Université de Montréal

Systématique moléculaire et biogéographie de trois genres malgaches menacés d’extinction *Delonix*, *Colvillea* et *Lemuropisum* (Caesalpinioideae : Leguminosae)

par

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Mémoire présenté à la Faculté des Arts et Sciences
en vue de l’obtention du grade de Maîtrise
en sciences biologiques

février 2013

Université de Montréal
Faculté des études supérieures et postdoctorales

Ce mémoire intitulé :

Systématique moléculaire et biogéographie de trois genres malgaches menacés d'extinction *Delonix, Colvillea* et *Lemuropisum* (Caesalpinioideae : Leguminosae)

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Anne Bruneau, directrice de recherche
Simon Joly, membre du jury
Luc Brouillet, président du jury

Résumé

Ce mémoire porte sur les relations phylogénétiques, géographiques et historiques du genre afro-malgache *Delonix* qui contient onze espèces et des genres monospécifiques et endémiques *Colvillea* et *Lemuropisum*. Les relations intergénériques et interspécifiques entre les espèces de ces trois genres ne sont pas résolues ce qui limite la vérification d'hypothèses taxonomiques, mais également biogéographiques concernant la dispersion de plantes depuis ou vers Madagascar. Une meilleure compréhension des relations évolutives et biogéographiques entre ces espèces menacées d'extinction permettrait une plus grande efficacité quant à leur conservation. L'objectif de ce mémoire est de reconstruire la phylogénie des espèces à l'aide de régions moléculaires des génomes chloroplastique et nucléaire, d'identifier les temps de divergences entre les espèces et de reconstruire l'aire géographique ancestrale pour chacun des groupes. Ce projet démontre que le genre *Delonix* n'est pas soutenu comme étant monophylétique et qu'une révision taxonomique s'impose. Les relations intergénériques demeurent floues quant à la position phylogénétique de *Colvillea* et nos résultats suggèrent de l'hybridation ou un assortiment incomplet de cette lignée. Les espèces sont apparues et se sont diversifiées au Miocène à partir d'un ancêtre commun du sud de Madagascar. La phylogénie montre deux clades associés aux aires géographiques de répartition des espèces opposant les espèces largement répandues à celles majoritairement restreintes au fourré aride. Différentes hypothèses afin d'expliquer la dispersion des *Delonix* africains au Miocène à partir de Madagascar sont discutées. Un point de mire sur les interactions biotiques et abiotiques, passées et présentes, dans le fourré aride de Madagascar est recommandé en terme de conservation.

Mots-clés : *Delonix*, *Colvillea*, *Lemuropisum*, loci nucléaire à faible copie, phylogénie, biogéographie, taxonomie, Madagascar, dispersion longue distance, conservation

Abstract

This thesis discusses the phylogenetic, geographic, and historic relationships of the eleven species of the afro-madagascan genus *Delonix*, as well as the endemic, monospecific genera *Colvillea* and *Lemuropisum*. The intergeneric and interspecific relationships of these taxa are unresolved which limits the evaluation of taxonomic changes suggested by some studies and of conflicting biogeographical hypotheses regarding plant dispersal around Madagascar. These species are all endangered, therefore a better understanding of their evolutionary pattern would benefit conservation efforts in Madagascar. The objective of this thesis is to reconstruct a phylogeny using multiple loci from two distinct genomes, and to use it to reconstruct the ancestral geographic range of species and estimate the times of divergence of the lineages. Results indicate that genus *Delonix* is not supported as monophyletic and a taxonomic revision is necessary. The exact position for *Colvillea* is ambiguous and suggests hybridization or incomplete lineage sorting for this taxon. Species evolved mostly during the Miocene from southern Madagascan ancestors. The phylogeny shows two clades associated with the geographic distribution of species, opposing the widespread species to the rare and mostly southern, endemic, spiny thicket species. The different hypotheses that could explain the Miocene long distance dispersal of the two African *Delonix* species from Madagascar are discussed. We recommend that conservation efforts should focus on past and present biotic and abiotic interactions in the Madagascar spiny thicket, considering that this unique habitat is an evolutionary cradle for at least three genera.

Keywords : *Delonix*, *Colvillea*, *Lemuropisum*, low-copy nuclear loci, phylogeny, biogeography, taxonomy, Madagascar, long distance dispersal, conservation

Table des matières

Liste des tableaux

Liste des figures

Liste des abréviations

ADN : acide désoxyribonucléique

AIGP : auxin-indpt growth promotor

ALLcp : matrice concaténée des loci chloroplastiques

ALLcpnc : matrice concaténée des loci chloroplastiques et nucléaires

ALLnc : matrice concaténée des loci nucléaires

BH : L. H. Bailey Hortorium Herbarium, New York

BM : Natural History Museum Herbarium, UK

BSA : bovine serum albumin

CTAB : cetyltrimethylammonium bromide

CTP : carboxyl-terminal peptidase

DMSO : dimethyl sulfoxide

DNA : desoxyribonucleic acid

DLEP : Boyce Thompson Desert Legume Program, Arizona

E : Royal Botanical Garden in Edinburgh Herbarium, UK

EIF3E : Translation initiation factor

FHO : Oxford University Herbarium, UK

ITS : ribosomal internal transcribed spacers *ITS1* and *ITS2* and 5.8S gene

IUCN: Union Internationale pour la Conservation de la Nature/International Union for the Conservation of Nature

K : Royal Botanical Gardens, Kew Herbarium, UK

LFY : LEAFY nuclear gene

MO : Missouri Botanical Garden Herbarium, St-Louis, USA

MT : Herbier Marie-Victorin, Montréal

NY : New-York Botanical Garden Herbarium, New York

P : Muséum d'Histoire Naturelle, Paris

PCR : polymerase chain reaction

PVP : polyvinylpyrrolidone

RNG : University of Reading Herbarium, UK

tRALs : Cytosolic tRNA-Ala synthetase

US : Smithsonian Institute Herbarium, Washington

USA : United States of America

UK : United Kingdom

WAG : Herbarium of Wageningen University, Netherland

Remerciements

Je tiens à remercier ma directrice, Anne Bruneau, tous mes collègues du laboratoire de Systématique moléculaire Bruneau/Brouillet: Carole Sinou, Edeline Gagnon, Erin Zimmerman, Aurélien Lauron-Moreau, Andriana et anciens du laboratoire : Sugir Seliah, Vincent Manzanilla, Annie Archambault. Membre de jury : Simon Joly et Luc Brouillet. Également, Isabelle Rocheleau pour l'obtention de séquences de plusieurs espèces.

Je remercie également les collaborateurs à Madagascar, Rolland Ranaivojaona† et Tefyharison Andriamihajarivo, au Royal Botanic Gardens de Kew, Félix Forest et Gwilym Lewis, ainsi que les herbiers suivants pour la permission d'utiliser le matériel végétal et des échantillons d'ADN: K, MO, MT, P, US, FHO, NY, WAG. Support technique : Micheline Fréchette, Ginette Hamel, Lucie Campeau et Anne Marie Gagné.

Je remercie Les Amis du Jardin Botanique de Montréal, le Centre de la Science de la Biodiversité du Québec (CSBQ), la bourse Jacques Rousseau, le Département de sciences biologiques de UdeM, et l'Association de Botanique du Canada (ABC) pour des bourses d'excellence et de voyage à l'étranger pour des conférences. Je tiens également à remercier mes parents qui m'ont soutenu à tous moments dans mes études.

Chapitre 1 : Introduction aux genres *Delonix, Colvillea* et *Lemuropisum*

Diversité des légumineuses à Madagascar

Les régions insulaires sont un des milieux terrestres les plus vulnérables aux changements environnementaux puisqu'elles sont isolées écologiquement et ainsi chaque organisme joue un rôle déterminant dans l'écosystème réduit que représente l'île. Mondialement, l'indice de richesse endémique pour les plantes vasculaires est dix fois plus élevé à l'intérieur des îles qu'en régions continentales (Kier et al 2009). L'île de Madagascar est l'une des îles les plus touchées par la déforestation et les changements climatiques et elle possède un taux d'espèces végétales endémiques de 96%, un des plus élevé au monde (Schatz 2001, Goodman & Benstead 2005, Kier et al 2009). Située à l'est du continent africain, cette île de 587 km² est désignée comme un point chaud de biodiversité (IUCN 2012). Avec ses forêts sempervirentes à l'est en passant par les déserts arides et les forêts décidues à l'ouest, Madagascar englobe un assemblage impressionnant d'écosystèmes donnant lieu à des formations végétales diversifiées (Koechlin et al 1974). La famille de plantes vasculaires la plus représentée à Madagascar est celle des Légumineuses (Fabaceae), avec 53 genres sur l'île dont 20 sont endémiques (Schatz 2001, Figure 1). La famille des Légumineuses est la troisième plus importante famille d'angiospermes en terme de nombre d'espèces, une des plus diversifiée morphologiquement et la deuxième famille en importance économique au monde. Malgré cette importance écologique et économique, les Légumineuses ont un indice d'extinction spécifique élevé avec 16,8% des espèces qui sont menacées de disparition (Hammer & Khoshbakht 2005, Lewis et al 2005).

