrostachys pertusa*** (Lindl.) Rchb.f., Africa, *Carlsward 399* (FLAS), DQ091384, –, DQ091509, DQ091637; ***Microcoelia gilpinae*** (Rchb.f. & Moore) Summerh., Md, *Carlsward 290* (FLAS), DQ091397, –, DQ091522, DQ091649; ***Microcoelia stolzii*** (Schltr.) Summerh., Africa, *Carlsward 287* (FLAS), DQ091405, –, DQ091530, DQ091657; ***Mystacidium capense*** (L.f.) Schltr., Africa, *Whitten 1781* (FLAS), DQ091362, –, DQ091487, DQ091573; ***Mystacidium flanaganii*** (Bolus) Bolus, Africa, *Kew 5084* (K), DQ091363, –, DQ091488, DQ091574; ***Neobathiea grandidierana*** (Rchb.f.) Garay, Md, *Carlsward 395* (FLAS), DQ091329, –, DQ091453, DQ091589; ***Oeonia rosea*†** Ridl., Md, *Andriananjamanantsoa & al. 00203385* (MT), KT826830*, KT826899*, –, DQ091736 (Carlsward 221, FLAS); ***Oeoniella polystachys*†** (Thouars) Schltr., Md, *Andriananjamanantsoa & al. 00203455* (MT), KT826831*, KT826900*, –, –; ***Pectinariella atlantica*** Stevart & Droissart, Africa, *Simo-Droissart & al. 2065* (BRLU), KF672274, KF672235, KF662349, –; ***Pectinariella doratophylla*** (Summerh.) Szlach., Africa, *Simo-Droissart & al. 1466* (BRLU), KF672261, KF672247, KF662351, –; ***Pectinariella gabonensis*** (Summerh.) Szlach., Africa, *Simo-Droissart & al. 1468* (BRLU), KF672279, KF672237, KF662333, –; ***Pectinariella pungens*** (Schltr.) Szlach., Africa, *Simo-Droissart & al. 1464* (BRLU), KF672278, KF672254, KF662329, –; ***Pectinariella subulata*** (Lindl.) Szlach., Africa, *Simo-Droissart & al. 1474* (BRLU), KF672285, KF672251, KF662355, –; ***Phalaenopsis cornu-cervi*** (Breda) Blume & Rchb.f., Asia, *Chase O-1356*

(K), KF558008, KF558082, –, –; ***Podangis dactyloceras*** (Rchb.f.) Schltr., Africa, *Kew 4999* (K), DQ091385, –, DQ091510, DQ091628; ***Polystachya fulvilabia*** Schltr., Africa, *Chase 17862* (K), KF558010, KF558057, KF558235, GU556680; ***Rangaeris amanuensis*** (Kraenzl.) Summerh., Africa, *Bytebier & Kirika 26* (EA), DQ091386, –, DQ091512, –; ***Rangaeris muscicola*** (Rchb.f.) Summerh., Africa, *Carlsward 169* (SEL), DQ091387, –, DQ091513, DQ091630; ***Rhipidoglossum subsimplex*** (Summerh.) Garay, Africa, *Bytebier 546* (EA), DQ091371, –, DQ091496, DQ091580; ***Rhipidoglossum xanthopollinium*** (Rchb.f.) Schltr., Africa, *Carlsward 384* (FLAS), DQ091370, –, DQ091495, DQ091582; ***Sobennikoffia humbertiana*** H.Perrier, Md, Kew 3044 (K), DQ091433, –, DQ091558, DQ091750; ***Solenangis clavata*** (Rolfe) Schltr., Africa, *Carlsward 397* (FLAS), DQ091409, –, DQ091534, DQ091666; ***Solenangis wakefieldii*** (Rolfe) P.J.Cribb & J.Stewart, Africa, *Bytebier 627* (EA), DQ091410, –, DQ091535, DQ091667; ***Sphyrarhynchus schliebenii*** Mansf., Africa, *Bytebier 393* (EA), DQ091359, –, DQ091484, –; ***Tridactyle bicaudata*** (Lindl.) Schltr., Africa, *Bytebier 348* (EA), DQ091388, –, DQ091514, DQ091638; ***Tridactyle filifolia*** (Schltr.) Schltr., Africa, *Bytebier 707* (EA), DQ091390, –, DQ091516, DQ091641; ***Vanda tricolor*** Lindl., Africa, *Chase 17970* (K), KC823021, –, KC985407, –; ***Ypsilopus longifolius*** (Kraenzl.) Summerh., Africa, *Bytebier 609* (EA), DQ091393, –, DQ091519, DQ091636; ***Ypsilopus viridiflorus*** P.J.Cribb & J.Stewart, Africa, *Bytebier 402* (EA), DQ091395, –, –, DQ091633.

Annexe 2. Morphological character description

Description of vegetative and floral morphological characters in Angraecinae

N°	Characters	Character states		Description of states	References
1	**habitat**	**1**	terrestrial	growing on the ground	Beentje, 2010
		2	lithophytic	growing on rock	Beentje, 2010
		3	epiphytic	growing on and attached to another plant without deriving nourishment from it	Beentje, 2010
2	**habit**	**1**	pendent	hanging	Beentje, 2010
		2	horizontal	parallel to the substrate	
		3	vertical	perpendicular to the substrate	
		4	erect	uprigth	Beentje, 2010
		5	climber	growing upwards by attaching itself to other structures which It uses as supports (tree or rock)	Beentje, 2010
3	**natural spread (height)**	**1**	tiny	0 cm to 3 cm	
		2	small	3 cm to 5 cm	
		3	medium	5 cm to 15 cm	
		4	large	15 cm to 35	
		5	very-large	> 35 cm	
		6	N/A	not applicable	
4	**natural spread (width)**	**1**	tiny	0 cm to 3 cm	
		2	small	3 cm to 5 cm	
		3	medium	5 cm to 15 cm	
		4	large	15 cm to 35	
		5	very-large	> 35 cm	
		6	N/A	not applicable	
5	**internode**	**1**	very-short	L < 0.3 cm	
		2	short	1 cm < L < 0.4 cm	
		3	medium	4 cm < L < 2 cm	
		4	long	L > 5 cm	
		5	N/A	not applicable	
6	**diameter of stem**	**1**	tiny	d < 0.2 cm	
		2	small	0.5 cm < d < 0.3 cm	
		3	medium	1.2 cm < d < 0.6 cm	
		4	large	d > 1.3 cm	
		5	N/A	not applicable	

7	**form of leaf**	**1**	acicular	needle-shaped; very narrow, stiff, and pointed	Beentje, 2010
		2	cylindrical	long and narrow with circular cross-section	Beentje, 2010
		3	linear	narrow and much longer than wide, with parallel margins	Beentje, 2010
		4	lanceolate	narrowly ovate and tapering to a point at the apex	Beentje, 2010
		5	oblong	longer than broad, with the margins parallel for most of their length (1.5 - 2 x as long as wide)	Beentje, 2010
		6	elliptic	broadest at the middle with two equal rounded ends, width ratio 1.5 - 2	Beentje, 2010
		7	triangular		
		8	N/A	not applicable	
8	**apex of leaf**	**1**	obtuse	not pointed, blunt, ending in an angle of between 90 - 180°	Beentje, 2010
		2	attenuate	gradually narrowing over a long distance	Beentje, 2010
		3	emarginate	with a distinct sharp notch, both ends not at the same level	Beentje, 2010
		4	incised	having straight to irregular lines of separation extending inward from the margin	Kiger & Porter, 2001
		5	N/A	not applicable	
9	**texture of leaf**	**1**	herbaceous	with the texture of a herb, soft and pliable	Beentje, 2010
		2	membraneous	like a membran, flexible and thin	Beentje, 2010
		3	fleshy	swollen largely because of high water content	Beentje, 2010
		4	succulent	thick, fleshy and swollen	Beentje, 2010
		5	coriaceous	moderately thick, and tough	Kiger & Porter, 2001
		6	fibrous	composed of fibers	Beentje, 2010
		7	N/A	not applicable	

10	**leaf-surface**	**1**	smooth	even or unrelieved overall	Kiger & Porter, 2001
		2	rugose	covered in reticulate lines, with the spaces in between convex	Beentje, 2010
		3	N/A	not applicable	
11	**numbre of leaves**	**1**	few	0 to 5	
		2	medium	5 to 10	
		3	many	10 to 25	
		4	dense	> 25	
		5	N/A	not applicable	
12	**leaf-width**	**1**	filiform	0 to 2 mm	
		2	very-narrow	3 to 5 mm	
		3	narrow	6 to 10 mm	
		4	medium	11 to 25 mm	
		5	large	26 to 40 mm	
		6	N/A	not applicable	
13	**leaf-length**	**1**	very-short	0 to 3 cm	
		2	short	3 to 7 cm	
		3	medium	7 to 15 cm	
		4	long	15 to 30 cm	
		5	very-long	> 30 cm	
		6	N/A	not applicable	
14	**type of inflorescence**	**1**	solitary type 1	1 to 3 solitary flowers	
		2	solitary type 2	more than 3 solitary flowers	
		3	raceme type 1	1 to 3 raceme bearing up to 5 flowers each	
		4	raceme type 2	more than 3 racemes bearing up to 5 flowers each	
		5	raceme type 3	1 to 3 raceme bearing more than 5 flowers each	
		6	raceme type 4	more than 3 racemes bearing more than 5 flowers each	
		7	N/A		
15	**length of inflorescence**	**1**	short	almost sessile	
		2	medium	1/3 of the size of the plant	
		3	long	1/2 of the size of the plant	
		4	very-long	longer than the plant	
16	**length of pedicel**	**1**	short	1/4 of the length of ovary	
		2	medium	1/2 of the length of ovary	
		3	long	same length as the ovary	

		4	very-long	longer than the ovary	
17	**position of inflorescence**	**1**	terminal	ending the axis	Beentje, 2010
		2	subterminal	lateral and just below the apex	Kiger & Porter, 2001
		3	suprafoliar	upon the stem directly above a leaf insertion	Kiger & Porter, 2001
		4	infrafoliar	upon the stem directly below a leaf insertion	Kiger & Porter, 2001
		5	N/A	not applicable	
18	**size of flower**	**1**	tiny	labellum (L) less than 0.3 cm (spur excluded)	
		2	small	0.4 cm < L < 0.9 cm	
		3	medium	2 cm < L < 1.9 cm	
		4	large	5 cm < L < 2 cm	
		5	very-large	L > 5 cm	
19	**form of sepal**	**1**	ovate	egg shaped (2-dimensional), about 1.5x as long as broad, with the wider part below the middle	Beentje, 2010
		2	linear		
		3	lanceolate		
		4	tubular	cylindrical and hollow	Beentje, 2010
		5	obovate	egg-shaped with the broadest part near the apex	Beentje, 2010
		6	elliptic		
20	**color of sepal**	**1**	green to yellowish		
		2	white to white green		
		3	ocher		
		4	other		
21	**apex of sepal**	**1**	acute	sharp, sharply pointed, the margins near the tip being almost straight and forming an angle of < 90°	Beentje, 2010
		2	attenuate		
		3	obtuse		
22	**form of petal**	**1**	ovate		
		2	linear		
		3	lanceolate		

		4	tubular		
		5	obovate		
		6	elliptic		
		7	N/A		
23	**color of petal**	**1**	green to yellowish		
		2	white to white green		
		3	ocher		
		4	other		
24	**apex of petal**	**1**	acute		
		2	attenuate		
		3	obtuse		
		4	lobed		
25	**orientation of dorsal sepal**	**1**	straight		
		2	inflexed	bent or curved inwards	Beentje, 2010
		3	reflexed	curved backwards	Beentje, 2010
26	**orientation of lateral sepal**	**1**	straight		
		2	inflexed		
		3	reflexed		
27	**orientation of lateral petal**	**1**	straight		
		2	inflexed		
		3	reflexed		
28	**2D form of labellum**	**1**	lanceolate		
		2	ovate		
		3	orbicular		
		4	elliptic		
		5	rhombic		
		6	trullate		
		7	obovate		
		8	linear		
29	**3D form of the labellum**	**1**	plane		
		2	scoop-shaped type 1	slightly concave from the base to the tip	
		3	scoop-shaped type 2	very concave from the base to the tip	
		4	navicular		
		5	infundibular		
		6	gibbous		
30	**position of labellum**	**0**	uppermost		
		1	lowermost		

31	**color of labellum**	**1**	greenish to yellowish	
		2	white to white green	
		3	other	
32	**apex of labellum**	**1**	round/obtuse	
		2	acute	
		3	attenuate	
		4	caudate	
		5	lobed	
		6	waved	
		7	dentate	
33	**texture of flower**	**1**	thin	
		2	thick	
		3	fleshy	
		4	succulent	
34	**spur-length**	**1**	very-short	L < 0.5 cm
		2	short	L < 3 cm
		3	medium	L < 7 cm
		4	long	L < 15 cm
		5	very-long	L > 15 cm
35	**form of spur**	**1**	straight	
		2	arcute type 1	arched at median (bow-like)
		3	arcute type 2	arched at the base (club-like)
		4	arcute type 3	very arched at median (swan neck-like)
		5	sigmoid	
		6	circinnate	
		7	N/A	not applicable
36	**base of spur**	**1**	isodiametric	same diameter form the base to the tip
		2	tapering type 1	large at the base, and become gradually isodiametric towards the tip
		3	tapering type 2	very large at the base, and become abruptley isodiametric towards the tip
		4	tapering type 3	very large at the base, and become narrow gradually towards the tip
		5	N/A	not applicable

37	**color of spur**	**1**	green to yellowish		
		2	white to white green		
		3	ocher		
		4	other		
		5	N/A	not applicable	
38	**apex of spur**	**1**	acute		
		2	obtuse		
		3	N/A	not applicable	
39	**orientation of spur**	**1**	straight	lacking significant curves or bends	Kiger & Porter, 2001
		2	ascending	spreading at the base and then curving upward or forward, the distal portion more or less parallel to the bearing structure	Kiger & Porter, 2001
		3	descending	salient at its base and then curving downward or backward	Kiger & Porter, 2001
		4	N/A	not applicable	

References:

Beentje, H.J. 2010. The Kew plant glossary: an illustrated dictionary of plant terms. Royal Botanic Gardens, Kew, 164 pages.

Kiger, R.W. & Porter, D.M. 2001. Categorical glossary for the flora of North America project. Pittsburgh, 165 pages.

Annexe 3. Morphological character matrix

Morphological character matrix used in the phylogenetic reconstruction.

Taxa	**123456789101112131415161718192021222324252627282930313233343536373839**
Acampe ochracea	335433535134362112543542222712313175334
Aerangis ellisii	235443615135454223321322333712212421223
Aerangis hariotiana	324413633125364131331322333112324111223
Aerangis hildebrandtii	323313633125364131331322333112324111223
Aerangis macrocentra	323412613115354233321322333712212321223
Aerangis punctata	322312613115334233321322333712212421223
Aeranthes aemula	334413332124414133112112222262131213123
Aeranthes arachnites	334413332124414133112112222262131213123
Aeranthes caudata	334413332124414133112112222262131213123
Aeranthes grandiflora	334413332124414133112112222262131213123
Aeranthes longipes	334413332124414133112112222262131213123
Aeranthes moratii	334413332124414133112112222262131213123
Aeranthes neoperrieri	334413332124414133112112222262131213123
Aeranthes strangulata	334413332124414133112112222262131213123
Aeranthes tenella	334413332124414133112112222262131213123
Aeranthes virginalis	334413332124414133112112222262131213123
Aerides odorata	345433335145464214341342111412352264323
Ancistrorhynchuscapitatus	315413335124471132623622222222214221223
Ancistrorhynchusrecurvus	315413335124571132623622222222214251123
Angraecoides cf. *moandense*	345342435143222123312312322111133211112
Angraecoides chevalieri	345342435143222123312312322111133211112
Angraecoides cultriformis	345342445133222123312312322111133211112
Angraecoides erecta	255342445143222123312312322111133211112
Angraecopsis parviflora	322312423124254132311312333511154211123
Angraecum amplexicaule	344332421133211413321321322441232451212
Angraecum ankeranense	343223635223111324321321121212222461213
Angraecum appendiculatum	344242635143112313432422333111232111112
Angraecum arachnites	344232632132122313432422333331242452212

Angraecum baronii	3153224232421211223113113224421341111 22
Angraecum borbonicum	33331353511431122432132111121222242121 3
Angraecum bracteosum	334524335125462123121121222222222211123
Angraecum cadetii	334524335125463123121121222222222211123
Angraecum calceolus	343422421124464232311311333441134211122
Angraecum caricifolium	315232332142263121311311222442134111121
Angraecum caulescens	343422421124464232311311333441134211121
Angraecum cf. *breve*	332313535213211323321321111212222421213
Angraecum cf. *elephantinum*	344423535114311124321321121212222421213
Angraecum cf. *germinyanum*	344222632143122313432422333331242452212
Angraecum cf. *humblotianum*	343111225241111112321321222212222111221
Angraecum cf. *humile*	311111323122131121121121222251222111121
Angraecum cf. *liliodorum*	333313535213111124321321121212222421213
Angraecum cf. *panicifolium*	243331431221211112321321222212222111221
Angraecum cf. *pauciramosum*	315332331143263121311311222442134111121
Angraecum cf. *peyrotii*	333313535113111124321321121212222421213
Angraecum cf. *pinifolium*	343322421121333232311311322442134111121
Angraecum cf. *rutenbergianum*	233313535123211124321321121212222421213
Angraecum cf. *sacculatum*	343312421133343121311311322442134111121
Angraecum cf. *zeylanicum*	343422421124464132311311333441134211122
Angraecum chaetopodium	323312421123214132311311333441134211122
Angraecum clareae	344424533234231325321322123232233443213
Angraecum compactum	344424533224331325321322123232223443213
Angraecum conchiferum	343342335222112313432422333431242352212
Angraecum conchoglossum	344222632133122313432422333331242452212
Angraecum cordemoyi	323312421123232122311311322442134111122
Angraecum cornigerum	344342235222212424321321121212222421213
Angraecum corrugatum	344332635144212313432422333111232111112
Angraecum costatum	355332421142121122311312322442134111122
Angraecum cucullatum	332213635122111323321322111212222414213
Angraecum danguyanum	243331421131312213321322222412212321213
Angraecum dasycarpum	243222615133111112321321222212222111221
Angraecum didieri	343223635223111324321322121212222461213
Angraecum dives	334413325134364131311312222142132111121

Angraecum drouhardii	332213635222111323321322111212222261213
Angraecum dryadum	343223635223111324321321121212222461213
Angraecum eburneum var. *eburneum*	235524335145564125311312111321243311111
Angraecum eburneum var. *superbum*	335524335145564125311312111321243411111
Angraecum eburneum var. *xerophilum*	235534335144464125311312111321243311111
Angraecum elephantinum	344423535124311124321322121212222421213
Angraecum expansum	344342635223112313432422333431242352212
Angraecum filicornu	244331431122312213321322222212222421213
Angraecum florulentum	315332435244232323321322322441232451212
Angraecum germinyanum	344222632143122313432422333331242452212
Angraecum hermannii	315322423242121122311312322442134111122
Angraecum huntleyoides	335514421134564432311312333441134211112
Angraecum lecomtei	332312235221111323321321111112222461213
Angraecum leonis	333414523115432325321322121232223443213
Angraecum letouzeyi	333312226221312323321322121212222321213
Angraecum liliodorum	333313535213111124321322121212222421213
Angraecum linearifolium	343322126131313313432422333431242452212
Angraecum longicalcar	235524335145564125311312111321243511111
Angraecum madagascariense	314122622143142121311311222442134111121
Angraecum magdalenae	234524535125331225321322121532233443213
Angraecum mauritianum	244322422134212213321322222212222421213
Angraecum mirabile	343342335222112313432422333431242452212
Angraecum moratii	315332635144222323321322333441232451212
Angraecum multiflorum	343422421132263122311312333441134211121
Angraecum musculiferum	315232332143263121311312222442134111121
Angraecum nanum	331111421122144131311312222142132111121
Angraecum obesum	344523335124311124321322121212232421213
Angraecum oblongifolium	314222635133111113321322333441232351212
Angraecum obversifolium	343312421133333122311312322442134111122
Angraecum ochraceum	333312421123314132311311333441124211122
Angraecum panicifolium	243331431222121111232132222221222211221
Angraecum pauciramosum	314132321142163121311312222442134111121
Angraecum pectinatum	243222535232111112321322222212222111221
Angraecum penzigianum	315432423144342323321322333441232321212

Angraecum perparvulum	3211116131131311311211222222251221111121
Angraecum peyrotii	3333135351133121243213221212122222421213
Angraecum pingue	3142227241331221122122121231521322111122
Angraecum pinifolium	3433224211213332323113123224421341111121
Angraecum praestans	2345243351254332252122121115322424443213
Angraecum pseudodidieri	3443233351232111243213221212122232421213
Angraecum pseudofilicornu	3133222251222122144314223333312324522212
Angraecum pterophyllum	3111113232221311211211222222251221111121
Angraecum ramosum	2542324252422122133213222222212222421213
Angraecum rhynchoglossum	3222114221142141322112123331511412111111
Angraecum sacciferum	3212114211242441313113123224421341111121
Angraecum scottianum	3143222251323122144314223333312324522212
Angraecum sedifolium	3331112241421221122122121231521322111122
Angraecum serpens	3154324231443123233213223334412322221212
Angraecum sesquipedale	2355243351454332253213221112122235211113
Angraecum setipes	3333124211233141323113123334411242111122
Angraecum sororium	2355245351454322253213221112122235211113
Angraecum sterophyllum	3433135352222132233213221212122222421213
Angraecum striatum	3345243351254641231211222222222222211123
Angraecum tenuifolium	3443324211222532223113123224421341111121
Angraecum tenuispica	3144224211432631213113122224421341111121
Angraecum teres	3334133251213644323113122221421322111121
Angraecum triangulifolium	3142227241331221122122121231521322111122
Angraecum viguieri	3454343321343221252322121215322424443213
Beclardia macrostachya	3444233351254542343215222227522522213121
Bolusiella iridifolia	3334135231222541313213222221122221121223
Bolusiella maudiae	3334135231231541313213222221122221121223
Calyptrochilum christyanum	3354445351343611223113122227121542121 23
Campylocentrum fasciola	3566558573566641511211222222322212111221
Cribbia brachyceras	3444233351243643222112122221221242111 23
Cribbia confusa	3434233351243643232112122221221242111 23
Cryptopus elatus	3454325351242541235237223338122521132 11
Cryptopus paniculatus	3454325351242541235137123338122521132 21
Cyrtorchis arcuata	3155236351254642242212223331122223221 13

Cyrtorchis chailluana	315523635125464224221222333112222422113
Dendrophylax funalis	356655857356623353311312111332252313111
Diaphananthe pellucida	315413635125564123321322222212271221213
Diaphananthe sarcophylla	315423443224464121331332333112324221313
Diaphananthe vesicata	315423443224564121311312333112124221123
Dolabrifolia bancoensis	313111612143121122321321111441231111213
Dolabrifolia cf. *aporoides*	313111612144121122321321111441231111213
Dolabrifolia disticha	313111612143121122321322111441231111213
Dolabrifolia podochiloides	315111612142121122321322111441231111213
Eichlerangraecum birrimense	355332435145422225311311333752233314123
Eichlerangraecum cf. *eichlerianum*	355332435145422225311311333752233444123
Eichlerangraecum infundibulare	355332435135422225311312333752233314123
Erasanthe henricii	323413635125454135322322222762272411113
Eurychone galeandrae	323413633124454134231222222332371214222
Eurychone rothschildiana	323413633124453134221222222232271214222
Jumellea anjouanensis	335413335134411323221222123612222431113
Jumellea arachnanta	335413335134411323221222123612222431113
Jumellea arborescens	335413335134411323221222123612222431113
Jumellea bosseri	335413335134411323221222123612222431113
Jumellea brevifolia	335413335134411323221222123612222431113
Jumellea densifoliata	335413335134411323221222123612222431113
Jumellea exilis	335413335134411323221222123612222431113
Jumellea francoisii	335413335134411323221222123612222431113
Jumellea hyalina	335413335134411323221222123612222431113
Jumellea jumelleana	335413335134411323221222123612222431113
Jumellea longivaginans	335413335134411323221222123612222431113
Jumellea majalis	335413335134411323221222123612222431113
Jumellea maxillarioides	335413335134411323221222123612222431113
Jumellea rigida	335413335134411323221222123612222431113
Jumellea spathulata	335413335134411323221222123612222431113
Jumellea tenuibracteata	335413335134411323221222123612222431113
Jumellea teretifolia	335413335134411323221222123612222431113
Lemurella pallidiflora	343322423124234122311312333152154212112
Lemurella papillosa	343322423124234122311312333152154212112

Lemurorchis madagascariensis	335524335145564122331332111252354211213
Listrostachys pertusa	34551433514446412212112233371222411123
Microcoelia gilpinae	35665585735666345213313222222314214323
Microcoelia stolzii	356655857356664151623622222412211124223
Mystacidium capense	323412433114364233321322333112221311213
Mystacidium flanaganii	322312433112164232311312333112121211113
Neobathiea grandidierana	314333635124232214321322333112222443113
Oeonia rosea	355341625122133123513512111852252111221
Oeoniella polystachys	254332535124364223311312222352242111112
Pectinariella atlantica	315221425242221122321321111441231111213
Pectinariella doratophylla	315221425242221122321322111441231211213
Pectinariella gabonensis	315221425242221122321322111441231111213
Pectinariella pungens	315221425242221122321322111441231111213
Pectinariella subulata	315331225241221122321322111441231111213
Phalaenopsis cornu-cervi	322412613115364224341342111212322175334
Podangis dactyloceras	3433134251243634222621622222422261224223
Polystachya fulvilabia	133312421122453342341342222221322175334
Rangaeris amaniensis	345514335124454323321322333512221421113
Rangaeris muscicola	344514335124354322321322333212221421213
Rhipidoglossum subsimplex	315343435133262122613112222712161211113
Rhipidoglossum xanthopollinium	315443435133363122613112222712161221113
Sobennikoffia humbertiana	234424335145564224321322323732252213122
Solenangis clavata	355342635134141222613612222112151221123
Solenangis wakefieldii	355342635134142222623622222112252324213
Sphyrarhynchus schliebenii	323313425113244332321322112222251221223
Tridactyle bicaudata	335444335124362122311212333512151211111
Tridactyle filifolia	315322225212341132311212333512151211111
Vanda tricolor	345433335144454315543542222712352175334
Ypsilopus longifolius	314313322123454332321322333512231321213
Ypsilopus viridiflorus	314313322123422132211212333112131221113

Annexe 4. Characters used in BAMM analyses

Continuous characters used for the phenotypic/evolution model, and fractioning used for the speciation/extinction model implemented in BAMM, values for flower size and spur length were log-transformed and fraction represents the percentage of samples used in the analyses for each clade (from 0 to 1).

Clade	**Taxa**	**Flower size**	**Spur length**	**Fraction**
Aeranthes	*Aeranthes aemula*	2.255272505	1.342422681	0.3
Aeranthes	*Aeranthes arachnites*	2.158362492	1.301029996	0.3
Aeranthes	*Aeranthes caudata*	2.021189299	1.397940009	0.3
Aeranthes	*Aeranthes grandiflora*	2.278753601	1.477121255	0.3
Aeranthes	*Aeranthes longipes*	2.130333768	1.301029996	0.3
Aeranthes	*Aeranthes moratii*	2.049218023	1.255272505	0.3
Aeranthes	*Aeranthes neoperrieri*	2.130333768	1.342422681	0.3
Aeranthes	*Aeranthes strangulata*	2.049218023	1.301029996	0.3
Aeranthes	*Aeranthes tenella*	2.158362492	1.176091259	0.3
Aeranthes	*Aeranthes virginalis*	2.049218023	1.342422681	0.3
Angraecum	*Angraecum amplexicaule*	1.77815125	1.954242509	0.8
Angraecum	*Angraecum ankeranense*	2.491361694	1.939519253	0.8
Angraecum	*Angraecum appendiculatum*	2.380211242	-1.958607315	0.8
Angraecum	*Angraecum arachnites*	1.77815125	2.041392685	0.8
Angraecum	*Angraecum baronii*	0	0.698970004	0.8
Angraecum	*Angraecum* cf. *germinyanum*	2.380211242	2.041392685	0.8
Angraecum	*Angraecum borbonicum*	2.342422681	1.84509804	0.8
Angraecum	*Angraecum bracteosum*	1.176091259	0.602059991	0.8
Angraecum	*Angraecum cadetii*	1.322219295	0.698970004	0.8
Angraecum	*Angraecum calceolus*	1.397940009	1.176091259	0.8
Angraecum	*Angraecum caricifolium*	0.477121255	0.477121255	0.8
Angraecum	*Angraecum caulescens*	0.954242509	1	0.8
Angraecum	*Angraecum* cf. *breve*	1.681241237	2.079181246	0.8
Angraecum	*Angraecum* cf. *peyrotii*	2.342422681	2.255272505	0.8
Angraecum	*Angraecum* cf. *elephantinum*	2.397940009	2.041392685	0.8
Angraecum	*Angraecum* cf. *germinyanum*	2.477121255	2.079181246	0.8
Angraecum	*Angraecum* cf. *pauciramosum*	0	0	0.8
Angraecum	*Angraecum* cf. *humblotianum*	1.544068044	0.477121255	0.8
Angraecum	*Angraecum* cf. *liliodorum*	2.653212514	1.77815125	0.8
Angraecum	*Angraecum* cf. *panicifolium*	1.544068044	2.004321374	0.8
Angraecum	*Angraecum* cf. *pinifolium*	0.602059991	0.602059991	0.8
Angraecum	*Angraecum* cf. *rutenbergianum*	2.498310554	2.146128036	0.8
Angraecum	*Angraecum* cf. *sacculatum*	0.602059991	0.477121255	0.8

Angraecum	*Angraecum* cf. *zeylanicum*	1.204119983	1	0.8
Angraecum	*Angraecum chaetopodium*	0.698970004	1.342422681	0.8
Angraecum	*Angraecum ochraceum*	0.698970004	1.342422681	0.8
Angraecum	*Angraecum clareae*	2.45484486	1.079181246	0.8
Angraecum	*Angraecum compactum*	2.318063335	2.041392685	0.8
Angraecum	*Angraecum conchiferum*	1.924279286	1.698970004	0.8
Angraecum	*Angraecum conchoglossum*	2.075546961	2.06069784	0.8
Angraecum	*Angraecum cordemoyi*	0.954242509	0.602059991	0.8
Angraecum	*Angraecum cornigerum*	2.290034611	2.113943352	0.8
Angraecum	*Angraecum corrugatum*	2.021189299	-1.958607315	0.8
Angraecum	*Angraecum costatum*	0.602059991	0.477121255	0.8
Angraecum	*Angraecum cucullatum*	1.875061263	1.079181246	0.8
Angraecum	*Angraecum danguyanum*	1.681241237	1.653212514	0.8
Angraecum	*Angraecum dasycarpum*	0.954242509	0.602059991	0.8
Angraecum	*Angraecum didieri*	2.167317335	1.929418926	0.8
Angraecum	*Angraecum dives*	0.698970004	0.903089987	0.8
Angraecum	*Angraecum drouhardii*	1.62324929	1.230448921	0.8
Angraecum	*Angraecum dryadum*	1.963787827	1.954242509	0.8
Angraecum	*Angraecum ebu.eburneum*	2.720159303	1.77815125	0.8
Angraecum	*Angraecum ebu.superbum*	2.670245853	1.84509804	0.8
Angraecum	*Angraecum ebu.xerophilum*	2.552668216	1.875061263	0.8
Angraecum	*Angraecum elephantinum*	2.698970004	2.041392685	0.8
Angraecum	*Angraecum expansum*	1.924279286	1.77815125	0.8
Angraecum	*Angraecum filicornu*	1.342422681	1.939519253	0.8
Angraecum	*Angraecum florulentum*	1.799340549	1.954242509	0.8
Angraecum	*Angraecum hermannii*	0.477121255	0.301029996	0.8
Angraecum	*Angraecum huntleyoides*	1.681241237	1.301029996	0.8
Angraecum	*Angraecum lecomtei*	1.62324929	1.977723605	0.8
Angraecum	*Angraecum leonis*	2.301029996	1.954242509	0.8
Angraecum	*Angraecum letouzeyi*	1.431363764	2.041392685	0.8
Angraecum	*Angraecum liliodorum*	2.653212514	1.77815125	0.8
Angraecum	*Angraecum linearifolium*	1.954242509	2	0.8
Angraecum	*Angraecum longicalcar*	2.684845362	2.431363764	0.8
Angraecum	*Angraecum madagascariense*	0	0	0.8
Angraecum	*Angraecum magdalenae*	2.498310554	1.986771734	0.8
Angraecum	*Angraecum mauritianum*	1.84509804	1.903089987	0.8
Angraecum	*Angraecum mirabile*	1.954242509	2.06069784	0.8
Angraecum	*Angraecum moratii*	2.130333768	2.06069784	0.8
Angraecum	*Angraecum multiflorum*	0.602059991	0.602059991	0.8
Angraecum	*Angraecum musculiferum*	0.477121255	0.477121255	0.8
Angraecum	*Angraecum obesum*	2.431363764	1.929418926	0.8
Angraecum	*Angraecum oblongifolium*	1.707570176	1.763427994	0.8