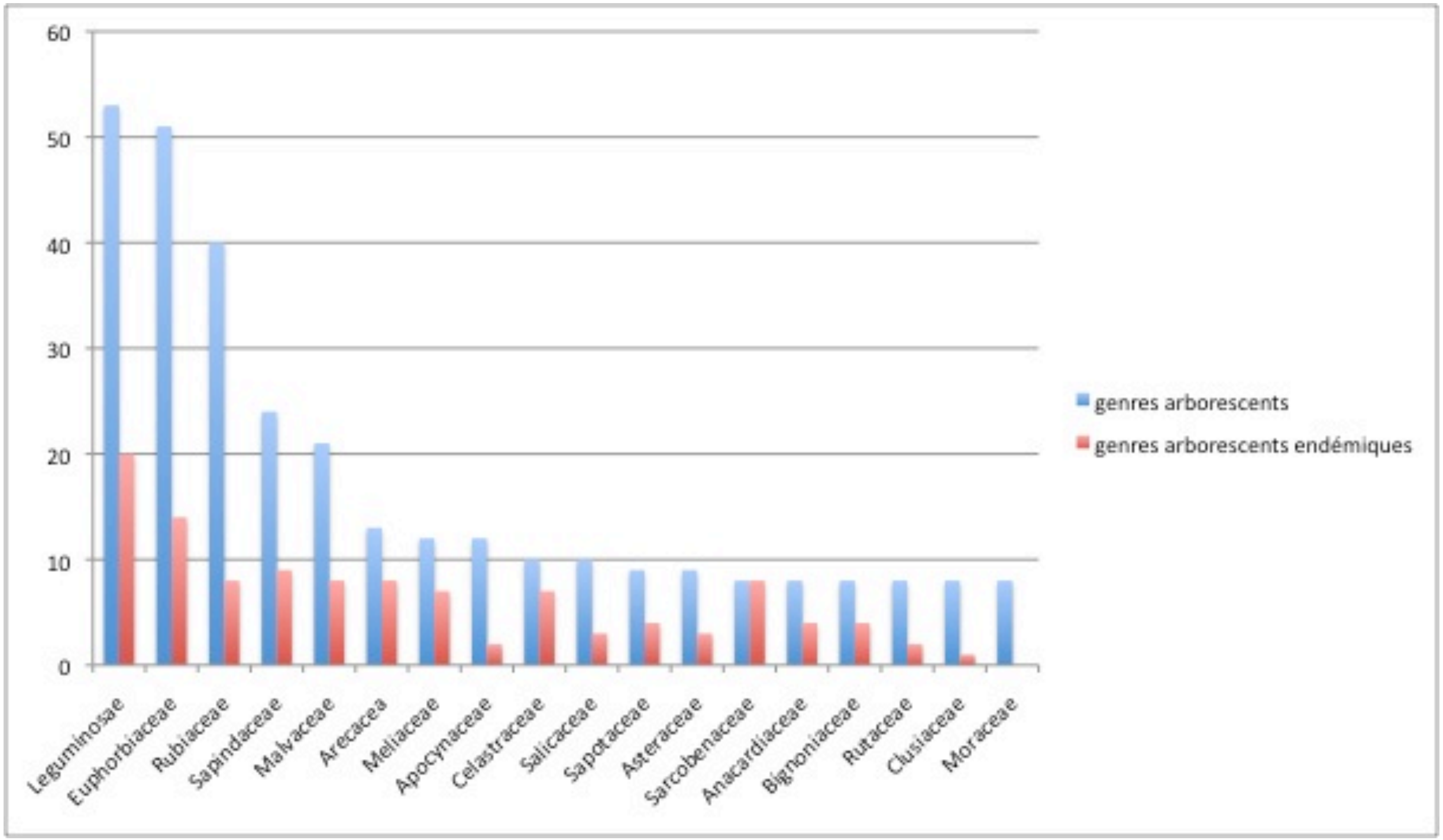


Figure 1. Histogramme de la diversité et de l'endémisme générique de la flore arborescente de Madagascar. Tiré de Schatz (2001).

Les Légumineuses se divisent en trois sous-familles, soit les Papilionoideae, les Mimosoideae et les Caesalpinioideae. Cette dernière est un assemblage paraphylétique de genres tropicaux composé majoritairement d'espèces arborescentes. Les Caesalpinioideae contiennent dix genres quasi endémiques à Madagascar et détiennent le plus grand nombre de genres monotypiques endémiques à Madagascar (DuPuy et al 2002). Un exemple est le genre afro-malgache *Delonix* Raf. dont la majorité des espèces est endémique à Madagascar et entretiennent de fortes affinités avec deux genres endémiques et monospécifiques malgaches, soit les espèces *Colvillea racemosa* Bojer ex Hook. et *Lemuropisum edule* H. Perrier (DuPuy et al 1995). Ces trois genres partagent plusieurs caractéristiques géographiques, biologiques, écologiques et économiques.

Delonix compte onze espèces, dont neuf sont endémiques à Madagascar dans des régions restreintes le long de la côte ouest (Figure 2) et deux se retrouvent en Afrique. L'une des deux espèces africaines, *Delonix elata* (L.) Gamble, est répartie de l'Afrique de l'est à l'Inde, alors que l'autre, *Delonix baccal* (Chiov.) Baker f., est endémique à la Somalie et au Kenya (DuPuy et al 1995, 2002). Trois centres de biodiversité sont identifiés à Madagascar pour les espèces du genre *Delonix* : 1) au nord à Antsiranana et sur le massif d'Ankarana, 2) à l'ouest sur le massif de Bemaraha et le plateau d'Ankara et 3) au sud-ouest à Toliara (DuPuy et al 1995). Ces espèces se trouvent surtout sur des sols calcaires, sableux et xérophytiques dans la forêt décidue sèche du nord et du nord-ouest, ainsi que dans le fourré aride du sud et de l'ouest. Ces deux types de végétation ont subi de sévères déforestations depuis les années 1950 pour faire place à l'agriculture et à pour production de charbon de bois (Harper et al 2007). *Colvillea racemosa* et *Lemuropisum edule* sont sympatriques aux espèces de *Delonix* de l'ouest, sur le plateau de Bemaraha, dans le sud-ouest près de Toliara et au sud près d'Itampolo (Figure 2).

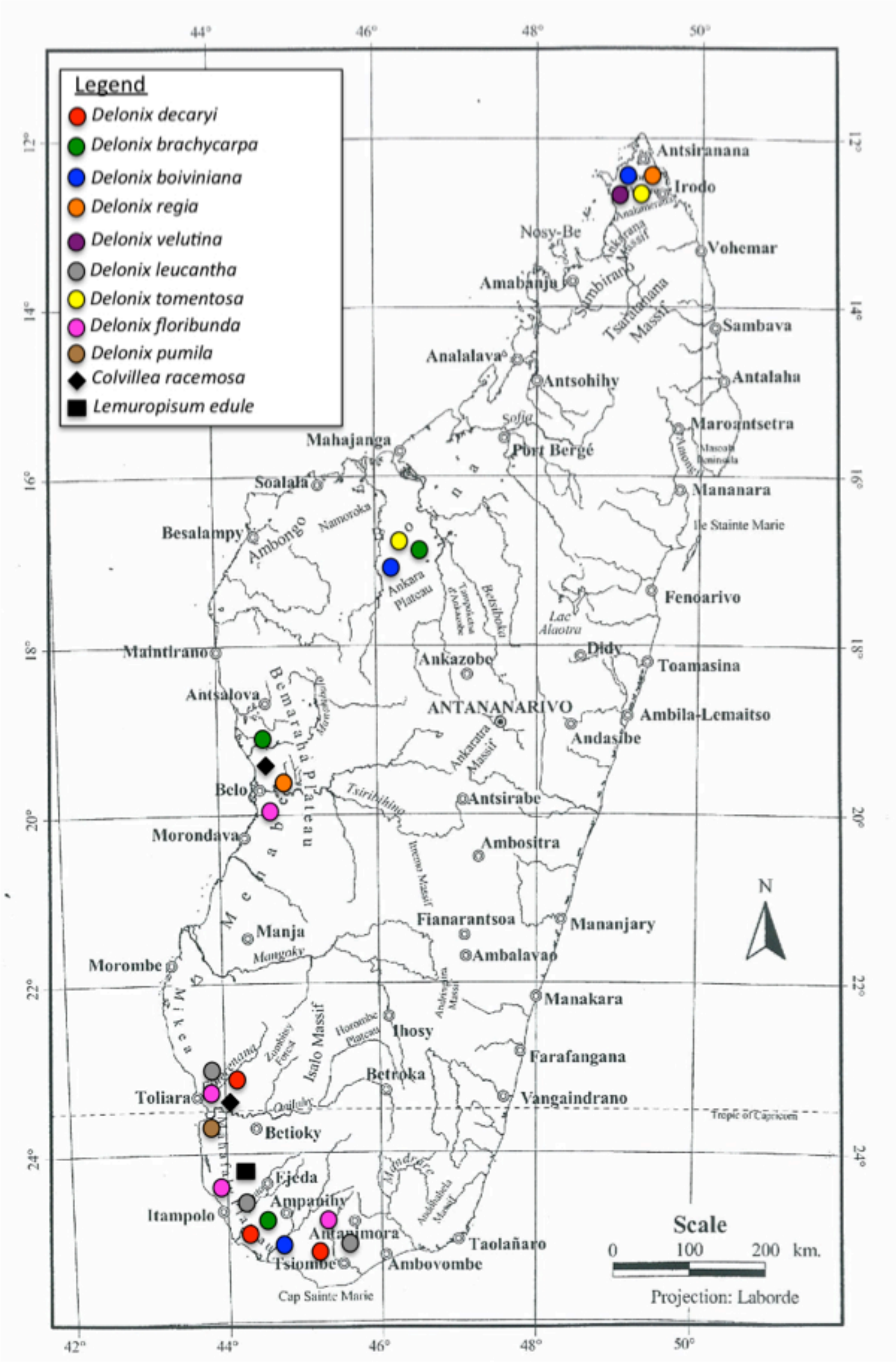


Figure 2. Répartition actuelle des espèces des genres *Delonix, Colvillea* et *Lemuropisum* à Madagascar. La carte provient de DuPuy et al (2002). Répartition établie par compilation de données de récoltes et d'observation provenant de DuPuy et al (1995), Rivers et al (2011) et de nouveaux échantillons récoltés par Anne Bruneau (2006).

Plusieurs espèces des genres *Delonix, Colvillea* et *Lemuropisum* sont d'intérêts socio-économiques à une échelle locale et internationale. Certaines sont utilisées comme arbres ornementaux dans plusieurs régions chaudes du globe (*Colvillea* et *Delonix regia* (Hook.) Raf: le Flamboyant), mais la majorité est tout particulièrement utilisée par les populations malgaches comme source d'alimentation (les graines de la majorité des espèces de *Delonix* et *Lemuropisum* sont comestibles) et comme matière première pour la construction d'habitation, de clôtures, de pirogues et de cercueils (Webb et al 1984, DuPuy et al 1995, Grant et al 1995, Kite et al 1995, Gordon & Gantz 2008, Orwa et al 2009). De plus, *D. regia* contient des toxines insecticides spécifiques aux coléoptères et est à l'étude afin de devenir un agent de lutte biologique (Alves et al 2009). L'espèce africaine *D. elata* est cultivée par le ministère de la foresterie indienne à des fins de reboisement urbain (Gagdil 2004) et *Lemuropisum edule* est à l'étude afin d'évaluer son potentiel en tant qu'espèce agricole en Afrique (Bosh et al 2009).

Statut de conservation précaire

Parmi les onze espèces de *Delonix*, neuf sont inscrites à la liste rouge d'espèces menacées de l'Union Internationale pour la Conservation de la Nature (IUCN 2012) : cinq sont considérées en danger d'être vulnérables (*Delonix boiviniana* (Baill.) Capuron*, D. brachycarpa* (R. Vig.) Capuron*, D. decaryi* (R. Vig.) Capuron*, D. floribunda* (Baill.) Capuron, *D. baccal* et également *Colvillea racemosa*), une espèce est considérée vulnérable (*D. regia*), deux sont en danger d'extinction (*D. pumila* Dupuy, Phillipson & R. Rabev. et *D. tomentosa* (R. Vig.) Capuron) et une (*D. velutina* Capuron) est en danger critique d'extinction. Le genre *Lemuropisum* n'apparaît plus sur la liste rouge pour 2012 mais est identifié comme étant menacé d'extinction dans une version précédente (IUCN 2009) et est identifié comme tel dans l'étude de Rivers et al (2011). L'espèce *D. leucantha* DuPuy, Phillipson & R. Rabev est identifiée comme vulnérable par DuPuy et al (1995) mais n'apparaît pas sur la liste rouge de l'IUCN (2012). Cependant, les informations utilisées pour déterminer le statut de conservation sont désuètes (Thulin, 1998) et ont besoin d'être mise à jour (IUCN, 2012). L'information manquante est de nature écologique, géographique, mais également phylogénétique. Le manque d'information phylogénétique et taxonomique est un obstacle important dans la gestion de la biodiversité tropicale (Schatz 2002, Callmander 2005). En effet, la mise en place de mesures de conservation basées sur une taxonomie erronée résulte trop souvent en l'inefficacité de telles mesures (Callmander 2005). Bien que des recherches récentes aient étudié la diversité génétique des populations de certaines espèces de *Delonix* (Rivers et al 2011), aucune étude jusqu'à présent n'a visé à acquérir des données phylogénétiques pour toutes les espèces des genres *Delonix, Colvillea* et *Lemuropisum* au niveau spécifique et générique nécessaires à une révision taxonomique et à l'évaluation de phénomènes géographiques, historiques et évolutifs pour ce groupe.

Systématique des genres *Delonix, Colvillea* et *Lemuropiusum* : revue de la littérature

La systématique du genre *Delonix* a d'abord été estimée à partir de comparaisons morphologiques (Polhill & Vidal 1981, Polhill 1994, DuPuy et al 1995, Lewis & Schrire 1995, DuPuy et al 2002). Lors de leur révision de la tribu Caesalpinieae, qui comprend le groupe Peltophorum dans lequel se trouvent les genres *Delonix*, *Colvillea* et *Lemuropisum*, Polhill & Vidal (1981) ont décrit le groupe Peltophorum comme étant très diversifié dans sa morphologie florale. Les genres *Delonix* et *Colvillea*, différant par la position des sépales et la position terminale de la panicule, exhibent les fleurs les plus complexes du groupe Peltophorum (DuPuy et al., 1995). Anciennement associé au groupe Caesalpinia, *Lemuropisum* est placé comme espèce proche des *Delonix* selon plusieurs caractères floraux, bien que *Lemuropisum* possède plusieurs caractères uniques comme des feuilles réduites paripinnées avec seulement deux ou trois paires de folioles, la présence de brachyblastes et l'absence de cloison individuelle entre les graines dans la gousse (Polhill 1994, DuPuy et al 1995). Le genre *Delonix* est jugé comme un groupe naturel monophylétique par sa possession de feuilles composées-pennées, de racèmes axillaires et de fruits en longues gousses dans lesquelles chaque graine est séparée par une cloison distincte (DuPuy et al 1995). Les espèces du genre *Delonix* sont des arbres hermaphrodites allant de un (*D. pumila*) à 30 mètres de hauteur, avec un tronc en forme de cigare pour certains, et une écorce mince et lisse. Les fleurs ont de longues étamines et sont composées généralement de cinq pétales libres pouvant être blancs, jaunes ou rouges. Les feuilles sont alternes, composées de 3 à 30 folioles. Le fruit est une gousse ligneuse et déhiscente courbée ou allongée (DuPuy et al 1995, Schatz 2001, Lewis et al 2005). Les deux espèces africaines se distinguent par leurs gousses plates et coriaces mais non ligneuses. La majorité des espèces ont d'abord été décrites sous le genre *Poinciana* L., tandis que les espèces *D. floribunda* et *D. velutina* ont d'abord été identifiées sous le genre *Aprevalia* Baill. basé sur la réduction de leurs pétales (Baillon 1884, Capuron 1968), mais cette distinction n'est pas retenue dans les plus récentes révisions. En général, les fleurs des espèces malgaches sont larges, blanches avec une tache jaune sur le pétal supérieur (étendard) ou rouge avec une tache blanche (*D. regia*), calice divisé en cinq segments charnus, valvulés se séparant de manière irrégulière, cinq pétals avec large lamelle, le pétal étendard étant le plus large, dix étamines de couleur rouge ou jaune, égales et longes et avec une exertion prononcé au delà des pétals. DuPuy et al (1995) distinguent quatre groupes à l'intérieur du genre *Delonix*, tous basés sur des caractères floraux : 1) *D. decaryi, D. floribunda*; *D. leucantha* et *D. pumila;* 2) *D. regia, D. tomentosa* et *D. velutina*; 3) *D. boiviniana* et *D. brachycarpa;* et finalement, 4) un groupe contenant les deux espèces africaines *D. baccal* et *D. elata*. Toutefois, une étude morphologique du pollen (Banks 1997) délimite quatre groupes très différents de ceux proposés par DuPuy et al (1995), soit 1) *D. baccal, D. elata* et *D. regia;* 2) *D. boiviniana,* *D. brachycarpa, D. decaryi, D. leucantha, D. pumila, D. tomentosa, D. velutina* et *Lemuropisum edule;* 3) *D. floribunda;* et finalement 4) *Colvillea racemosa*. La diversité morphologique des espèces de *Delonix* est apparente par l'absence de consensus, avec ou sans l'inclusion de *Colvillea* et *Lemuropisum*, tel qu'illustrée par les différences proposées dans les regroupements taxonomiques des études précédentes.