Angraecum	*Angraecum obversifolium*	0.301029996	0.301029996	0.8
Angraecum	*Angraecum panicifolium*	1.544068044	2.004321374	0.8
Angraecum	*Angraecum pauciramosum*	0	0.301029996	0.8
Angraecum	*Angraecum pectinatum*	1.113943352	0.301029996	0.8
Angraecum	*Angraecum penzigianum*	1.755874856	1.662757832	0.8
Angraecum	*Angraecum peyrotii*	2.45484486	1.986771734	0.8
Angraecum	*Angraecum pingue*	0.84509804	1.544068044	0.8
Angraecum	*Angraecum pinifolium*	0.602059991	0.602059991	0.8
Angraecum	*Angraecum praestans*	2.737192643	1.986771734	0.8
Angraecum	*Angraecum pseudodidieri*	2.155336037	1.740362689	0.8
Angraecum	*Angraecum pseudofilicornu*	2.176091259	2.096910013	0.8
Angraecum	*Angraecum ramosum*	1.903089987	1.544068044	0.8
Angraecum	*Angraecum sacciferum*	0.301029996	0.301029996	0.8
Angraecum	*Angraecum scottianum*	2.73239376	2.176091259	0.8
Angraecum	*Angraecum sedifolium*	1.301029996	1.113943352	0.8
Angraecum	*Angraecum serpens*	1.944482672	1.568201724	0.8
Angraecum	*Angraecum sesquipedale*	2.812913357	2.431363764	0.8
Angraecum	*Angraecum setipes*	0.602059991	0.954242509	0.8
Angraecum	*Angraecum sororium*	3.021189299	2.505149978	0.8
Angraecum	*Angraecum sterophyllum*	2.008600172	2	0.8
Angraecum	*Angraecum striatum*	1.322219295	0.903089987	0.8
Angraecum	*Angraecum tenuifolium*	0.477121255	0.602059991	0.8
Angraecum	*Angraecum tenuispica*	0.217483944	0.301029996	0.8
Angraecum	*Angraecum teres*	0.477121255	1.380211242	0.8
Angraecum	*Angraecum triangulifolium*	1.255272505	1.397940009	0.8
Angraecum	*Angraecum viguieri*	2.77815125	2.079181246	0.8
Angraecum	*Angraecum nanum*	0	0	0.8
Angraecum	*Angraecum zaratananae*	1.602059991	1.544068044	0.8
Angraecum	*Oeoniella polystachys*	2.397940009	0.698970004	0.8
Angraecum	*Sobennikoffia humbertiana*	2.531478917	1.477121255	0.8
CladeIA	*Angraecum* cf. *humile*	0.301029996	0.301029996	0.4
CladeIA	*Angraecum perparvulum*	0	0	0.4
CladeIA	*Angraecum pterophyllum*	0.77815125	0.477121255	0.4
CladeIA	*Angraecum rhynchoglossum*	0.477121255	1.431363764	0.4
CladeIA	*Beclardia macrostachya*	2.903089987	1.414973348	0.4
CladeIA	*Cryptopus elatus*	2.62324929	0.698970004	0.4
CladeIA	*Cryptopus paniculatus*	2.720159303	0.698970004	0.4
CladeIA	*Erasanthe henricii*	2.829303773	2.176091259	0.4
CladeIA	*Lemurella pallidiflora*	1.380211242	1.414973348	0.4
CladeIA	*Lemurella papillosa*	1.380211242	1.380211242	0.4
CladeIA	*Neobathiea grandidierana*	2.459392488	2.079181246	0.4
CladeIA	*Oeonia rosea*	2.397940009	0.602059991	0.4

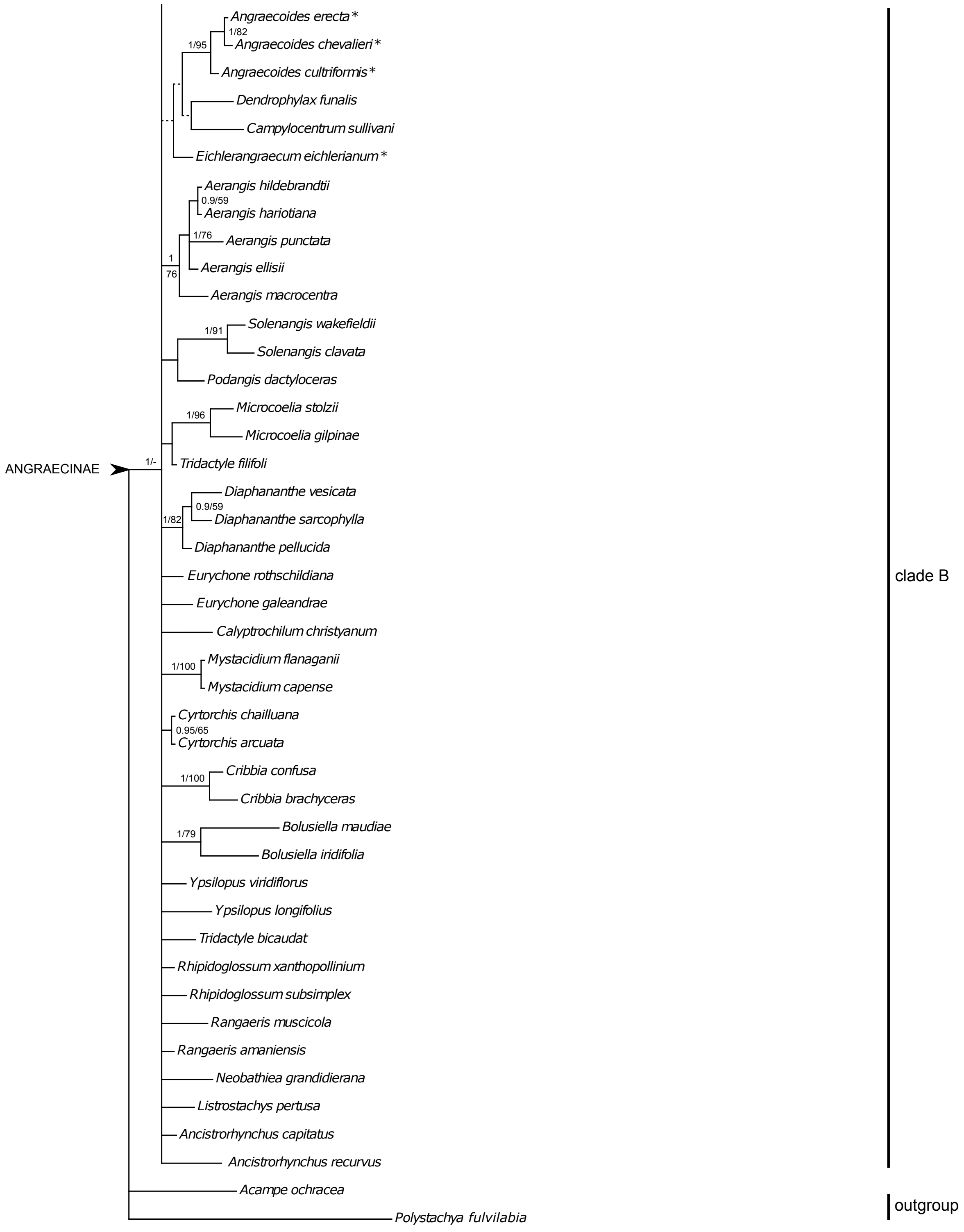
CladeIB	*Ancistrorhynchus capitatus*	1.204119983	1.414973348	0.4
CladeIB	*Ancistrorhynchus recurvus*	1.079181246	1.380211242	0.4
CladeIB	*Angraecoides chevalieri*	1.079181246	1.204119983	0.4
CladeIB	*Angraecoides cultriformis*	1.477121255	1.342422681	0.4
CladeIB	*Angraecoides erecta*	1.230448921	1.278753601	0.4
CladeIB	*Angraecoides* cf. *moandense*	1.176091259	1.301029996	0.8
CladeIB	*Angraecopsis parviflora*	1.301029996	1.301029996	0.4
CladeIB	*Bolusiella iridifolia*	0.477121255	0.602059991	0.4
CladeIB	*Bolusiella maudiae*	0.477121255	0.698970004	0.4
CladeIB	*Calyptrochilum christyanum*	1.477121255	1.301029996	0.4
CladeIB	*Campylocentrum fasciola*	0.301029996	0.602059991	0.4
CladeIB	*Cribbia brachyceras*	1.380211242	1.176091259	0.4
CladeIB	*Cribbia confusa*	2.158362492	1.204119983	0.4
CladeIB	*Cyrtorchis arcuata*	2.544068044	1.77815125	0.4
CladeIB	*Cyrtorchis chailluana*	2.431363764	1.954242509	0.4
CladeIB	*Dendrophylax funalis*	2.255272505	1.740362689	0.4
CladeIB	*Diaphananthe pellucida*	2.049218023	1.380211242	0.4
CladeIB	*Diaphananthe sarcophylla*	0.477121255	1.176091259	0.4
CladeIB	*Diaphananthe vesicata*	0.477121255	1.414973348	0.4
CladeIB	*Dolabrifolia bancoensis*	0.477121255	0.301029996	0.4
CladeIB	*Dolabrifolia* cf. *aporoides*	0	0.84509804	0.4
CladeIB	*Dolabrifolia disticha*	0	0.84509804	0.4
CladeIB	*Dolabrifolia podochiloides*	1.447158031	1.146128036	0.4
CladeIB	*Eichlerangraecum birrimense*	2.908485019	1.278753601	0.4
CladeIB	*Eichlerangraecum* cf. *eichlerianum*	3.096910013	1.477121255	0.4
CladeIB	*Eichlerangraecum infundibulare*	3.342422681	2.301029996	0.4
CladeIB	*Eurychone galeandrae*	2.954242509	1.397940009	0.4
CladeIB	*Eurychone rothschildiana*	2.880813592	1.361727836	0.4
CladeIB	*Aerangis ellisii*	2.176091259	2.146128036	0.4
CladeIB	*Aerangis hariotiana*	0.77815125	0.602059991	0.4
CladeIB	*Aerangis hildebrandtii*	0.77815125	0.698970004	0.4
CladeIB	*Aerangis macrocentra*	1.77815125	1.812913357	0.4
CladeIB	*Aerangis punctata*	2.176091259	2.079181246	0.4
CladeIB	*Listrostachys pertusa*	1.322219295	0.698970004	0.4
CladeIB	*Microcoelia gilpinae*	0.903089987	1.146128036	0.4
CladeIB	*Microcoelia stolzii*	0.77815125	0.698970004	0.4
CladeIB	*Mystacidium capense*	1.857332496	1.77815125	0.4
CladeIB	*Mystacidium flanaganii*	1.477121255	1.397940009	0.4
CladeIB	*Pectinariella atlantica*	1.602059991	0.602059991	0.4
CladeIB	*Pectinariella doratophyllum*	0.477121255	1	0.4
CladeIB	*Pectinariella gabonensis*	0.77815125	0.477121255	0.4

CladeIB	*Pectinariella pungens*	1	0.698970004	0.4
CladeIB	*Pectinariella subulata*	0.77815125	0.698970004	0.4
CladeIB	*Podangis dactyloceras*	1.380211242	-1.958607315	0.4
CladeIB	*Rangaeris amaniensis*	1.903089987	2.041392685	0.4
CladeIB	*Rangaeris muscicola*	1.602059991	2.079181246	0.4
CladeIB	*Rhipidoglossum subsimplex*	1.380211242	1.414973348	0.4
CladeIB	*Rhipidoglossum xanthopollinium*	1.447158031	1.397940009	0.4
CladeIB	*Solenangis clavata*	1.431363764	1.431363764	0.4
CladeIB	*Solenangis wakefieldii*	1.301029996	1.740362689	0.4
CladeIB	*Sphyrarhynchus schliebenii*	1.204119983	1.397940009	0.4
CladeIB	*Tridactyle bicaudata*	1.146128036	1.380211242	0.4
CladeIB	*Tridactyle filifolia*	1.301029996	1.361727836	0.4
CladeIB	*Ypsilopus longifolius*	1.301029996	1.698970004	0.4
CladeIB	*Ypsilopus viridiflorus*	1.146128036	1.477121255	0.4
Jumellea	*Jumellea anjouanensis*	1.397940009	1.301029996	0.3
Jumellea	*Jumellea arachnantha*	2.352182518	1.77815125	0.3
Jumellea	*Jumellea arborescens*	2.225309282	2.041392685	0.3
Jumellea	*Jumellea bosseri*	2.336459734	1.477121255	0.3
Jumellea	*Jumellea brevifolia*	2.322219295	2.06069784	0.3
Jumellea	*Jumellea densifoliata*	1.857332496	2	0.3
Jumellea	*Jumellea exilis*	1.748188027	1.792391689	0.3
Jumellea	*Jumellea francoisii*	2	1.397940009	0.3
Jumellea	*Jumellea hyalina*	1.832508913	1.342422681	0.3
Jumellea	*Jumellea jumelleana*	1.880813592	2.041392685	0.3
Jumellea	*Jumellea longivaginans*	1.832508913	1.977723605	0.3
Jumellea	*Jumellea majalis*	2.459392488	2.079181246	0.3
Jumellea	*Jumellea maxillarioides*	2.556302501	1.819543936	0.3
Jumellea	*Jumellea rigida*	2.534026106	2	0.3
Jumellea	*Jumellea spathulata*	1.908485019	1.301029996	0.3
Jumellea	*Jumellea tenuibracteata*	2.352182518	2.041392685	0.3
Jumellea	*Jumellea teretifolia*	2.350248018	2.079181246	0.3
Lemurorchis	*Lemurorchis madagascariensis*	1.255272505	1.176091259	1
Outgroup	*Acampe ochracea*	0.477121255	-1.958607315	1
Outgroup	*Aerides odorata*	2.602059991	1.397940009	1
Outgroup	*Phalaenopsis cornu-cervi*	2.447158031	-1.958607315	1
Outgroup	*Polystachya fulvilabia*	1.447158031	1.477121255	1
Outgroup	*Vanda tricolor*	2.90579588	1	1

Annexe 5. ITS2 phylogenetic tree

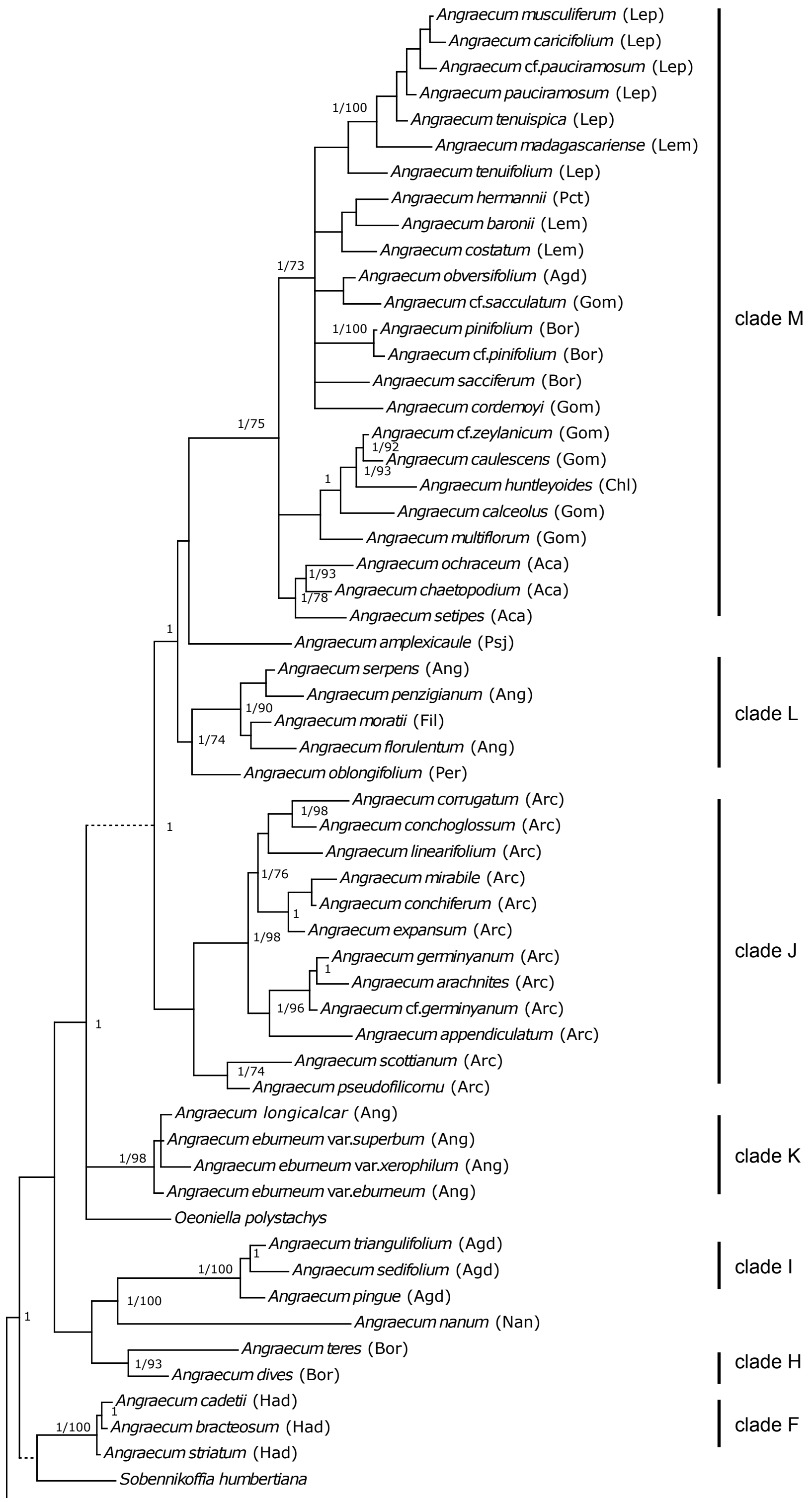
ITS2 phylogenetic relationships of Angraecinae. 50% Bayesian majority-rule consensus tree from the nuclear ribosomal sequences (ITS2). Values above branches or at nodes represent posterior probability (PP) and bootstrap percentage (BP) support. Dashes represent branches that collapsed in the MP strict consensus tree. Taxa with an asterisk are *Angraecum* sensu Garay species. Abbreviations in brackets denote sections sensu Garay (1973): Aca = *Acaulia*, Ang = *Angraecum*, Arc = *Arachnangraecum*, Bor = *Boryangraecum*, Fil = *Filangis*, Hum = *Humblotiangraecum*, Pct = *Pectinaria*, Per = *Perrierangraecum*, Psj = *Pseudojumellea*.

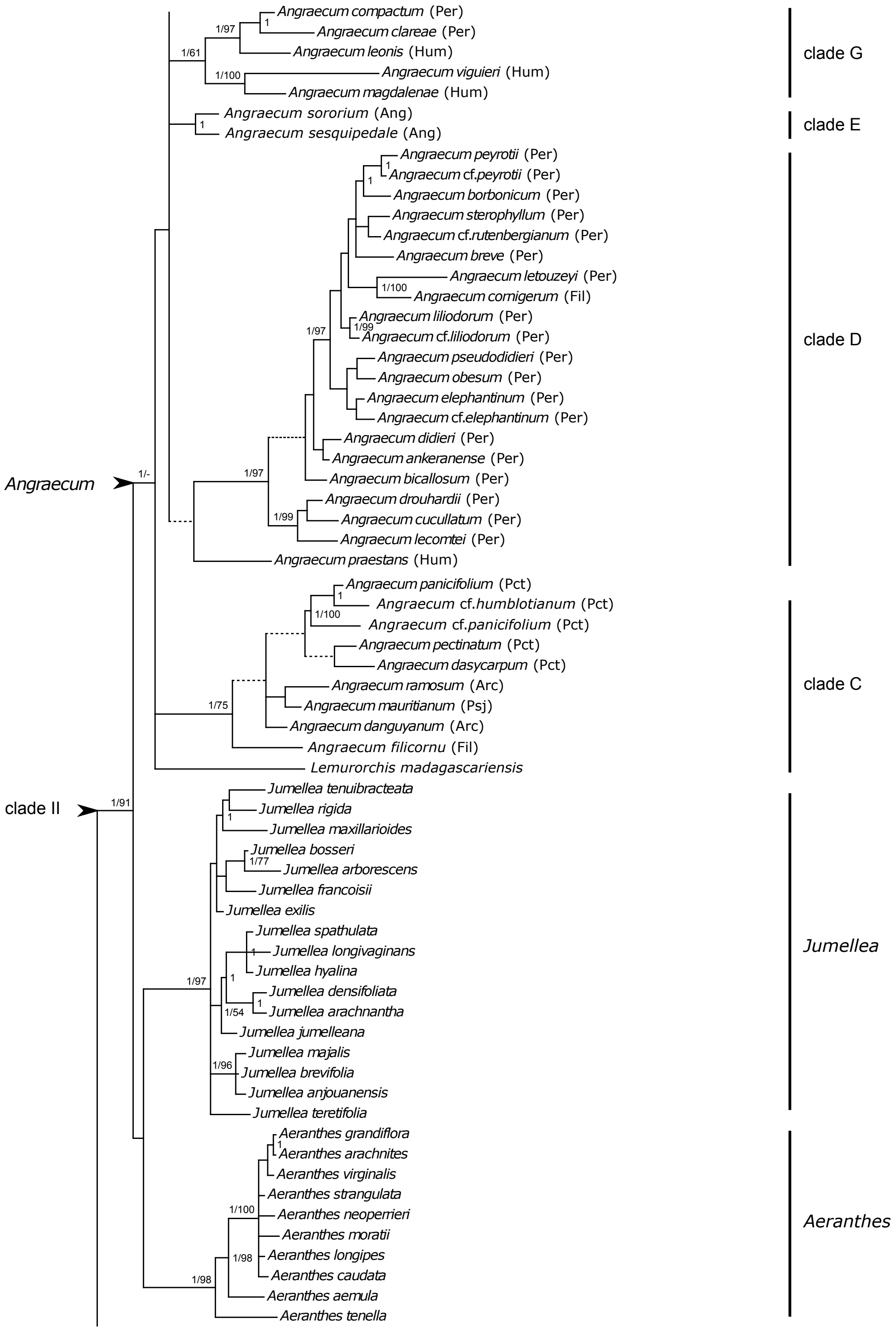

Angm scottianum (Arc)
0.9
51
Angm conchiferum (Arc)
Angraecum cf. germinyanum (Arc)
0.9/63
Angm germinyanum (Arc)
0.9/76
Angraecum mirabile (Arc)
Angm appendiculatum (Arc)
clade J
1/100
Aeranthes grandiflora
Aeranthes arachnites
0.8/62
Angm rutenbergianum (Per)
Angm elephantinum (Per)
clade D
Sobennikoffia humbertiana
Angm longicalcar (Ang)
1/99
Angm eburneum var. superbum (Ang)
Angm eburneum var. eburneum (Ang)
clade K
Angraecum chaetopodium (Aca)
1/90
Angraecum ochraceum (Aca)
1/98
Angraecum setipes (Aca)
clade M
Angraecum lecomtei (Per)
0.7/83
Angraecum praestans (Hum)
0.7/59
Angraecum drouhardii (Per)
clade D
Jumellea rigida
0.7/55
Jumellea francoisii
Lemurorchis madagascariensis
Angm florulentum (Ang)
Oeoniella polystachys
Jumellea teretifolia
Jumellea spathulata
Jumellea majalis
Jumellea longivaginans
Jumellea jumelleana
clade II
Jumellea hyalina
Jumellea brevifolia
Jumellea bosseri
Jumellea arachnantha
Jumellea densifoliata
Angum dives (Bor)
clade H
Angm teres (Bor)
Angraecum danguyanum (Arc)
1/77
Angraecum panicifolium (Pct)
1/66
Angraecum mauritianum (Psj)
1/98
Angraecum filicornu (Fil)
clade C
Cryptopus paniculatus
1/68
Cryptopus elatus
Neobathiea grandidierana
clade A
Angraecum pterophyllum *


Angraecoides erecta*
1/82
Angraecoides chevalieri*
1/95
Angraecoides cultriformis*
-
Dendrophylax funalis
-
Campylocentrum sullivani
Eichlerangraecum eichlerianum*
Aerangis hildebrandtii
0.9/59
Aerangis hariotiana
1/76
Aerangis punctata
1
76
Aerangis ellisii
Aerangis macrocentra
1/91
Solenangis wakefieldii
Solenangis clavata
Podangis dactyloceras
1/96
Microcoelia stolzii
Microcoelia gilpinae
Tridactyle filifoli
ANGRAECINAE
1/-
Diaphananthe vesicata
0.9/59
Diaphananthe sarcophylla
1/82
Diaphananthe pellucida
Eurychone rothschildiana
Eurychone galeandrae
Calyptrochilum christyanum
1/100
Mystacidium flanaganii
Mystacidium capense
Cyrtorchis chailluana
0.95/65
Cyrtorchis arcuata
1/100
Cribbia confusa
Cribbia brachyceras
1/79
Bolusiella maudiae
Bolusiella iridifolia
Ypsilopus viridiflorus
Ypsilopus longifolius
Tridactyle bicaudat
Rhipidoglossum xanthopollinium
Rhipidoglossum subsimplex
Rangaeris muscicola
Rangaeris amaniensis
Neobathiea grandidierana
Listrostachys pertusa
Ancistrorhynchus capitatus
Ancistrorhynchus recurvus
Acampe ochracea
Polystachya fulvilabia
clade B
outgroup

Annexe 6. cpDNA + morphology phylogenetic tree

Phylogenetic relationships within subtribe Angraecinae. 50% Bayesian majority-rule consensus tree from the combined plastid (*mat*K, *rps*16 and *trn*L) and morphological data. Values above branches or at nodes represent posterior probability (PP) and bootstrap percentage (BP) support. Dashes represent branches that collapsed in the MP strict consensus tree. Taxa with an asterisk are *Angraecum* sensu Garay species. Abbreviations in brackets denote sections sensu Garay (1973): Aca = *Acaulia*, Agd = *Angraecoides*, Ang = *Angraecum*, Arc = *Arachnangraecum*, Bor = *Boryangraecum*, Chl = *Chlorangraecum*, Fil = *Filangis*, Gom = *Gomphocentrum*, Had = *Hadrangis*, Hum = *Humblotiangraecum*, Lem = *Lemurangis*, Lep = *Lepervenchea*, Nan = *Nana*, Pct = *Pectinaria*, Per = *Perrierangraecum*, Psj = *Pseudojumellea*.


Angraecum musculiferum (Lep)
Angraecum caricifolium (Lep)
Angraecum cf.pauciramosum (Lep)
Angraecum pauciramosum (Lep)
Angraecum tenuispica (Lep)
Angraecum madagascariense (Lem)
Angraecum tenuifolium (Lep)
Angraecum hermannii (Pct)
Angraecum baronii (Lem)
Angraecum costatum (Lem)
Angraecum obversifolium (Agd)
Angraecum cf.sacculatum (Gom)
Angraecum pinifolium (Bor)
Angraecum cf.pinifolium (Bor)
Angraecum sacciferum (Bor)
Angraecum cordemoyi (Gom)
Angraecum cf.zeylanicum (Gom)
Angraecum caulescens (Gom)
Angraecum huntleyoides (Chl)
Angraecum calceolus (Gom)
Angraecum multiflorum (Gom)
Angraecum ochraceum (Aca)
Angraecum chaetopodium (Aca)
Angraecum setipes (Aca)
Angraecum amplexicaule (Psj)
Angraecum serpens (Ang)
Angraecum penzigianum (Ang)
Angraecum moratii (Fil)
Angraecum florulentum (Ang)
Angraecum oblongifolium (Per)
Angraecum corrugatum (Arc)
Angraecum conchoglossum (Arc)
Angraecum linearifolium (Arc)
Angraecum mirabile (Arc)
Angraecum conchiferum (Arc)
Angraecum expansum (Arc)
Angraecum germinyanum (Arc)
Angraecum arachnites (Arc)
Angraecum cf.germinyanum (Arc)
Angraecum appendiculatum (Arc)
Angraecum scottianum (Arc)
Angraecum pseudofilicornu (Arc)
Angraecum longicalcar (Ang)
Angraecum eburneum var.superbum (Ang)
Angraecum eburneum var.xerophilum (Ang)
Angraecum eburneum var.eburneum (Ang)
Oeoniella polystachys
Angraecum triangulifolium (Agd)
Angraecum sedifolium (Agd)
Angraecum pingue (Agd)
Angraecum nanum (Nan)
Angraecum teres (Bor)
Angraecum dives (Bor)
Angraecum cadetii (Had)
Angraecum bracteosum (Had)
Angraecum striatum (Had)
Sobennikoffia humbertiana
1/100
1/73
1/100
1/75
1/92
1/93
1
1/93
1/78
1
1/90
1/74
1
1/98
1/76
1
1/98
1
1/96
1/74
1
1/98
1
1/100
1/100
1/93
1
1
1/100
clade M
clade L
clade J
clade K
clade I
clade H
clade F