Les études de systématique moléculaire montrent toutes que le clade formé par les genres *Delonix, Colvillea* et *Lemuropisum* est bien soutenu comme monophylétique (Bruneau et al 2001 et 2008, Kajita et al 2001, Haston et al 2003 et 2005, Simpson et al 2003, Hawkins et al 2007, Manzanilla & Bruneau 2012). Les genres les plus près évolutivement de ce clade trigénérique sont *Conzattia* Rose (Haston et al 2003 et 2005, Hawkins et al 2007) et *Parkinsonia* L. (Lewis & Schrire 1995, Bruneau et al 2001 et 2008, Kajita et al 2001). Les relations phylogénétiques entre les espèces de *Delonix* ont fait l'objet de peu d'études à l'exception de celles de Haston et al (2003, 2005) qui trouvent les deux espèces africaines avec *D. regia*, et un groupe formé de *D. brachycarpa, D. floribunda*, *D. pumila* et *Lemuropisum edule.* L'étude plus récente de Rivers et al (2011), portant sur la diversité génétique des populations de huit espèces de *Delonix,* a identifié trois regroupements, soit 1) *D. velutina* et *D. regia*, 2) *Colvillea* et *D. boiviniana*, et 3) un clade regroupant d'un côté *D. pumila* et *Lemuropisum* et de l'autre *D. floribunda* avec *D. decaryi*. Cette même étude recense plus de variation génétique à l'intérieur des espèces (entre populations) qu'entre les espèces reflétant peut-être ainsi les regroupements conflictuels au niveau morphologique. Cependant, dans toutes les analyses, et, peu importe le marqueur moléculaire étudié, *Delonix* n'est jamais soutenu comme un groupe naturel et se retrouve généralement avec les genres *Colvillea* et *Lemuropisum* nichés en son intérieur (Haston et al 2003 avec *trnL-F*, Simpson et al 2003 avec *trnL-F,* Haston et al 2005 avec *trnL-F, rbcL* et *rps16,* Hawkins et al 2007 avec *trnL-F, rbcL* et *rps16,* Rivers et al 2011 avec AFLP nucléaire, Manzanilla &

Bruneau 2012 avec *SucS*). C'est le paraphylétisme récurent, statut non naturel en terme évolutif, du genre *Delonix* qui a incité Haston et al (2005) à proposer une révision taxonomique du genre afin de respecter l'aspiration d'identifier des groupes monophylétiques en systématique. Cette étude propose d'inclure les espèces *Colvillea racemosa* et *Lemuropisum edule* à l'intérieur du genre *Delonix*.

Toutes ces études se sont butées à une grande complexité génomique et morphologique inhérente aux Légumineuses et plus particulièrement à la sous-famille Caesalpinioideae (Herendeen et al 2003, Wojciechowski et al 2004, Lewis et al 2005), mais ont également des lacunes intrinsèques qui pourraient expliquer la résolution peu satisfaisante obtenue jusqu'à présent. D'abord, l'échantillonnage le plus exhaustif dans une analyse phylogénétique est de six espèces de *Delonix* (Haston et al 2005, Hawkins et al 2007), huit espèces dans une étude de génétique des populations (Rivers et al 2011), sur un total de onze. Ceci illustre la difficulté d'échantillonnage des espèces et d'analyse de données moléculaires. Deuxièmement, le choix du génome ciblé pourrait expliquer le manque de résolution interspécifique et intergénérique puisqu'une majorité des loci séquencés proviennent du génome chloroplastique, ce dernier évoluant plus lentement que le génome nucléaire (Moore 1995, Small et al 1998, Aïnouche & Bayer 1999, Small et al 2004, Li et al 2008). Les deux études ayant inclus des séquences du génome nucléaire n'ont obtienu aucune résolution phylogénétique (Rivers et al 2011, Manzanilla & Bruneau 2012). Les espèces des genres *Delonix, Colvillea* et *Lemuropisum* démontrent une étonnante homogénéité génétique. La difficulté à développer des marqueurs phylogénétiques applicables à un large éventail de taxa tout en procurant une forte résolution interspécifique chez les Légumineuses, est une limitation technique importante.

Biogéographie

Le manque de résolution obtenu pour les espèces des genres *Delonix*, *Colvillea* et *Lemuropisum* limite les interprétations possibles concernant le passé évolutif et l'histoire biogéographique de ces espèces. Deux évènements biogéographiques peuvent être abordés : la disjonction, plus ancienne, entre le genre sud-américain *Conzattia* et les genres africains et malgaches *Delonix, Colvillea* et *Lemuropisum*, et la disjonction entre les espèces de *Delonix* africaines et malgaches.

La disjonction Amérique du Sud - Madagascar/Afrique ne peut pas être attribuée à la vicariance puisque l'apparition des genres en question est beaucoup trop récente (Bruneau et al 2008) considérant que la séparation du Gondwana a débuté il y a 170 Ma et que la disparition du pont terrestre Kerguelen qui reliait Madagascar, l'Inde et l'Amérique du Sud a eu lieu il y a environ 100-90 Ma (Jokat et al 2005, Master 2006). Comme pour plusieurs autres familles d'angiospermes, cette disjonction est donc expliquée par une dispersion transatlantique des espèces végétales durant l'Oligocène (Renner et al 2001). Le moyen de dispersion le plus probable est le fort courant marin nord équatorial qui voyage du nord du Brésil vers l'Afrique de l'Ouest (Fratantoni 2000). De forts vents sont également épisodiquement enregistrés entre l'Amérique et l'Afrique/Madagascar (Grodsky et al 2003), mais sont une cause moins probable puisque les graines de *Conzattia, Colvillea, Delonix* et *Lemuropisum* sont grosses, lourdes et ne démontrent aucune adaptation au transport aérien. Une dispersion par des oiseaux migrateurs est également moins vraisemblable puisqu'aucun oiseau ne migre entre ces deux continents et que les oiseaux marins mangent rarement des graines (Renner 2001). Cet évènement biogéographique, quoique significatif, est antérieur à l'apparition des genres *Delonix, Colvillea* et *Lemuropisum* et ainsi n'est pas l'objectif de ce projet.

La disjonction plus récente Madagascar - Afrique pour les espèces du genre *Delonix* est celle qui sera explorée plus en détail dans ce projet et tout spécialement au chapitre 2. Madagascar s'est dissociée du continent africain il y a 120 Ma et de l'Inde vers 88 Ma (Rabinowitz et al 1983). Les hypothèses de radeaux végétatifs (Simpson 1940, Ali & Huber 2010), de pont terrestre (McCall 1997), de dispersion par les vents ou par des animaux (Muñoz et al 2004, Renner et al 2004a, Nathan et al 2008), ainsi que la vicariance seront étudiées.

Une étude biogéographique des genres *Delonix, Colvillea* et *Lemuropisum* permettrait de savoir si ces Légumineuses malgaches sont apparues à Madagascar et que par la suite deux espèces se seraient dispersées en Afrique (Yuan et al 2005, Wikström et al 2010, Krüger et al 2012) ou si, au contraire, les espèces africaines sont ancestrales, se sont retrouvées à Madagascar, et auraient ensuite subi un événement de radiation adaptative comme c'est le cas pour beaucoup d'espèces endémiques à Madagascar (Schatz 2001, Yoder & Nowak 2006, Tsy et al 2009, Anthony et al 2010). Malheureusement, l'origine, le patron ainsi que le nombre de dispersions demeurent inconnus jusqu'à présent pour ce groupe trigénérique, mais pourraient être résolus par l'obtention d'un patron phylogénétique robuste. L'obtention de telles données permettrait de mieux comprendre l'histoire évolutive, les relations génétiques et morphologiques, entre les espèces du genre *Delonix,* et permettrait une meilleure compréhension des mécanismes sous-tendant la biodiversité malgache.

Problématique, hypothèses et objectifs du mémoire

Plusieurs questions restent sans réponses concernant ces trois genres malgaches. Les genres et les espèces, sont-ils monophylétiques ? Quelles sont les relations évolutives entre les espèces ? Quel âge ont-elles ? Sur quel continent sont-elles apparues ? À quel moment, et combien de fois, y a-t-il eu dispersion entre l'Afrique et Madagascar ? Quelle a été la directionalité de cette (ces) dispersion(s) ? *Colvillea racemosa* et *Lemuropisum edule* seraient-elles en fait des espèces mal identifiées du genre *Delonix* ? Considérant leur rôle économique local et international, leur statut de conservation précaire, et la destruction rapide de leur habitat, un patron précis et robuste des relations phylogénétiques est impératif.

J'émets les hypothèses suivantes concernant cette problématique.

1) Relations intergénériques

La différence morphologique ainsi que la présence d'allèles uniques pour *Colvillea racemosa* par rapport à *Delonix* indique une certaine différentiation évolutive entre ces deux genres, du moins plus grande qu'entre *Delonix* et *Lemuropisum* (DuPuy et al 1995, Banks 1997, Rivers et al 2011). Il est donc possible que *Colvillea* soit le groupe frère d'un clade formé des espèces des genres *Delonix* et *Lemuropisum*. Dans ce cas, *Delonix* est paraphylétique si l'on continue de considérer *Lemuropisum* comme un genre distinct. Certains changements taxonomiques devront sans doute être considérés (Haston et al 2005).

2) Relations interspécifiques

L'observation d'un groupe formé de *Lemuropisum, D. pumila, D. floribunda* et *D. brachycarpa* dans les analyses phylogénétiques moléculaires de Haston et al (2005) est congruente avec leurs répartitions géographiques commune. Il est également possible de supposer que les deux espèces africaines se regrouperont ensemble (DuPuy et al 1995, Haston et al 2005, Hawkins et al 2007) dénotant une unicité génétique et morphologique des espèces africaines qui pourraient s'avérer être ancestrales aux espèces malgaches.

3) Biogéographie

> Une ou plusieurs dispersions vers Madagascar auraient pu être effectuées par l'intermédiaire d'oiseaux qui auraient ingéré les graines en Afrique pour les rejeter à Madagascar (Renner 2004a). En effet, certaines espèces de *Delonix* sont pollinisées par des oiseaux migrateurs (DuPuy et al 1995, Banks 1997) et les graines de plusieurs espèces sont comestibles (Webb et al 1984, Gordon & Gantz 2008). Ce mode de dispersion pourrait être couplé avec l'hypothèse d'un pont terrestre entre l'Afrique et Madagascar (McCall 1997). Cette dernière hypothèse dépend de la date de la dispersion puisque le pont terrestre n'est présent qu'entre 45 et 26 Ma. J'émets l'hypothèse que l'ancetre des deux espèces africaines ont été transporté par des oiseaux migrateurs à partir de Madagascar vers l'Afrique de l'est.

Avec l'échantillonnage complet des espèces du genre *Delonix* et avec l'utilisation d'information génétique provenant des génomes nucléaire et chloroplastique, l'objectif de notre étude est de d'éclaircir le statut taxonomique des genres *Delonix, Colvillea et Lemuropisum*, ainsi que les relations phylogénétiques entre ces treize espèces.

Pour ce faire, quatre régions chloroplastiques (*rps16, trnD-trnT, trnL-trnF, matK*) et six loci nucléaires (*LEAFY, CTP, tRALs, ITS, AIGP, EIF3E*) ont été séquencés pour onze espèces du genre *Delonix* et également chez *Colvillea racemosa* et *Lemuropisum edule*. Ces loci ont également été séquencés chez des espèces des groupes Peltophorum, Tachigali et Dimorphandra (Bruneau et al 2008), et de la sous-famille Mimosoideae afin d'inclure l'information temporelle de fossiles. Les alignements de séquences nucléotidiques de chacun des loci ont été analysés individuellement ainsi que dans des analyses de matrices combinées à l'aide de méthodes de parcimonie et bayésiennes. Les topologies résultantes ont été comparées avec le calcul de valeurs de support. Grâce au positionnement des fossiles, la topologie bayésienne a été soumise à deux types d’analyses de datation et à une analyse biogéographique d’estimation d’aires géographiques ancestrales.

Le chapitre 2 est sous forme d’article scientifique et décrit en détails la méthodologie utilisée, les résultats des analyses ainsi que l’interprétation au niveau phylogénétique, taxonomique, biologiques et biogéographique. Le chapitre 3 contient une discussion concernant les perspectives futures de cette étude.

Chapitre 2 :

Miocene long distance dispersal and evolution in the spiny thicket habitat: Multilocus phylogeny and biogeography of the endangered Madagascan genera *Delonix*, *Colvillea* and *Lemuropisum* (Leguminosae)

Cet article sera soumis sous peu à *American Journal of Botany*

Contribution respective des auteurs :

Marielle Babineau : sélection et exécution de la méthodologie en laboratoire et de l'analyse des données, interprétation et rédaction.

Anne Bruneau : Supervision, conseils, recommandations, corrections et direction générale.

Abstract

- *Premise of the study*: The intergeneric and interspecific relationships of the afro-madagascan genus *Delonix*, and of the endemic madagascan genera *Colvillea* and *Lemuropisum* are unresolved. This group includes a large number of endangered species; our lack of knowledge regarding their relationships limits accurate conservation assessment and hampers testing of conflicting biogeographical hypotheses explaining plant dispersal around Madagascar.
- *Methods*: A phylogeny was reconstructed using nucleotide sequences from *ITS*, five low-copy nuclear genes and four plastid regions. The loci were analyzed under a parsimony and Bayesian framework. The resulting topology was analyzed using two molecular dating methods with fossil calibrations and used to reconstruct the ancestral geographic range.
- *Key Results*: *Colvillea racemosa*, which diverged approximately 21 (± 5) Ma in south-west Madagascar, is most likely a distinct genus sister to the *Delonix/Lemuropisum* clade but may have undergone hybridization or be subject to incomplete lineage sorting. Around 19-18 (± 5) Ma, two major clades diverged from a southern Madagascan ancestor: one contains the five most geographically widespread species with one ancestral Madagascar to Africa dispersal around 17 (± 6) Ma. The second clade contains mostly southern species and includes *Lemuropisum edule*.
- *Conclusions*: *Delonix* is not monophyletic due to the supported nested position of *Lemuropisum*. Morphological and ecological characteristics support the observed species relationships. A single Miocene long distance dispersal possibly by vegetative rafting explains the dispersal of the two African *Delonix* species. This study highlights the need of conservation efforts directed to the unique Madagascar spiny thicket habitat along with the integration of knowledge from past and present biotic interactions.

Key Words: *Delonix*, *Colvillea*, *Lemuropisum*, Madagascar, low-copy nuclear genes, phylogeny, conservation, long distance dispersal, spiny thicket, taxonomy

Introduction

Over 90% of all vascular plants in Madagascar are endemic and the Leguminosae family, suggested as a proxy for the study of global patterns of angiosperm diversity (Nic Lughadha et al 2005), has the highest number of endemic genera on this island (Schatz 2001, DuPuy et al 2002, Kier et al 2009). Intense deforestation, overexploitation and climate change has put nearly every endemic plant species in Madagascar in danger of extinction (DuPuy & Moat 1998, Schatz 2001, Harper et al 2007). The tropical rainforest habitat of Madagascar has received more consideration (better survey, more national parks) while drier habitats, such as the southern spiny thicket, are left mostly unprotected (DuPuy & Moat 1998, Ganzhorn et al 2001, Madagascar National Parks Association 2013). The Madagascar spiny thicket habitat is unique in the world due to increased rainfall allowing for its evergreen characteristic, which leads to higher plant biodiversity (Grubb 2003). Indeed, the spiny thicket harbors the highest endemism in Madagascar (Davis et al 1994, Phillipson 1996). The disappearance and reduced population sizes of southern endemic species will only make it more difficult to answer evolutionary and biogeographical questions. Conservation efforts also depend heavily on up to date biological information and accurate taxonomic assessments of endangered species (Schatz 2002, Callmander 2005, IUCN 2012). Poor taxonomic and phylogenetic data often result in an unknown conservation status for many tropical species thus limiting biodiversity management. Conservation efforts based on incorrect taxonomy often end in fallacious assessments (Callmander 2005). This creates a vicious loop where, because species are endangered, it becomes difficult to obtain biological information, yet the absence of information limits not only conservation efficiency but also the correct assessment of the conservation status of species.

An example of this situation, and the focus of our study, is a closely related trigeneric clade found in Madagascar formed by the near-endemic genus *Delonix*, and two closely related, monospecific, and endemic genera, *Colvillea* and *Lemuropisum*. Genus *Delonix* includes eleven species, two of which are found in Africa: *Delonix baccal* is restricted to eastern

Somalia and Kenya, while *D. elata* is widespread. The nine other species are endemic to Madagascar and are distributed along the West coast of the island in the northern dry deciduous forest and the southern spiny thicket (Fig 2). *Colvillea racemosa*, found in western and southern Madagascar, and *Lemuropisum edule*, restricted to the south, are both sympatric with *Delonix* species. Three biodiversity centers, one in the North, another in the West and a third in the Southwest, have been identified in Madagascar for *Delonix*, all on dry, xerophytic, and limestone soil conditions (DuPuy et al 1995).