Angraecum compactum (Per)
Angraecum clareae (Per)
Angraecum leonis (Hum)
Angraecum viguieri (Hum)
Angraecum magdalenae (Hum)
Angraecum sororium (Ang)
Angraecum sesquipedale (Ang)
Angraecum peyrotii (Per)
Angraecum cf.peyrotii (Per)
Angraecum borbonicum (Per)
Angraecum sterophyllum (Per)
Angraecum cf.rutenbergianum (Per)
Angraecum breve (Per)
Angraecum letouzeyi (Per)
Angraecum cornigerum (Fil)
Angraecum liliodorum (Per)
Angraecum cf.liliodorum (Per)
Angraecum pseudodidieri (Per)
Angraecum obesum (Per)
Angraecum elephantinum (Per)
Angraecum cf.elephantinum (Per)
Angraecum didieri (Per)
Angraecum ankeranense (Per)
Angraecum bicallosum (Per)
Angraecum drouhardii (Per)
Angraecum cucullatum (Per)
Angraecum lecomtei (Per)
Angraecum praestans (Hum)
Angraecum panicifolium (Pct)
Angraecum cf.humblotianum (Pct)
Angraecum cf.panicifolium (Pct)
Angraecum pectinatum (Pct)
Angraecum dasycarpum (Pct)
Angraecum ramosum (Arc)
Angraecum mauritianum (Psj)
Angraecum danguyanum (Arc)
Angraecum filicornu (Fil)
Lemurorchis madagascariensis
Jumellea tenuibracteata
Jumellea rigida
Jumellea maxillarioides
Jumellea bosseri
Jumellea arborescens
Jumellea francoisii
Jumellea exilis
Jumellea spathulata
Jumellea longivaginans
Jumellea hyalina
Jumellea densifoliata
Jumellea arachnantha
Jumellea jumelleana
Jumellea majalis
Jumellea brevifolia
Jumellea anjouanensis
Jumellea teretifolia
Aeranthes grandiflora
Aeranthes arachnites
Aeranthes virginalis
Aeranthes strangulata
Aeranthes neoperrieri
Aeranthes moratii
Aeranthes longipes
Aeranthes caudata
Aeranthes aemula
Aeranthes tenella
clade G
clade E
clade D
clade C
Jumellea
Aeranthes
Angraecum
clade II
1/97
1
1/61
1/100
1
1
1
1/100
1/99
1/97
1/97
1/99
1
1/100
1/75
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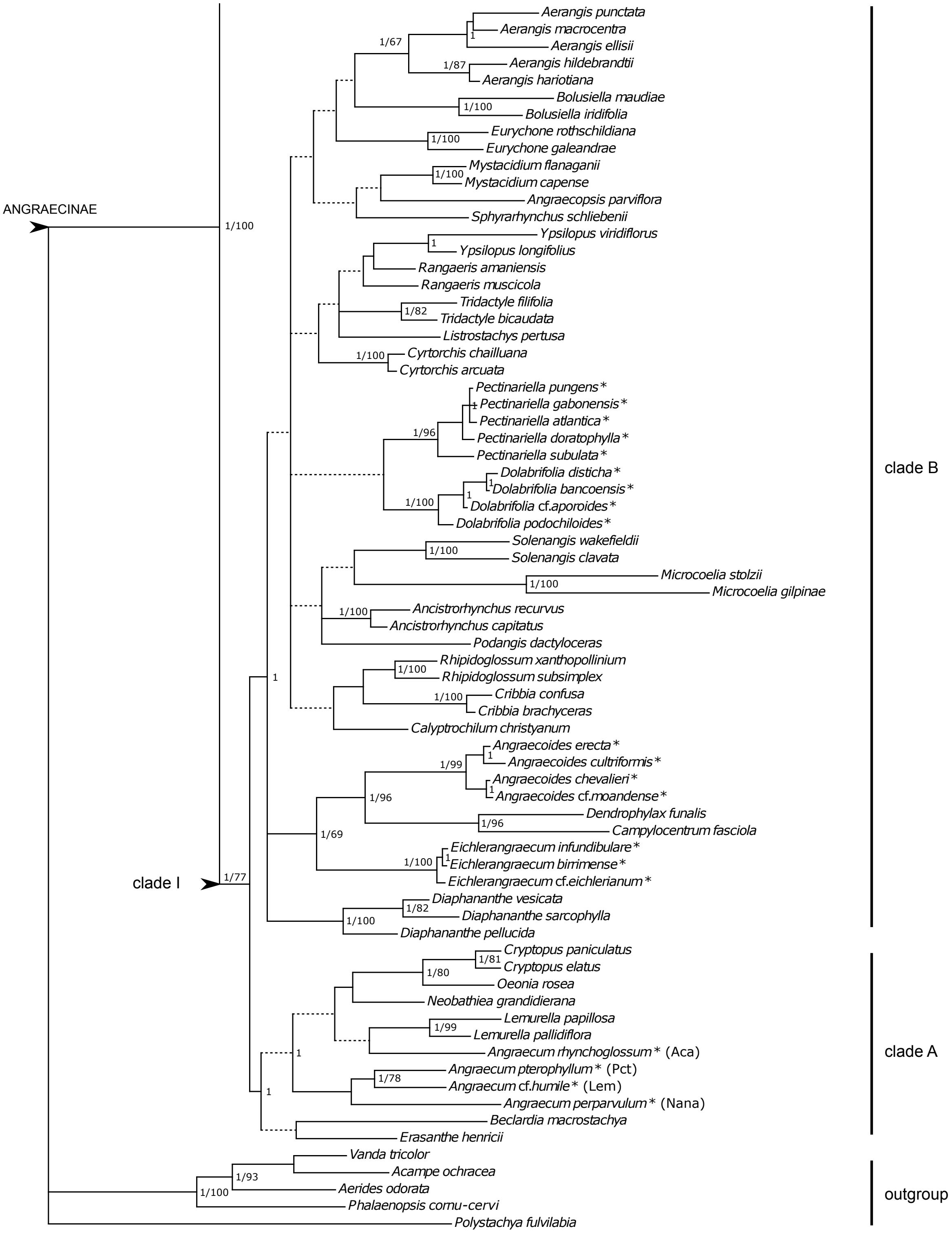
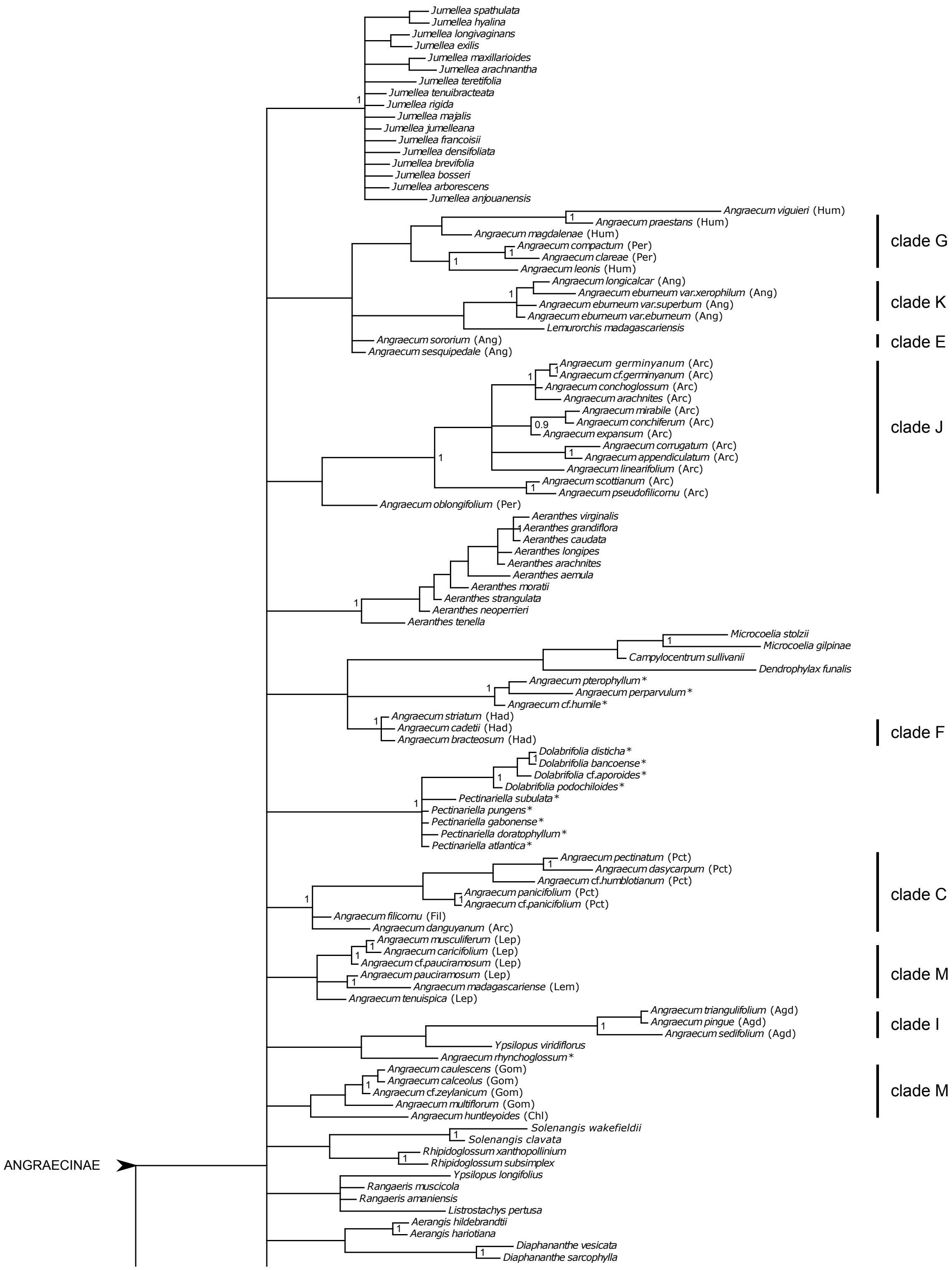
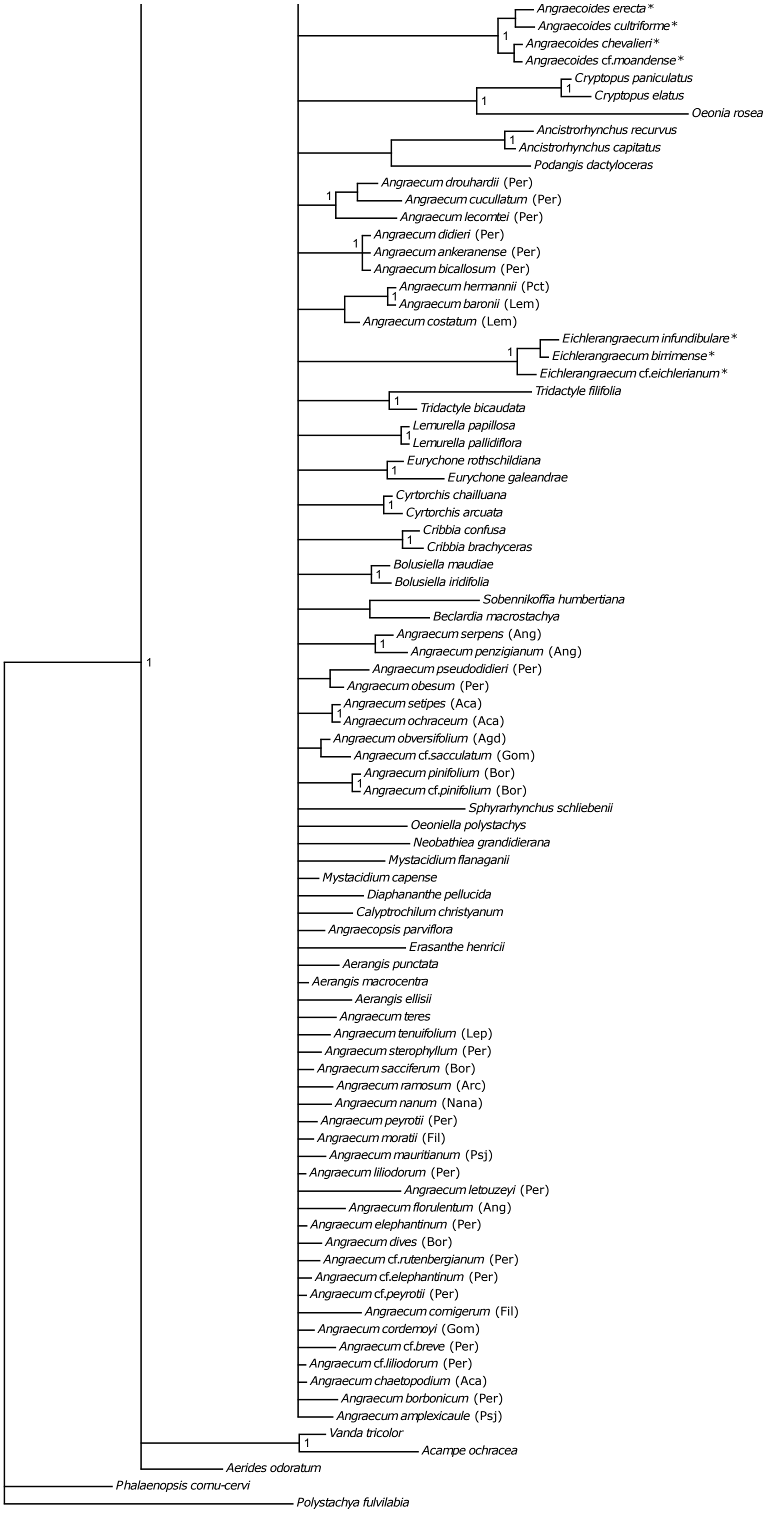
1
1/100
1/98
1/98


ANGRAECINAE
1/100
clade I
1/77
clade B
clade A
outgroup
1/67
Aerangis punctata
Aerangis macrocentra
1
Aerangis ellisii
1/87
Aerangis hildebrandtii
Aerangis hariotiana
Bolusiella maudiae
1/100
Bolusiella iridifolia
1/100
Eurychone rothschildiana
Eurychone galeandrae
1/100
Mystacidium flanaganii
Mystacidium capense
Angraecopsis parviflora
Sphyrarhynchus schliebenii
1
Ypsilopus viridiflorus
Ypsilopus longifolius
Rangaeris amaniensis
Rangaeris muscicola
1/82
Tridactyle filifolia
Tridactyle bicaudata
Listrostachys pertusa
1/100
Cyrtorchis chailluana
Cyrtorchis arcuata
Pectinariella pungens*
1
Pectinariella gabonensis*
Pectinariella atlantica*
1/96
Pectinariella doratophylla*
Pectinariella subulata*
Dolabrifolia disticha*
1
Dolabrifolia bancoensis*
1
1/100
Dolabrifolia cf.aporoides*
Dolabrifolia podochiloides*
1/100
Solenangis wakefieldii
Solenangis clavata
1/100
Microcoelia stolzii
Microcoelia gilpinae
1/100
Ancistrorhynchus recurvus
Ancistrorhynchus capitatus
Podangis dactyloceras
1
1/100
Rhipidoglossum xanthopollinium
Rhipidoglossum subsimplex
1/100
Cribbia confusa
Cribbia brachyceras
Calyptrochilum christyanum
Angraecoides erecta*
1/99
1
Angraecoides cultriformis*
Angraecoides chevalieri*
1
Angraecoides cf.moandense*
1/96
1/96
Dendrophylax funalis
Campylocentrum fasciola
1/69
Eichlerangraecum infundibulare*
1
1/100
Eichlerangraecum birrimense*
Eichlerangraecum cf.eichlerianum*
1/82
Diaphananthe vesicata
Diaphananthe sarcophylla
1/100
Diaphananthe pellucida
1/81
Cryptopus paniculatus
Cryptopus elatus
1/80
Oeonia rosea
Neobathiea grandidierana
1/99
Lemurella papillosa
Lemurella pallidiflora
Angraecum rhynchoglossum* (Aca)
1
Angraecum pterophyllum* (Pct)
1/78
Angraecum cf.humile* (Lem)
Angraecum perparvulum* (Nana)
1
Beclardia macrostachya
Erasanthe henricii
Vanda tricolor
1/93
Acampe ochracea
Aerides odorata
1/100
Phalaenopsis cornu-cervi
Polystachya fulvilabia

Annexe 7. Morphological phylogenetic tree

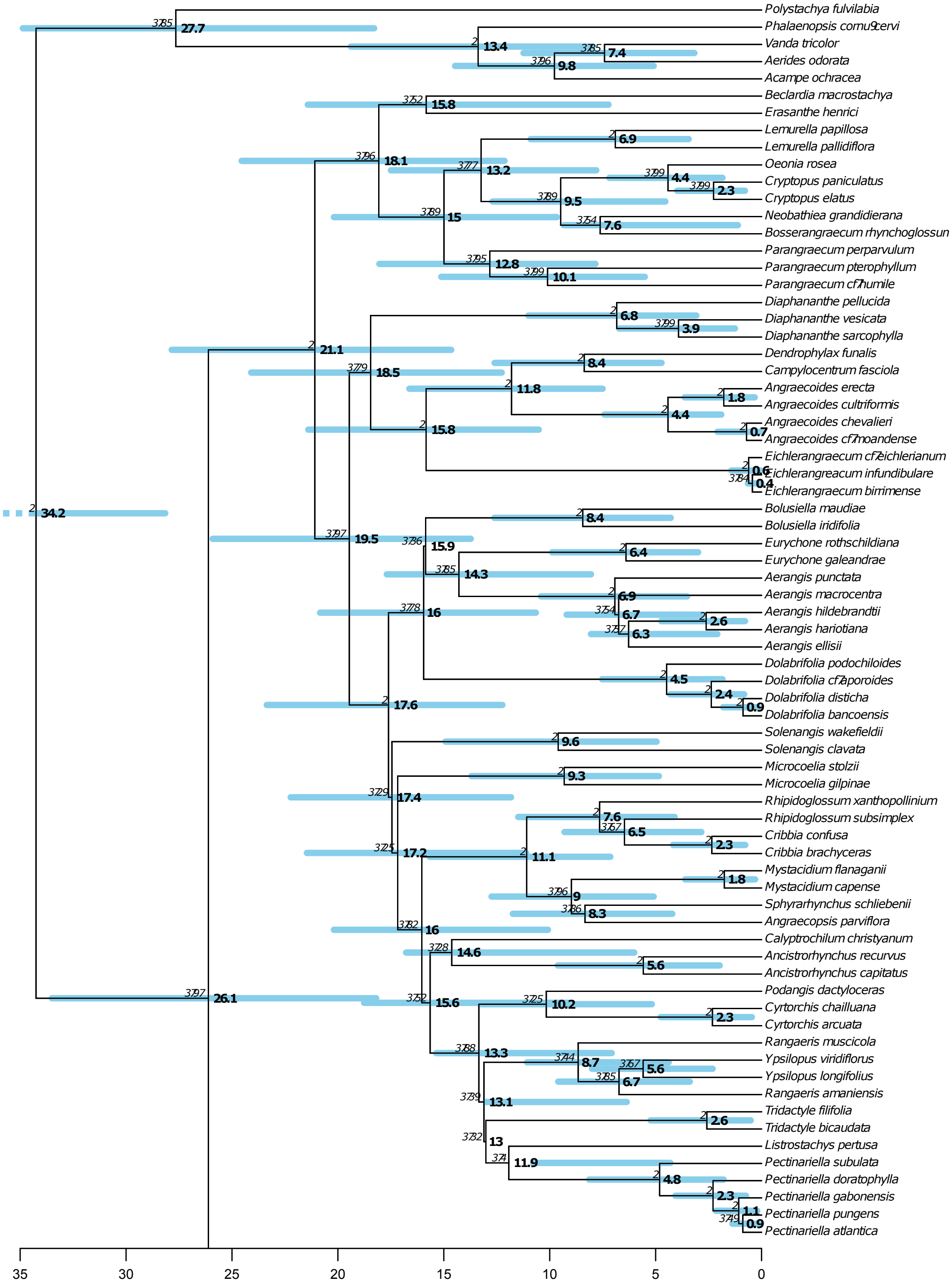
Phylogenetic relationships of Angraecinae. 50% Bayesian majority-rule consensus tree from morphological data. Values above branches or at nodes represent posterior probability (PP). Dashes represent branches that collapsed in MP strict consensus trees. Taxa with an asterisk are *Angraecum* sensu Garay species. Abbreviations in brackets denote sections sensu Garay (1973): Aca = *Acaulia*, Agd = *Angraecoides*, Ang = *Angraecum*, Arc = *Arachnangraecum*, Bor = *Boryangraecum*, Chl = *Chlorangraecum*, Fil = *Filangis*, Gom = *Gomphocentrum*, Had = *Hadrangis*, Hum = *Humblotiangraecum*, Lem = *Lemurangis*, Lep = *Lepervenchea*, Pct = *Pectinaria*, Per = *Perrierangraecum*, Psj = *Pseudojumellea*.