As with many other legume species, the thirteen species of *Delonix*, *Colvillea* and *Lemuropisum* have many local uses – edible seeds, boats, fences - and are of international economic importance – horticulture, insecticide development, potential crop and urban forestry (DuPuy et al 1995, Grant et al 1995, Kite et al 1995, Gagdil 2004, Gordon & Gantz 2008, Alves et al 2009, Bosh et al 2009, Orwa et al 2009). These species are all currently endangered (IUCN 2012). However, their conservation status has not been revised for the last 14 years due to a lack of biological information (Thulin 1998), which considerably compromises conservation efforts. Although work is underway to correctly assess their conservation status using population genetics and herbarium specimens (Rivers et al 2010, 2011), attempts at resolving phyletic, historic and geographic relationships have yielded few conclusive results (DuPuy et al 1995, Haston et al 2005). The morphological diversity within the *Delonix/Colvillea/Lemuropisum* group is impressive and has complicated previous taxonomic studies. Indeed, previous studies described two genera for *Delonix* species: genus *Poinciana* L., described the majority of species, while genus *Aprevalia* Baill., described *D. velutina* and *D. floribunda* (Baillon 1884, Capuron 1968). The species of genera *Delonix, Colvillea* and *Lemuropisum* have been grouped into multiple, inconsistent and incongruent subdivisions based on different vegetative and floral characters (Polhill & Vidal 1981, Polhill 1994, DuPuy et al 1995, Banks et al 1997).

Despite this diversity, molecular analyses show surprisingly low levels of genetic variation among species. Previous studies found a lack of resolution between the three genera, which most often appear in a polytomy (Simpson et al 2003, Haston et al 2005, Hawkins et al 2007, Manzanilla & Bruneau 2012). When some resolution is recovered, *Lemuropisum* occurs as nested within *Delonix* (Haston et al 2005, Hawkins et al 2007, Rivers et al 2011) while *Colvillea* can be weakly supported as external to a *Delonix/Lemuropisum* group (Bruneau et al 2008). However, several *Delonix* species have never been sampled, clouding even more the phylogenetic relationships. The lack of resolution, caused in large part by the comparison of plastid genome loci with low variation, may also explain the observed phylogenetic uncertainty. Given that low-copy nuclear genes tend to show more variability between species than plastid regions (Doyle et al 1999, Choi et al 2006, Hughes et al 2006, Chapman 2007, Li et al 2008, Steele et al 2008, Duarte et al 2010, Ilut et al 2012), it is not suprising that they have been increasingly used in phylogenetics. Two recent studies used nuclear loci in *Delonix, Colvillea* and *Lemuropisum*, with no conclusive phylogenetic results (Manzanilla & Bruneau 2012), but still found considerable genetic diversity (Rivers et al 2011) within species populations, illustrating the potential of nuclear markers for this group.

The absence of resolved generic and species relationships limits our ability to evaluate historical and geographical aspects of species dispersals to, or from, Madagascar. Endemic Madagascan legumes often show morphological, ecological, and genetic affinities with species found on neighboring landmasses such as Africa and India, and on islands and archipelagos such as Comoros, Reunion, Seychelles and Mauritius. However, the evolutionary relationships between many endemic Madagascar legume species and their closest non-madagascan relatives are poorly known (DuPuy et al 2002). The elucidation of evolutionary relationships for madagascan plants would allow a better understanding of plant evolution on isolated islands and their dispersal mecanisms. Phylogenetic information is needed to decipher plant temporal and spatial dispersal patterns and to identify factors involved in legume dispersal around Madagascar. For many near-endemic Madagascan legume genera, such as *Delonix* that has African and Madagascan species, the time in history, continent of origin, directionality, and number of dispersal events remains unknown or disputed (DuPuy et al 2002, Lavin et al 2004). Many hypotheses have been put forward to explain the distribution of plants between Madagascar and Africa. In the case of *Delonix*, vicariance is unlikely as a previous study (Bruneau et al 2008) estimated the Peltophorum group node at 40 Ma old while Africa and Madagascar separated around 120

Ma ago and Madagascar and India separated around 88 Ma ago (Rabinowitz et al 1983, Jokat et al 2005, Master et al 2006). The presence of a land bridge from 45 to 26 Ma linking Madagascar and Africa is hypothesized from the existence of underwater mountains that would have emerged when sea levels dropped (McCall 1997). Long distance dispersal of plants and other organisms via animals and winds is more common than previously assumed and has been suggested many times in Leguminosae evolutionary history (Hagen et al 2001, Bremer et al 2004, Givnish & Renner 2004, Renner 2004b, Lavin et al 2004, Davis et al 2005, Lavin et al 2005, de Queiroz 2005, Knapp et al 2005, Clayton et al 2009). The sweepstake hypothesis implies the dispersal of organisms across water on floating vegetative rafts (Simpson 1940, Thiel & Haye 2006). It recently has received support as sea currents in the Mozambique Channel were shown to have flowed from Africa to Madagascar during the Cenozoic-Oligocene, and then to have reversed to the present Madagascar to Africa flow direction during the early Miocene (Ali & Huber 2010). Strong winds connect Africa and Madagascar and can be efficient dispersal vectors (Muñoz et al 2004). Animals can also easily transport seeds externally or internally (Yoder & Nowak 2006). Tracking the exact mechanism for one specific dispersal event in time is almost impossible, but the accumulation of evidence allows the identification of possible dispersal vectors around the estimated time of dispersal, consistent with knowledge of the species biology, and can help to identify the most probable cause (Nathan 2006).

To respond to the conservation urgency, as well as to address the evolutionary and biogeography uncertainty, the objectives of this study are to 1) determine the intergeneric and interspecific phylogenetic relationships for the eleven species of the genus *Delonix,* and of *Colvillea racemos*a and *Lemuropisum edule* using multiple plastid and low-copy nuclear loci; 2) estimate the age of divergence of the different clades; and 3) reconstruct the ancestral geographic range of clades. The results will give the evolutionary data needed to assess the most suitable conservation strategy for the species, and will also identify ecological and morphological characteristics possessed by closely related endemic species that could lead to a better understanding of plant evolution in Madagascar, particularly in dry deciduous forests and spiny thicket habitats. Our results will allow the testing of island biogeography hypotheses on the origin, timing, directionality and possible means of dispersal in this trigeneric clade.

Materials and methods

Taxon sampling

A total of 100 samples was studied. Of these, 51 represent the 13 species of genera *Delonix, Colvillea* and *Lemuropisum*. The majority of the *Delonix* and *Colvillea* samples were collected in Madagascar in 2006. The other 49 samples are part of a large subset of outgroup taxa necessary to include the fossil calibration points for the divergence time estimates. The choice of the outgroup taxa was guided by the phylogenetic analyses of Bruneau et al (2008). The outgroup includes 15 samples from seven genera of the Dimorphandra group representing ten species (*Mora, Moldenhawera, Dimorphandra, Diptychandra, Dinizia, Erythrophleum* and *Campsiandra*), nine samples from two genera of the Tachigali group representing eight species (*Tachigali* and *Jacqueshuberia*), 15 samples from five genera of the Peltophorum group representing seven species (*Conzattia, Parkinsonia, Peltophorum, Bussea* and *Schizolobium*) and ten samples from six genera of subfamily Mimosoideae representing seven species (*Inga, Parkia, Pentaclethra, Mimosa, Calliandra* and *Acacia*) (Table I). Leaf material for some taxa was obtained from the following herbaria: MO, K, MT, P, US, NY, WAG.

Table 1. Species of *Delonix, Colvillea* and *Lemuropisum*, and outgroup taxa from subfamily Mimosoideae and from the Dimorphandra, Tachigali, and Peltophorum Groups of subfamily Caesalpinioideae sequenced for the plastid *trnL-trnF, trnD-trnT, matK-trnK, rps16*, and for the nuclear *ITS, CTP, AIGP, tRALs, EIF3E* and *Leafy* loci. Number in parenthesis indicates the number of clones for each sequence. Previously published sequences : [a] Bruneau et al 2001, [b] Bruneau et al 2008, [c] Haston et al 2005, [d] Luckow et al 2000, [e] Luckow et al 2003, [f] Miller & Bayer 2001, [g] Särkinen et al 2011. * indicates selected sequences for reduced sampling in the concatenated matrices when two or more sequences were available for a particular molecular marker in a genus, ** identified as *Sclerolobium guianense* Aubl. in GenBank and on specimen, *** identified as *Sclerolobium densiflorum* Benth. in GenBank, **** identified as *Tachigali myrmecophila* (Ducke) Ducke in GenBank.