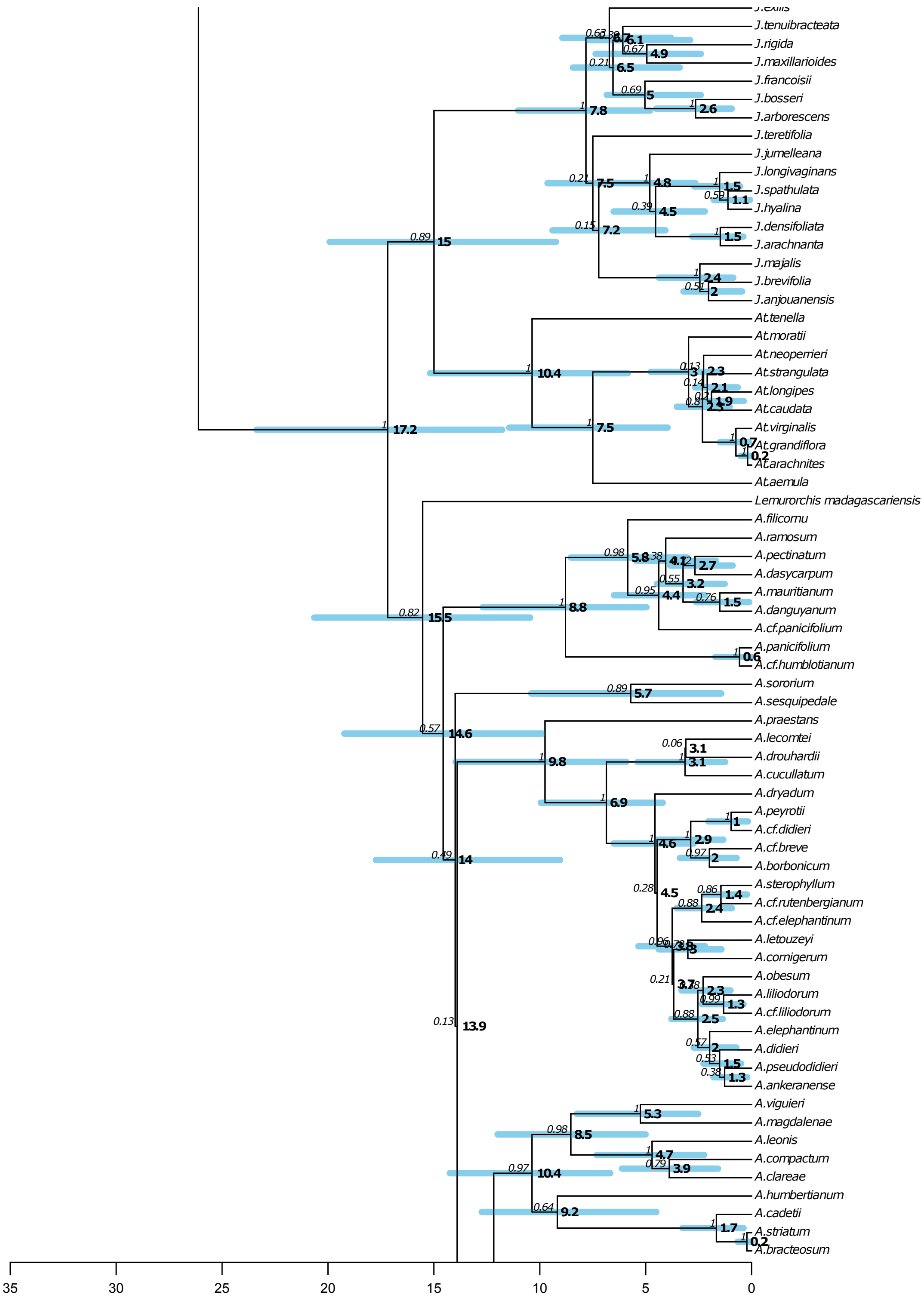

Jumellea spathulata
Jumellea hyalina
Jumellea longivaginans
Jumellea exilis
Jumellea maxillarioides
Jumellea arachnantha
Jumellea teretifolia
Jumellea tenuibracteata
1
Jumellea rigida
Jumellea majalis
Jumellea jumelleana
Jumellea francoisii
Jumellea densifoliata
Jumellea brevifolia
Jumellea bosseri
Jumellea arborescens
Jumellea anjouanensis
Angraecum viguieri (Hum)
1
Angraecum praestans (Hum)
Angraecum magdalenae (Hum)
Angraecum compactum (Per)
1
Angraecum clareae (Per)
1
Angraecum leonis (Hum)
clade G
Angraecum longicalcar (Ang)
1
Angraecum eburneum var.xerophilum (Ang)
Angraecum eburneum var.superbum (Ang)
Angraecum eburneum var.eburneum (Ang)
Lemurorchis madagascariensis
clade K
Angraecum sororium (Ang)
Angraecum sesquipedale (Ang)
clade E
Angraecum germinyanum (Arc)
1
Angraecum cf.germinyanum (Arc)
1
Angraecum conchoglossum (Arc)
Angraecum arachnites (Arc)
Angraecum mirabile (Arc)
0.9
Angraecum conchiferum (Arc)
Angraecum expansum (Arc)
1
Angraecum corrugatum (Arc)
1
Angraecum appendiculatum (Arc)
Angraecum linearifolium (Arc)
Angraecum scottianum (Arc)
1
Angraecum pseudofilicornu (Arc)
Angraecum oblongifolium (Per)
clade J
Aeranthes virginalis
1
Aeranthes grandiflora
Aeranthes caudata
Aeranthes longipes
Aeranthes arachnites
Aeranthes aemula
Aeranthes moratii
Aeranthes strangulata
1
Aeranthes neoperrieri
Aeranthes tenella
Microcoelia stolzii
1
Microcoelia gilpinae
Campylocentrum sullivanii
Dendrophylax funalis
Angraecum pterophyllum*
1
Angraecum perparvulum*
Angraecum cf.humile*
Angraecum striatum (Had)
1
Angraecum cadetii (Had)
Angraecum bracteosum (Had)
clade F
Dolabrifolia disticha*
1
Dolabrifolia bancoense*
Dolabrifolia cf.aporoides*
1
Dolabrifolia podochiloides*
Pectinariella subulata*
1
Pectinariella pungens*
Pectinariella gabonense*
Pectinariella doratophyllum*
Pectinariella atlantica*
Angraecum pectinatum (Pct)
1
Angraecum dasycarpum (Pct)
Angraecum cf.humblotianum (Pct)
Angraecum panicifolium (Pct)
1
Angraecum cf.panicifolium (Pct)
1
Angraecum filicornu (Fil)
Angraecum danguyanum (Arc)
clade C
Angraecum musculiferum (Lep)
1
Angraecum caricifolium (Lep)
1
Angraecum cf.pauciramosum (Lep)
Angraecum pauciramosum (Lep)
1
Angraecum madagascariense (Lem)
Angraecum tenuispica (Lep)
clade M
Angraecum triangulifolium (Agd)
1
Angraecum pingue (Agd)
Angraecum sedifolium (Agd)
Ypsilopus viridiflorus
Angraecum rhynchoglossum*
clade I
Angraecum caulescens (Gom)
1
Angraecum calceolus (Gom)
Angraecum cf.zeylanicum (Gom)
Angraecum multiflorum (Gom)
Angraecum huntleyoides (Chl)
clade M
1
Solenangis wakefieldii
Solenangis clavata
1
Rhipidoglossum xanthopollinium
Rhipidoglossum subsimplex
ANGRAECINAE
Ypsilopus longifolius
Rangaeris muscicola
Rangaeris amaniensis
Listrostachys pertusa
Aerangis hildebrandtii
1
Aerangis hariotiana
Diaphananthe vesicata
1
Diaphananthe sarcophylla

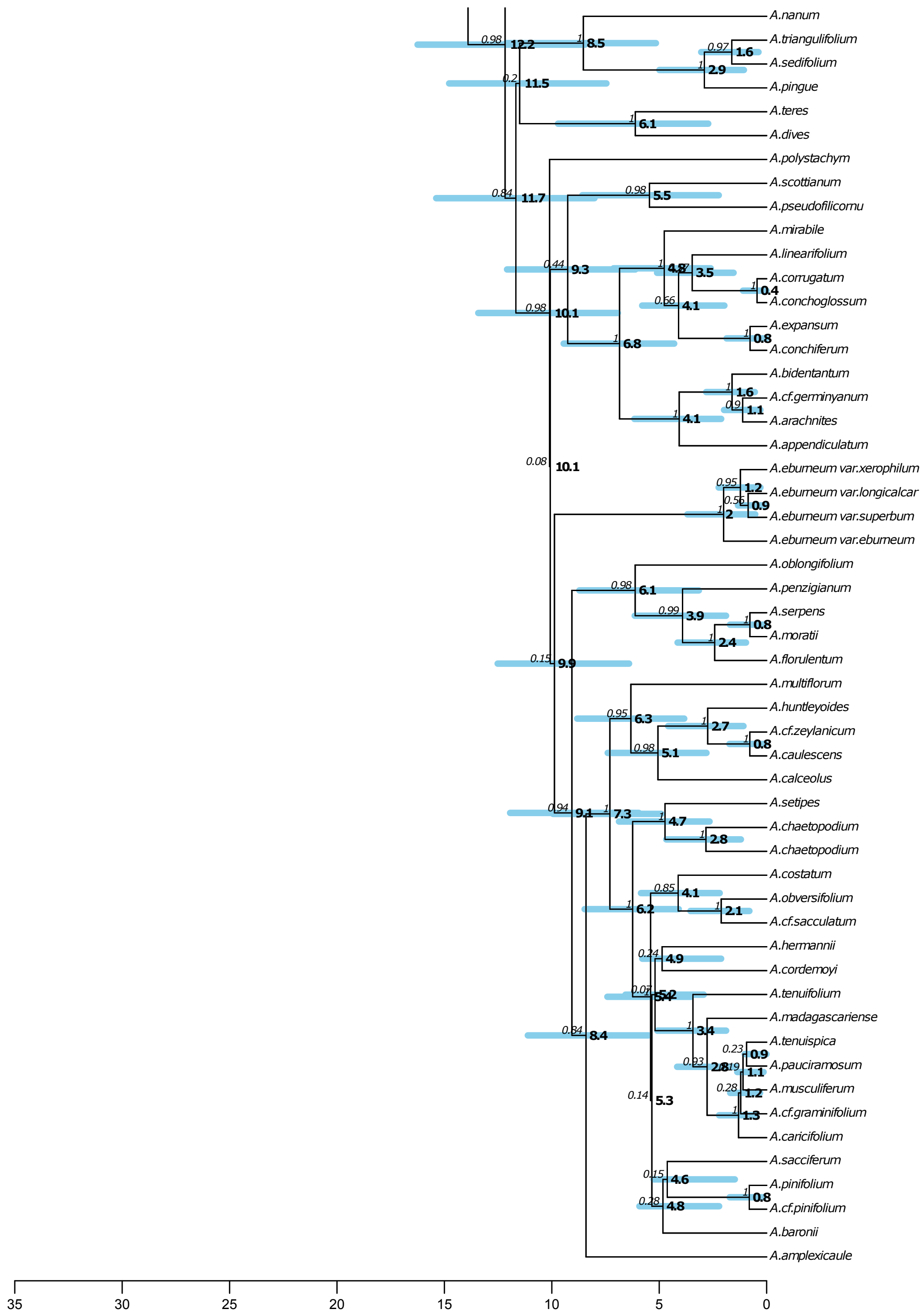

Angraecoides erecta*
Angraecoides cultriforme*
Angraecoides chevalieri*
Angraecoides cf.moandense*
Cryptopus paniculatus
Cryptopus elatus
Oeonia rosea
Ancistrorhynchus recurvus
Ancistrorhynchus capitatus
Podangis dactyloceras
Angraecum drouhardii (Per)
Angraecum cucullatum (Per)
Angraecum lecomtei (Per)
Angraecum didieri (Per)
Angraecum ankeranense (Per)
Angraecum bicallosum (Per)
Angraecum hermannii (Pct)
Angraecum baronii (Lem)
Angraecum costatum (Lem)
Eichlerangraecum infundibulare*
Eichlerangraecum birrimense*
Eichlerangraecum cf.eichlerianum*
Tridactyle filifolia
Tridactyle bicaudata
Lemurella papillosa
Lemurella pallidiflora
Eurychone rothschildiana
Eurychone galeandrae
Cyrtorchis chailluana
Cyrtorchis arcuata
Cribbia confusa
Cribbia brachyceras
Bolusiella maudiae
Bolusiella iridifolia
Sobennikoffia humbertiana
Beclardia macrostachya
Angraecum serpens (Ang)
Angraecum penzigianum (Ang)
Angraecum pseudodidieri (Per)
Angraecum obesum (Per)
Angraecum setipes (Aca)
Angraecum ochraceum (Aca)
Angraecum obversifolium (Agd)
Angraecum cf.sacculatum (Gom)
Angraecum pinifolium (Bor)
Angraecum cf.pinifolium (Bor)
Sphyrarhynchus schliebenii
Oeoniella polystachys
Neobathiea grandidierana
Mystacidium flanaganii
Mystacidium capense
Diaphananthe pellucida
Calyptrochilum christyanum
Angraecopsis parviflora
Erasanthe henricii
Aerangis punctata
Aerangis macrocentra
Aerangis ellisii
Angraecum teres
Angraecum tenuifolium (Lep)
Angraecum sterophyllum (Per)
Angraecum sacciferum (Bor)
Angraecum ramosum (Arc)
Angraecum nanum (Nana)
Angraecum peyrotii (Per)
Angraecum moratii (Fil)
Angraecum mauritianum (Psj)
Angraecum liliodorum (Per)
Angraecum letouzeyi (Per)
Angraecum florulentum (Ang)
Angraecum elephantinum (Per)
Angraecum dives (Bor)
Angraecum cf.rutenbergianum (Per)
Angraecum cf.elephantinum (Per)
Angraecum cf.peyrotii (Per)
Angraecum cornigerum (Fil)
Angraecum cordemoyi (Gom)
Angraecum cf.breve (Per)
Angraecum cf.liliodorum (Per)
Angraecum chaetopodium (Aca)
Angraecum borbonicum (Per)
Angraecum amplexicaule (Psj)
Vanda tricolor
Acampe ochracea
Aerides odoratum
Phalaenopsis cornu-cervi
Polystachya fulvilabia

Annexe 8. Dated phylogenetic tree

Maximum credibility tree of the calibrated relaxed molecular clock analysis of Angraecinae inferred from combined plastid *mat*K, *rps*16 and *trn*L sequences. Posterior probabilities are displayed above branches in italics; node ages are indicated in bold, with blue bars representing the 95% highest height probability densities (HPD) of the node. Abbreviations: A, *Angraecum*; At, *Aeranthes*; J, *Jumellea*.

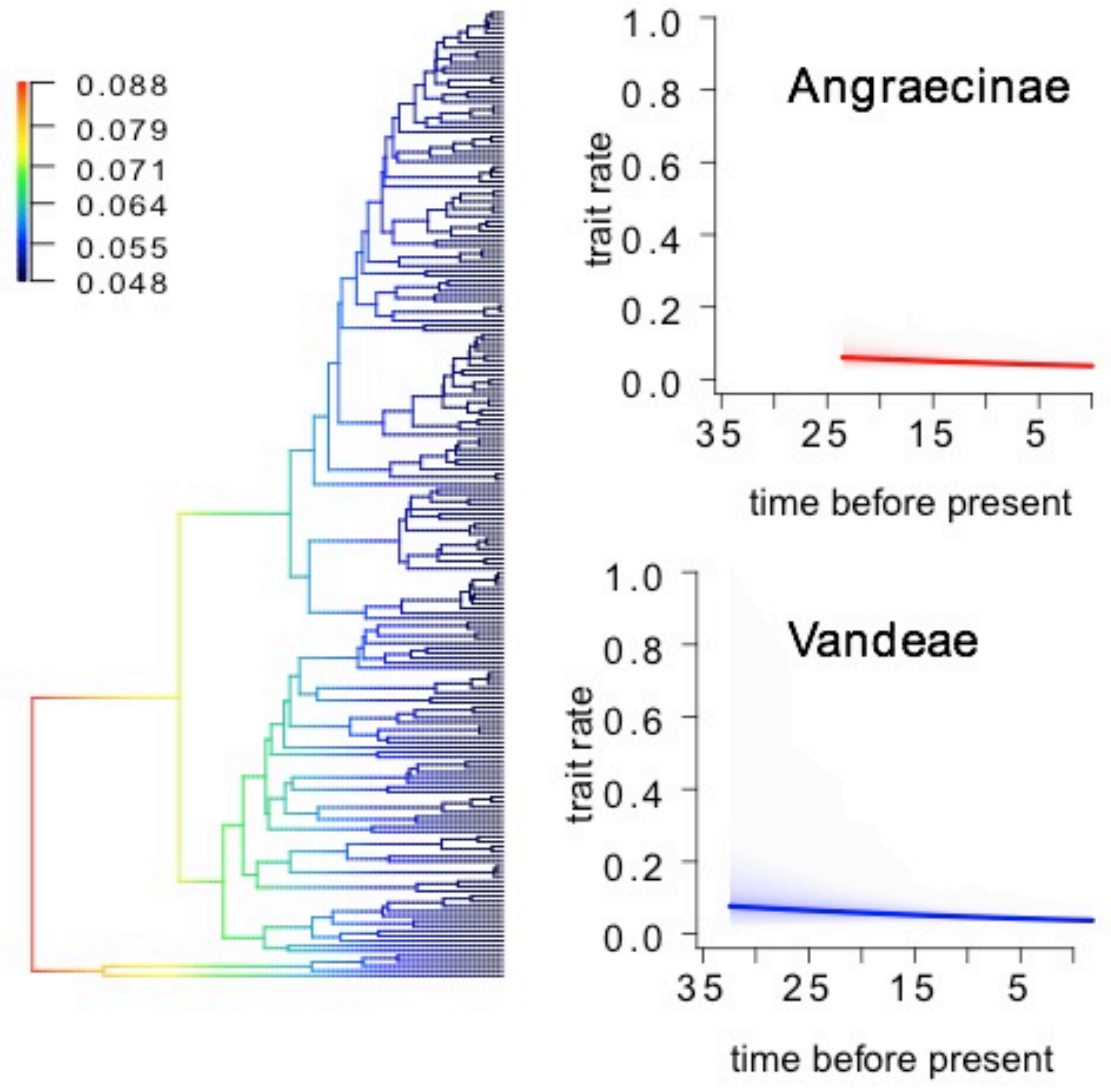

Polystachya fulvilabia
Phalaenopsis cornu-cervi
Vanda tricolor
Aerides odorata
Acampe ochracea
Beclardia macrostachya
Erasanthe henrici
Lemurella papillosa
Lemurella pallidiflora
Oeonia rosea
Cryptopus paniculatus
Cryptopus elatus
Neobathiea grandidierana
Bosserangraecum rhynchoglossum
Parangraecum perparvulum
Parangraecum pterophyllum
Parangraecum cf. humile
Diaphananthe pellucida
Diaphananthe vesicata
Diaphananthe sarcophylla
Dendrophylax funalis
Campylocentrum fasciola
Angraecoides erecta
Angraecoides cultriformis
Angraecoides chevalieri
Angraecoides cf. moandense
Eichlerangraecum cf. eichlerianum
Eichlerangraecum infundibulare
Eichlerangraecum birrimense
Bolusiella maudiae
Bolusiella iridifolia
Eurychone rothschildiana
Eurychone galeandrae
Aerangis punctata
Aerangis macrocentra
Aerangis hildebrandtii
Aerangis hariotiana
Aerangis ellisii
Dolabrifolia podochiloides
Dolabrifolia cf. aporoides
Dolabrifolia disticha
Dolabrifolia bancoensis
Solenangis wakefieldii
Solenangis clavata
Microcoelia stolzii
Microcoelia gilpinae
Rhipidoglossum xanthopollinium
Rhipidoglossum subsimplex
Cribbia confusa
Cribbia brachyceras
Mystacidium flanaganii
Mystacidium capense
Sphyrarhynchus schliebenii
Angraecopsis parviflora
Calyptrochilum christyanum
Ancistrorhynchus recurvus
Ancistrorhynchus capitatus
Podangis dactyloceras
Cyrtorchis chailluana
Cyrtorchis arcuata
Rangaeris muscicola
Ypsilopus viridiflorus
Ypsilopus longifolius
Rangaeris amaniensis
Tridactyle filifolia
Tridactyle bicaudata
Listrostachys pertusa
Pectinariella subulata
Pectinariella doratophylla
Pectinariella gabonensis
Pectinariella pungens
Pectinariella atlantica
34.2
27.7
13.4
7.4
9.8
15.8
18.1
6.9
13.2
4.4
2.3
9.5
15
7.6
12.8
10.1
21.1
6.8
3.9
18.5
8.4
11.8
1.8
4.4
15.8
0.7
0.6
0.4
19.5
8.4
15.9
6.4
14.3
6.9
16
6.7
2.6
6.3
4.5
2.4
0.9
17.6
9.6
9.3
17.4
7.6
6.5
2.3
17.2
11.1
1.8
9
8.3
16
14.6
5.6
26.1
15.6
10.2
2.3
13.3
8.7
5.6
6.7
13.1
2.6
13
11.9
4.8
2.3
1.1
0.9
35
30
25
20
15
10
5
0


J.exilis
J.tenuibracteata
J.rigida
J.maxillarioides
J.francoisii
J.bosseri
J.arborescens
J.teretifolia
J.jumelleana
J.longivaginans
J.spathulata
J.hyalina
J.densifoliata
J.arachnanta
J.majalis
J.brevifolia
J.anjouanensis
At.tenella
At.moratii
At.neoperrieri
At.strangulata
At.longipes
At.caudata
At.virginalis
At.grandiflora
At.arachnites
At.aemula
Lemurorchis madagascariensis
A.filicornu
A.ramosum
A.pectinatum
A.dasycarpum
A.mauritianum
A.danguyanum
A.cf.panicifolium
A.panicifolium
A.cf.humblotianum
A.sororium
A.sesquipedale
A.praestans
A.lecomtei
A.drouhardii
A.cucullatum
A.dryadum
A.peyrotii
A.cf.didieri
A.cf.breve
A.borbonicum
A.sterophyllum
A.cf.rutenbergianum
A.cf.elephantinum
A.letouzeyi
A.cornigerum
A.obesum
A.liliodorum
A.cf.liliodorum
A.elephantinum
A.didieri
A.pseudodidieri
A.ankeranense
A.viguieri
A.magdalenae
A.leonis
A.compactum
A.clareae
A.humbertianum
A.cadetii
A.striatum
A.bracteosum
35
30
25
20
15
10
5
0


A.nanum
A.triangulifolium
A.sedifolium
A.pingue
A.teres
A.dives
A.polystachym
A.scottianum
A.pseudofilicornu
A.mirabile
A.linearifolium
A.corrugatum
A.conchoglossum
A.expansum
A.conchiferum
A.bidentantum
A.cf.germinyanum
A.arachnites
A.appendiculatum
A.eburneum var.xerophilum
A.eburneum var.longicalcar
A.eburneum var.superbum
A.eburneum var.eburneum
A.oblongifolium
A.penzigianum
A.serpens
A.moratii
A.florulentum
A.multiflorum
A.huntleyoides
A.cf.zeylanicum
A.caulescens
A.calceolus
A.setipes
A.chaetopodium
A.chaetopodium
A.costatum
A.obversifolium
A.cf.sacculatum
A.hermannii
A.cordemoyi
A.tenuifolium
A.madagascariense
A.tenuispica
A.pauciramosum
A.musculiferum
A.cf.graminifolium
A.caricifolium
A.sacciferum
A.pinifolium
A.cf.pinifolium
A.baronii
A.amplexicaule
35
30
25
20
15
10
5
0

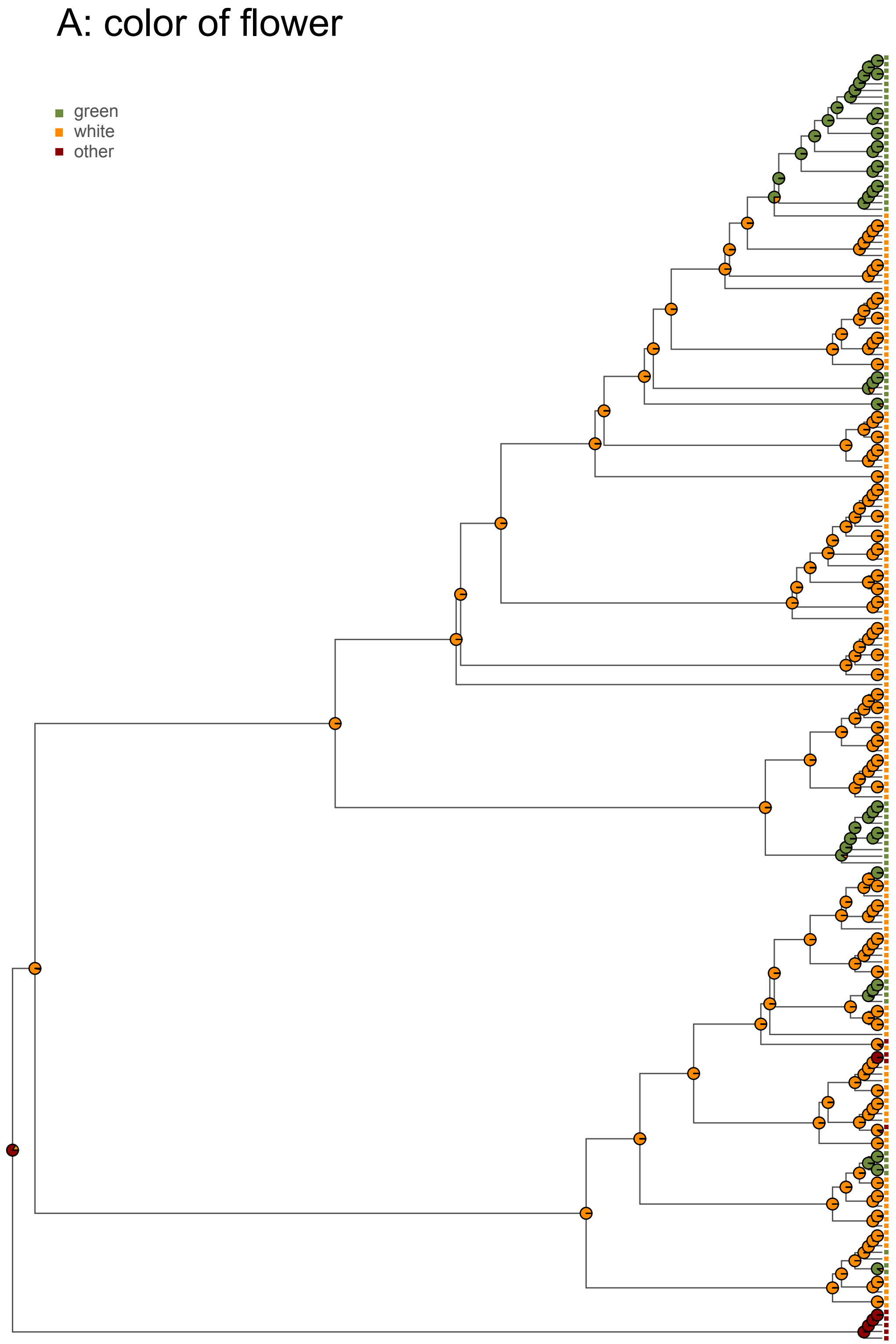
Annexe 9. Spur length BAMM analysis

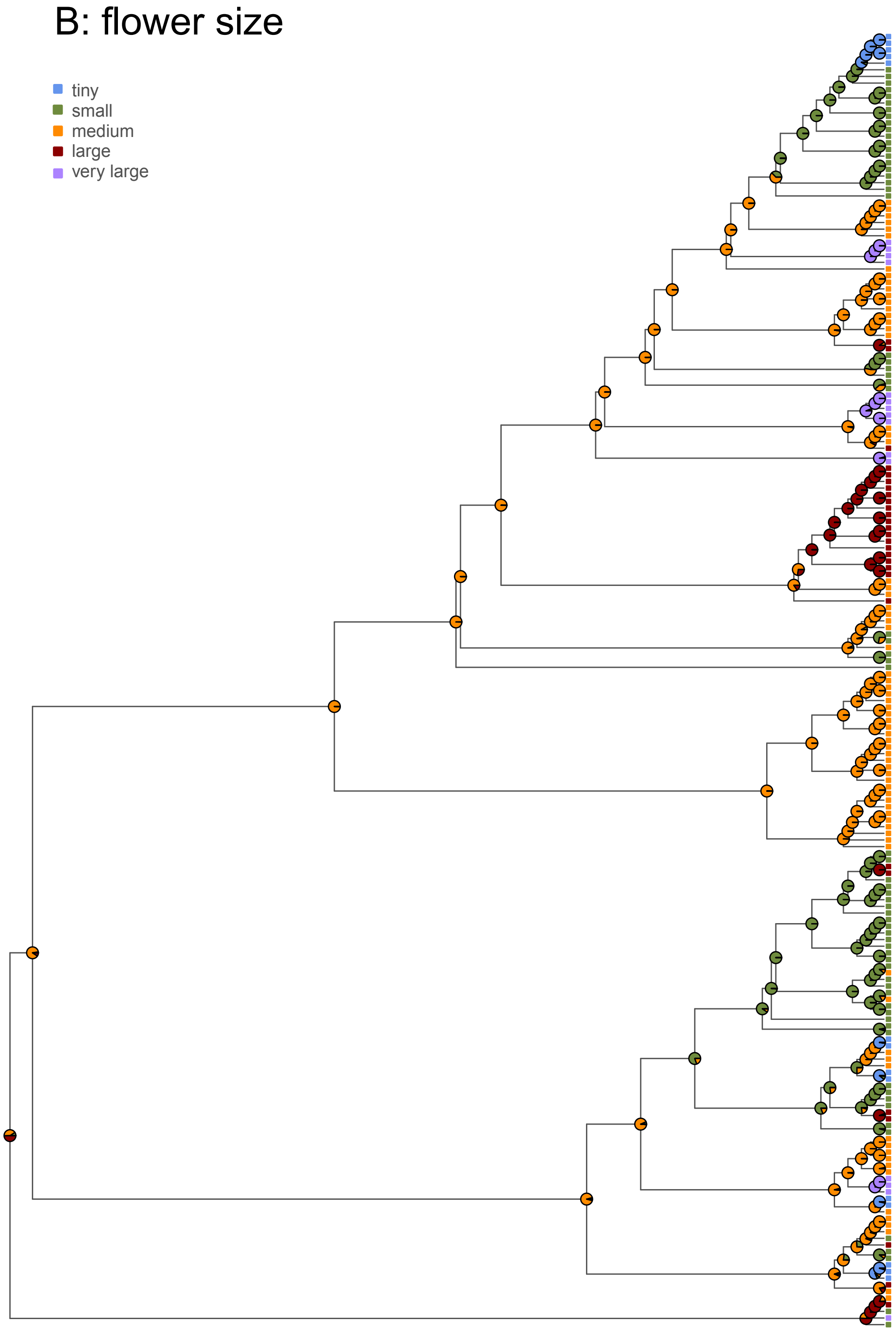
Configuration shifts from the 95% credible set sampled by BAMM for the evolution of spur length across a phylogenetic tree of Angraecinae and evolutionary rates through time. The intensity of colors on branches reflects the instantaneous rate of phenotypic evolution (cool colors = slow, warm = fast). The red curve illustrates the mean speciation rate-through-time trajectory of Angraecinae in million years, and blue that of Vandeae.

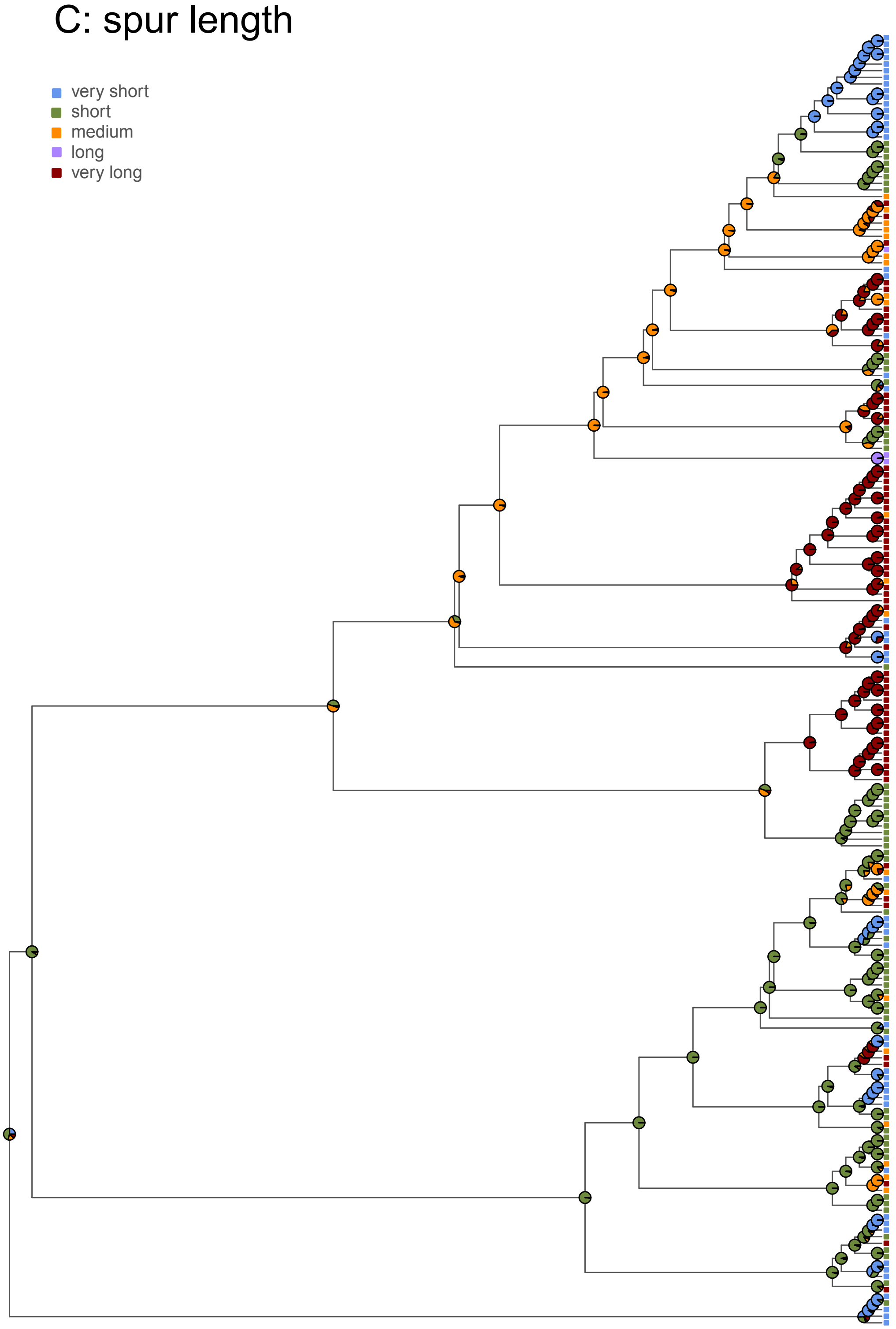


Annexe 10. Ancestral state reconstructions of floral traits

Ancestral state reconstructions of floral traits in Angraecinae implemented in ‘diversitree’; colors represent character states and pie charts represent the probability of ancestral states at node. Characters: A, flower color; B, flower size; C, spur length.