Taxon	Locality	Voucher information	*trnL-trnF*	*matK*	*rps16*	*trnD-trnT*	*ITS*	*tRALs*	*AIGP*	*CTP*	*EIF3E*	*Leafy*
Peltophorum Group												
Colvillea racemosa Bojer ex Hook.	Madagascar	Bruneau 1397 (MT)*		x	x	x	x	x	x	x	x (4)	x (4)
	Madagascar	Bruneau 1360 (MT)*		x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau 1403 (MT)*		x	x	x	x	x	x	x	x (3)	x (4)
	Madagascar	Lewis 2147 (K)	AY899739[c]	EU361916[b]	AY899794[c]							
Delonix baccal (Chiov.) Baker f.	Somalia	Aronson 12 (MO)	x	x		x	x	x	x	x		x (3)
	Kenya	Gillett 13717 (K)			AY899792[c]							
Delonix boiviniana (Baill.) Capuron	Madagascar	Bruneau 1365 (MT)	x		x	x		x	x	x	x (8)	
	Madagascar	Bruneau 1381 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau 1357 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau 1378 (MT)	x	x	x	x	x		x			
	Madagascar	Bruneau 1401 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (4)
	Madagascar	Andriamihajarivo 919 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (6)
	Madagascar	DJ DuPuy M515 (P)					x					
	Madagascar	Phillipson 2452 (P)		x								
Delonix brachycarpa (R. Vig.) Capuron	Madagascar	Phillipson 3081 (P)*	x		AY899790[c]	x	x					

	Madagascar	Randriantafika 232 (P)	x				x					
	Madagascar	Rakotovao 5671 (P)										x (3)
	Madagascar	Randriamiera 7745 (P)										x (7)
Delonix decaryi (R. Vig.) Capuron	Madagascar	Rivers MR37 (MO)*				x	x					
	Madagascar	DJ & BP DuPuy M94 (MO)			x	x			x	x		
	Madagascar	DJ DuPuy M448 (P)	x									
	Madagascar	Capuron 28520-SF (K)*	x				x					
Delonix elata (L.) Gamble	Tanzania	Herendeen 20-XII-97-1 (US)*	x	x		x	x	x	x	x	x (4)	x (4)
	Tanzania	Herendeen 17-XII-97-2 (US)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Ethiopia	Friis et al 10280 (K)				x		x				x (3)
	Yemen	JRI Wood Y/74/449 (BM)			AY899787[c]							
Delonix floribunda (Baill.) Capuron	Madagascar	Bruneau 1393 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau 1398 (MT)	x	x	x	x	x	x	x			x (4)
	Madagascar	Bruneau 1405 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau 1409 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
Delonix leucantha var gracilis DuPuy, Phillipson & R. Rabev.	Madagascar	DJ DuPuy M87 (MO)					x					
	Madagascar	McWhirter 237 (K)	x									
	Madagascar	Allorge 904 (P)*				x	x	x	x	x		
Delonix pumila Dupuy, Phillipson & R. Rabev.	Madagascar	Bruneau 1394 (MT)*	x	x	x	x	x	x	x	x	x (3)	
	Madagascar	Bruneau 1411 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau 1412 (MT)*	x	x	x	x	x	x	x	x	x (3)	
Delonix regia (Hook) Raf.	Madagascar	Bruneau 1320 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (4)
	Madagascar	Bruneau 1355 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
	Madagascar	Bruneau s.n. A (MT)*	x	x	x	x	x	x	x	x	x (3)	
	Madagascar	Bruneau s.n. B (MT)*	x	x	x	x	x	x	x	x	x (3)	
	Madagascar	Bruneau s.n.C (MT)	x	x	x	x	x		x	x		
	Mauritius	Archambault 9 (MT)		x	x	x		x	x	x		
	Mauritius	Archambault 69 (MT)*	x	x	x	x	x	x	x	x	x (4)	x (3)
	Madagascar	T. Bradley 31902 (MT)	x	x	x	x	x		x			
Delonix tomentosa (R.Vig.)	Madagascar	Perrier de la Bâthie	x		x	x	x	x	x	x		x (3)

Capuron		1325 (K)										
Delonix velutina Capuron	Madagascar	Bruneau 1349 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (3)
		Bruneau 1354 (MT)*	x	x	x	x	x	x	x	x	x (3)	x (5)
Lemuropisum edule H. Perrier	Madagascar	DuPuy M1033 (K)*	EU361818[b]			x	x	x	x		x (4)	x (3)
	Madagascar	Phillipson 3460 (K)	AF365070[a]	EU361991[b]								
	Madagascar	T. Willings sn (K)			AY899795[c]							
Peltophorum outgroup												
Bussea perrieri R. Vig	Madagascar	Randrianasolo 527 (P)	EU361757[b]	EU361896[b]		x		x	x	x	x (3)	
Bussea sakalava DuPuy & Rabevohitra	Madagascar	DuPuy M500 (NY)					x					
	Madagascar	R. Capuron 23.331_SF (K)	AY899708[c]		AY899766[c]							
Conzattia multiflora Robinson	Mexico	Hughes 1815 (NY)*				x	x	x	x	x	x (4)	x (7)
	Mexico	Sahagun sn (RNG)	AY899729[c]	AY386918[b]	AY899786[c]							
Parkinsonia aculeata L.	New Mexico	Spellenberg 12704 (MT)*	AF365072[a]	EU362019[b]		x	x	x	x	x	x (3)	x (3)
	Mexico	Hawkins 94/5 (FHO)	AY899715[c]		AY899772[c]							
Peltophorum pterocarpum (DC.) K.Heyne	Tanzania	Goyder 3719 (K)		EU362023[b]				x		x		
	Tanzania	Herendeen 12-XII-97-2 (US)							x			
		Grierson & Long 2884 (E)	AY899713[c]		AY899770[c]							
Peltophorum dubium dubium (Spreng.) Taub.	Bolivia	Hughes 2436 (K)				x				x	x (3)	x (3)
	Brazil	Savassi et al 1303*				x	x					x (3)
	Mexico	Hughes 1685 (FHO)	AY904432[c]	AY386846[c]	AY899769[c]							
Schizolobium parahyba (Vell.) Blake	South Africa	Klitgaard 694 (K)		EU362036[b]		x	x	x	x	x	x (7)	x (3)
	Mexico	Hughes 1880 (FHO)*	AY899710[c]		AY899767[c]							
Tachigali Group												
Jacqueshuberia brevipes (Barneby)	Guyana	Redden 1240 (US)	EU361815[b]	EU361984[b]		x	x	x	x	x	x (3)	x (3)
Jacqueshuberia loretensis	Peru	M Rimachi Y 9050 (NY)			AY899761[c]							

R.S. Cowan												
Tachigali guianense (Benth.) Zarucchi & Herend.**	French Guyana	S. Mori et al 22791 (NY)		x		x	x	x	x	x		x (6)
	South Africa	Klitgaard 687 (K)	AF365111^{a}									
Tachigali densiflorum (Benth.) L.F Gomes da Silva & H.C Lima.***	Brazil	de Carvalho 4095 (K)			AY899763^{c}							
Tachigali vasquezii Pipoly	Ecuador	Neill 13998 (K)*	AF365113^{a}	EU362054^{b}				x	x	x	x (5)	x (3)
Tachigali amplifolia (Ducke) Barneby	French Guyana	S. Mori et al 23868 (NY)					x	x		x	x (3)	
Tachigali paniculata Aubl.	Guyana	T.W. Henkel 657 (NY)				x		x	x			x (5)
Tachigali glauca Tul.****	Brazil	R.S. Cowan 38220 (K)	AY899706^{c}		AY899764^{c}							
Dimorphandra Group												
Campsiandra comosa Benth.		T. Pennington 341 (K)			x				x			
	Guyana	Maas et al 5431 (MO)					x					
	Guyana	Redden 1100 (US)*	EU361780^{b}	EU361908^{b}	x	x		x		x	x (4)	
Dinizia excelssa Ducke	Guyana	Jansen-Jacob 1900 (NY)			x	x	x	x		x		x (4)
		Sergio de Faria sn (BH)	AF278479^{d}	AF521827^{e}								
Dinizia sp (Taxon nov)	Brazil	Folli 4889 (K)	EU361809^{b}	EU361952^{b}	x				x	x		x (3)
	Brazil	Folli 4884 (K)*	EU361808^{b}	EU361951^{b}	x	x	x	x	x	x	x (3)	x (3)
Dimorphandra conjugata Sandwith	Guyana	Breteler 13800 (WAG)	AF365099^{a}	EU361934^{b}	x	x		x	x	x	x (8)	
Diptychandra aurantiaca Tul.	Brazil	Klitgaard 70 (NY)	EU361799^{b}	EU361935^{b}	x	x	x	x	x	x	x (0)	
Erythrophleum suavolense (Guill. & Perr.) Brenan	Tanzania	Herendeen 17-XII-97-3 (US)*	AF365103^{a}	EU361949^{b}		x		x			x (5)	
	Tanzania	Herendeen 17-XII-97-2 (US)			x					x	x (4)	
Erythrophleum ivorense A. Chev.	Gabon	Breteler 15446 (WAG)						x	x			
Moldenhawera floribunda Schroder.	Brazil	Klitgaard 30 (K)			x	x			x			
Moldenhawera brasiliensis Yakovlev	Brazil	Queiroz 5530 (K)	EU361824^{b}	EU362004^{b}								
Mora gonggrijpii (Kleinh.) Sandwith	Guyana	Breteler 13792 (WAG)	AF365104^{a}	EU62005^{b}	x	x	x	x	x	x		
Mimosoideae												