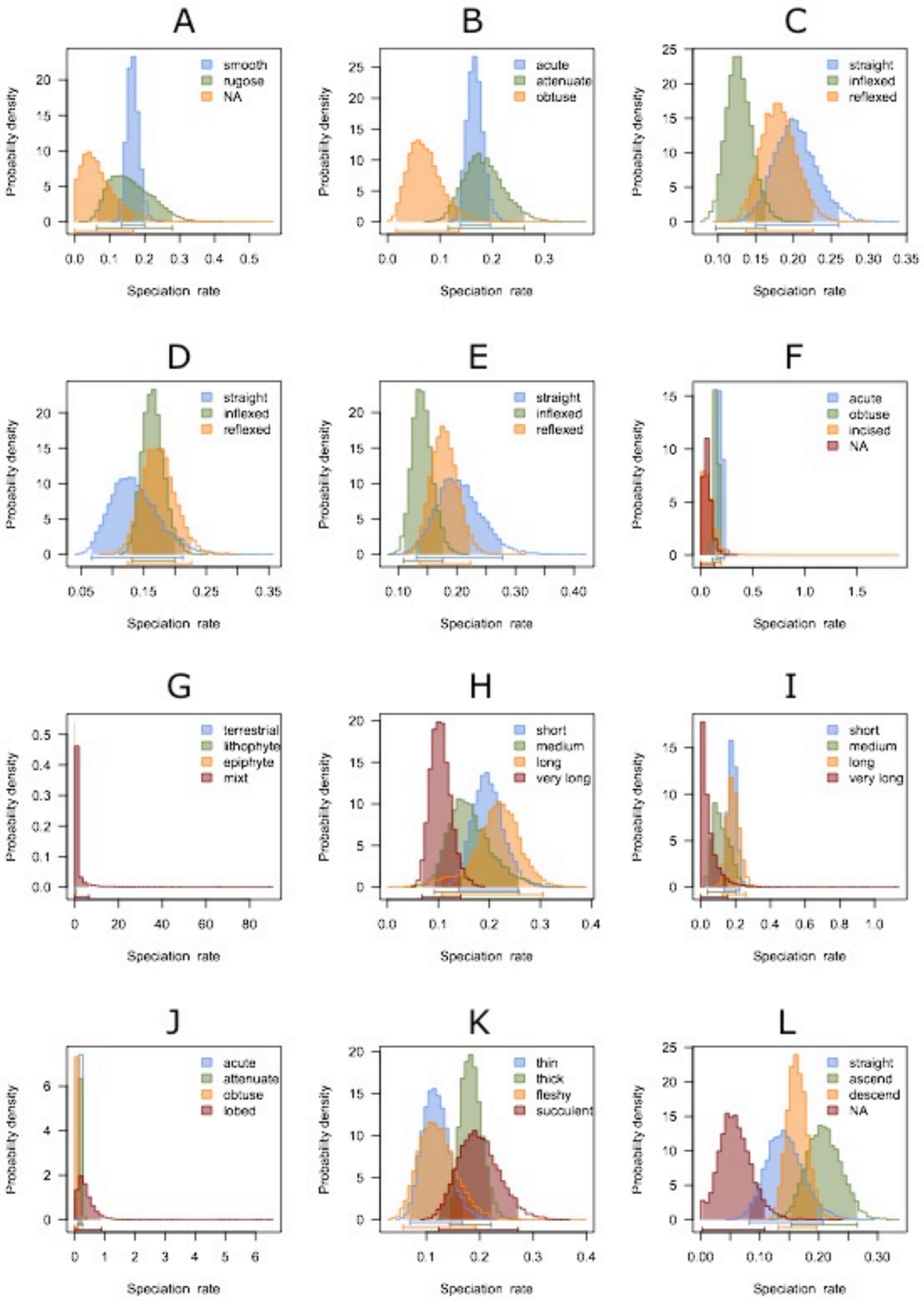

A: color of flower
green
white
other

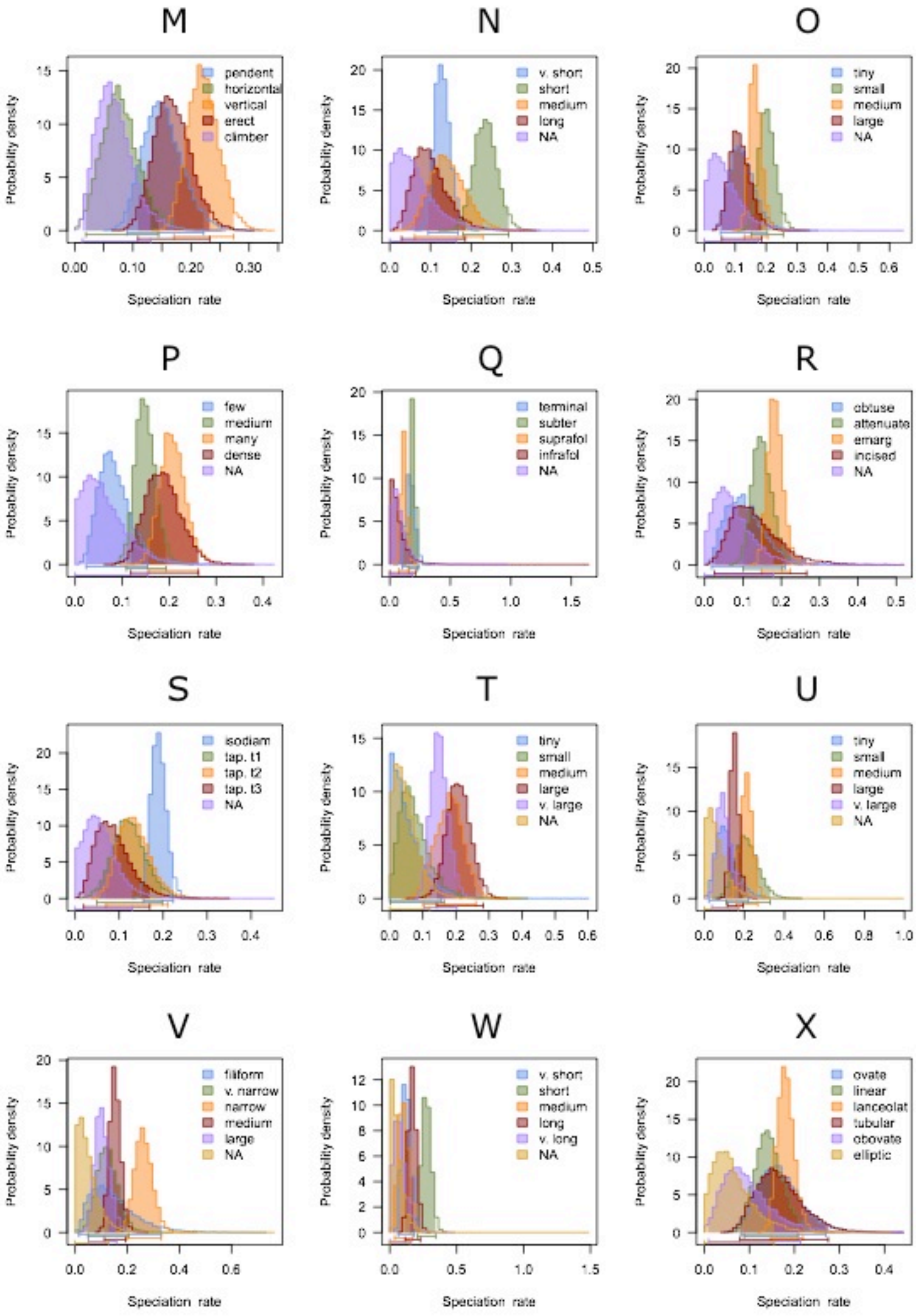

B: flower size
🟦 tiny
🟩 small
🟧 medium
🟥 large
🟪 very large


C: spur length
very short
short
medium
long
very long

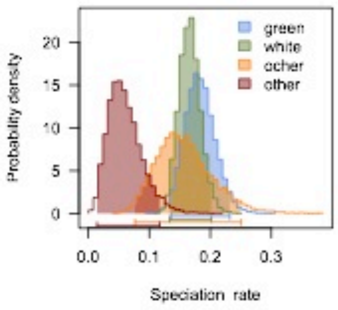
Annexe 11. Speciation analyses

Posterior probability distributions for the speciation rates (in Ma) of morphological characters of subtribe Angraecinae inferred by the MuSSE model. A, leaf surface; B, sepal apex; C, orientation of dorsal sepal; D, orientation of lateral sepal; E, orientation of lateral petal; F, spur apex; G, habitat; H, inflorescence length; I, pedicel length; J, petal apex; K, flower texture; L, spur orientation; M, habit; N, internode; O, stem diameter; P, number of leaves; Q, position of inflorescence; R, leaf apex; S, spur base; T, natural spread (vertical); U, natural spread (horizontal); V, leaf width; W, leaf-length; X, sepal shape; AA, sepal color; AB, petal color; AC, spur color; AD, 3D labellum shape; AE, leaf texture; AF, petal shape; AG, type of inflorescence; AH, spur shape; AI, labellum apex; AJ, 2D labellum shape; AK, leaf shape. Abbreviations: circin, circinnate; coriac, coriaceous; herb, herbaceous; infrafol, infrafoliar; infundi, infundibular; lanceo, lanceolate; memb, membraneous; NA, not applicable; race, raceme; scoop, scoop-shaped; sol, solitary; subter, subterminal; succ, succulent; suprafol, suprafoliar; t, type; tap, tapering; triang, triangular; v, very.


A
smooth
rugose
NA
Probability density
0
5
10
15
20
0.0
0.1
0.2
0.3
0.4
0.5
Speciation rate
B
acute
attenuate
obtuse
Probability density
0
5
10
15
20
25
0.0
0.1
0.2
0.3
Speciation rate
C
straight
inflexed
reflexed
Probability density
0
5
10
15
20
25
0.10
0.15
0.20
0.25
0.30
0.35
Speciation rate
D
straight
inflexed
reflexed
Probability density
0
5
10
15
20
0.05
0.15
0.25
0.35
Speciation rate
E
straight
inflexed
reflexed
Probability density
0
5
10
15
20
0.10
0.20
0.30
0.40
Speciation rate
F
acute
obtuse
incised
NA
Probability density
0
5
10
15
0.0
0.5
1.0
1.5
Speciation rate
G
terrestrial
lithophyte
epiphyte
mixt
Probability density
0.0
0.1
0.2
0.3
0.4
0.5
0
20
40
60
80
Speciation rate
H
short
medium
long
very long
Probability density
0
5
10
15
20
0.0
0.1
0.2
0.3
0.4
Speciation rate
I
short
medium
long
very long
Probability density
0
5
10
15
0.0
0.2
0.4
0.6
0.8
1.0
Speciation rate
J
acute
attenuate
obtuse
lobed
Probability density
0
2
4
6
0
1
2
3
4
5
6
Speciation rate
K
thin
thick
fleshy
succulent
Probability density
0
5
10
15
20
0.1
0.2
0.3
0.4
Speciation rate
L
straight
ascend
descend
NA
Probability density
0
5
10
15
20
25
0.00
0.10
0.20
0.30
Speciation rate


M
Probability density
0
5
10
15
pendent
horizontal
vertical
erect
climber
0.00
0.10
0.20
0.30
Speciation rate
N
Probability density
0
5
10
15
20
v. short
short
medium
long
NA
0.0
0.1
0.2
0.3
0.4
0.5
Speciation rate
O
Probability density
0
5
10
15
20
tiny
small
medium
large
NA
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Speciation rate
P
Probability density
0
5
10
15
few
medium
many
dense
NA
0.0
0.1
0.2
0.3
0.4
Speciation rate
Q
Probability density
0
5
10
15
20
terminal
subter
suprafol
infrafol
NA
0.0
0.5
1.0
1.5
Speciation rate
R
Probability density
0
5
10
15
20
obtuse
attenuate
emarg
incised
NA
0.0
0.1
0.2
0.3
0.4
0.5
Speciation rate
S
Probability density
0
5
10
15
20
isodiam
tap. t1
tap. t2
tap. t3
NA
0.0
0.1
0.2
0.3
0.4
Speciation rate
T
Probability density
0
5
10
15
tiny
small
medium
large
v. large
NA
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Speciation rate
U
Probability density
0
5
10
15
tiny
small
medium
large
v. large
NA
0.0
0.2
0.4
0.6
0.8
1.0
Speciation rate
V
Probability density
0
5
10
15
20
filiform
v. narrow
narrow
medium
large
NA
0.0
0.2
0.4
0.6
Speciation rate
W
Probability density
0
2
4
6
8
10
12
v. short
short
medium
long
v. long
NA
0.0
0.5
1.0
1.5
Speciation rate
X
Probability density
0
5
10
15
20
ovate
linear
lanceolat
tubular
obovate
elliptic
0.0
0.1
0.2
0.3
0.4
Speciation rate

AA



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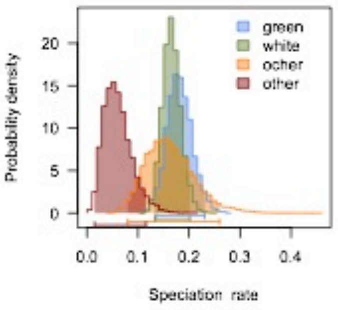

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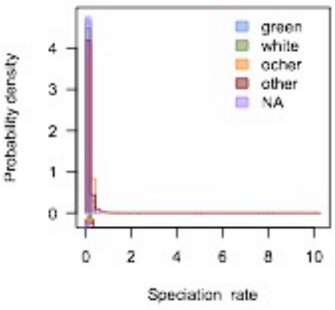

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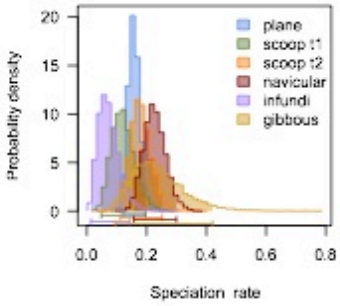

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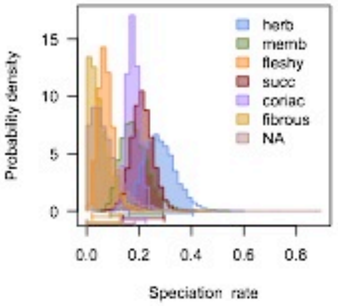

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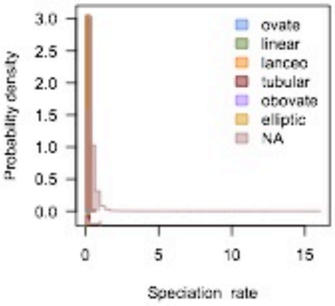

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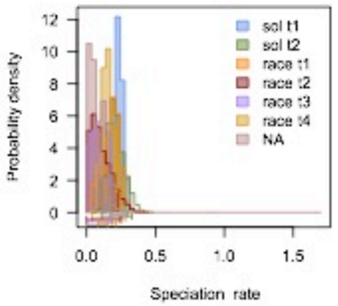

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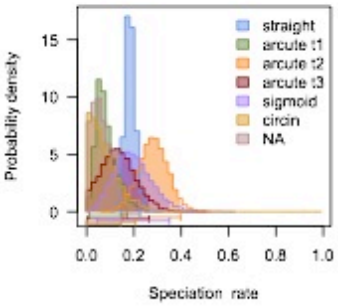

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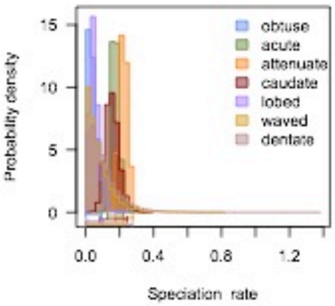

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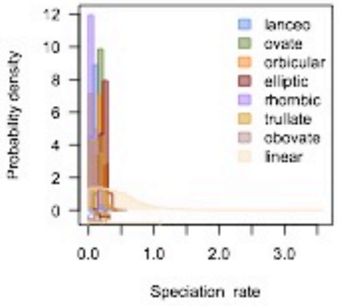

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