Acacia caven (Molina) Molina	Canada	JBM no.386-89 (MT)	AF365041[a]		x	x	x	x	x	x	x (0)
	Argentina	CANB 615552 (DLEP)		AF274131[f]							
Calliandra inaequilatera Rusby	Canada	Archambault sn (MT)*			x	x	x		x	x	x (4)
Calliandra trinervia var. carbonaria (Benth.) Barneby	Ecuador	Klitgaard 622 (K)	AF278516[a]	AF521815[e]	x				x		x (3)
Inga sp	Ecuador	Klitgaard 677 (K)	AF365046[a]	EU361980[b]	x	x	x	x	x	x	x (3)
Mimosa quitensis Benth.	Ecuador	Klitgaard 647 (K)	AF278514[a]	AF521844[e]	x		x		x		x (3)
		Lewis 2856 (K)				HM353116[g]					
Parkia multijuga Benth.	South Africa	Klitgaard 697 (K)	AF365050[a]	EU362018[b]	x	x	x	x	x		x (5)
Pentaclethra macrophylla Benth.	Gabon	Wilde & Maessen 11496 (WAG)	AF278485[a]		x	x	x	x	x	x	
	Congo	BNBG 87-1143 (BR)		AF521853[e]							

Selection of molecular markers

A total of ten molecular markers were studied, four from the plastid genome (*trnL-trnF*, *matk, rps16* and *trnD-trnT*) and six from the nuclear genome (*ITS, Leafy, CTP, AIGP, tRALs* and *EIF3E*). Previously published sequences from plastid loci *matK*, *rps16, and trnL-trnF* for the genera *Delonix, Colvillea* and *Lemuropisum* were included to evaluate the position and effect on resolution of newly added species (Haston et al 2003, Simpson et al 2003, Haston et al 2005, Hawkins et al 2007, Bruneau et al 2008). The *trnD-trnT* region contains three introns (Shaw et al 2005) and has been successful in resolving the phylogeny of closely related legume species (Scherson et al 2008, Dexter et al 2010, Simon et al 2011). The two ribosomal internal transcribed spacers *ITS1* and *ITS2,* (including the 5.8S gene) are known to be variable among closely related Leguminosae species (Turchetto-Zolet et al 2012). The low copy nuclear gene *Leafy,* of which we amplified the second intron and part of exon 2 and 3, was chosen for its high level of variability between closely related plant species and for their phylogenetic utility in Leguminosae (Archambault & Bruneau 2004, Oh & Potter 2005, Scherson et al 2008). The four remaining low-copy nuclear genes were selected from the studies of Choi et al (2006) and Li et al (2008). Recent analyses by Babineau et al (unpublished) suggest the intron-spanning regions *AIGP* and *EIF3E* to be among the most useful regions of the 19 loci tested for resolving relationships among *Delonix* species, whereas *tRALs* and *CTP* were selected for their ability to resolve intergeneric relationships.

Molecular methods

DNA extraction of dried material was done using a modified cetyltrimethylammonium bromide (CTAB) protocol from Joly et al (2006). A total of 1% polyvinylpyrrolidone (PVP) and 0.2% of β-mercaptoethanol was added to the total volume of the extraction buffer. A concentration of 0.05 mg/mL of RNase A was then added to each sample before incubation at 65ºC.

Plastid and nuclear loci were amplified and sequenced using the same primer pairs. Internal primers were designed for *rps16, trnD-trnT, Leafy, CTP* and *tRALs* to amplify and sequence recalcitrant samples (Table II). Polymerase chain reaction (PCR), in a total volume of 25 µL, contained 10% PCR reaction buffer 10x (Roche Diagnostics, Laval, Quebec, Canada), 2.5 mmol/L $MgCl_2$ (Promega, Maddison, Wisconsin, USA), 200 mmol/L of each dNTP (MBI Fermentas, Burlington, Ontario, Canada), 0.4 mmol/L of each primer (Alpha DNA, Montreal, Quebec, Canada), 2 units of *Taq* Polymerase, and approximately 200 ng of genomic DNA. For recalcitrant samples with potential PCR inhibitors, 0.1 mg/mL bovine serum albumin (BSA) (New England Biolabs, Pickering, Ontario, Canada), 0.03% Tween 20 (J-T. Baker, Phillipsburg, New Jersey, USA), and 4% of pure dimethyl sulfoxide (DMSO) (Fisher Scientific, Ottawa, Ontario, Canada) were also added to the mix. PCR cycle settings for *matK* and *rps16* are the same as described in Bruneau et al (2008) and Oxelman et al (1997) respectively. The PCR cycle profile for *trnL-trnF, trnD-trnT, Leafy, ITS, AIGP, CTP, tRALs* and *EIF3E* were modified from their original source (Table II). Nested PCR were performed for *trnL-trnF, matK, rps16, trnD-trnT, ITS, Leafy, CTP* and *tRALs* when the first PCR failed to produce sufficient amount of product to be sequenced or cloned using the conditions described above. PCR products were purified using a PEG protocol (Joly et al 2006). Sequencing was performed on Applied Biosystems 3730xl DNA Analyzer (McGill University Genome Quebec) using BigDye chemistry (version 1.1) and following the manufacturer protocol.

Sequences were assembled and edited with Sequencher 4.7 (GeneCodes Corporation, Ann Arbor, Michigan, USA). After direct sequencing, nuclear loci sequences that were observed to be polymorphic were cloned. These samples were amplified in triplicate reactions to help reduce PCR recombinants and *Taq* induced errors (Judo et al 1998, Cronn et al 2002, Joly et al 2006). Cloning was performed using the CloneJET PCR cloning kit (Fermentas (now Thermo

Fisher Scientific), Ottawa, Ontario, Canada), following manufacturer instructions, but with lower concentrations of reagents for the sticky-end protocol and ligation reactions. All sequenced transformed colonies were visually inspected upon alignment; sequences were submitted to a BLAST search (www.ncbi.nlm.nih.gov/blast) and eliminated if they did not correspond to published sequences in GenBank. A minimum of three clones per sequence was included in the alignment. *Colvillea racemosa* was randomly chosen as a representative of the *Delonix/Colvillea/Lemuropisum* group, and its *ITS* sequences were cloned to verify the nucleotide sequence homogeneity, presence of pseudogene and copy number because *ITS* has been reported as possibly having paralogous copies that escape concerted evolution (Alvarez & Wendel 2003, Feliner & Roselló 2007).

Table II. Primers used to amplify each of the ten loci in the phylogenetic study of genera *Delonix*, *Colvillea* and *Lemuropisum*. Full oligonucleotide sequences are given for newly developped primers, as well as modified PCR cycle.

	Foward primer	Reverse primer	PCR cycle	Source
trnL (UAA)-trnF (GAA)	c, e	f, d	95°C for 3 min; 95°C for 30 s, 50°C for 30 s, 72°C for 45 s, for 35 cycles; 72°C for 10 min	Taberlet 1991
matk-3’trnK	trnK685F, matK4La, matK1100L, MIF	trnK2R*, KC6, matK1932Ra	See Bruneau et al 2008	Hu et al 2000, Lavin et al 2000, Wojciechowski et al 2004, Bruneau et al 2008
rps16	F, rps16F61: 5’- GGG TGC TCT TGG CTC GAC AT	R2, rps16R8 : 5’-CTC CTC GTA CGG CTC GAG	See Oxelman et al 1997	Oxelman et al 1997, This study
trnD-trnT	D, E, TrnD2	T, Y, trnT2del : 5’-ATC CGC ATA GCG GCT CAT TGC TC	80°C for 5 min; 94°C for 45 s, 57°C for 45 s, 72°C for 110 s, for 35 cycles; 72°C for 7 min	Demesure et al 1995, Simon et al 2009, Shaw et al 2005, This study
ITS	AB101, ITS5, ITS3	AB102, ITS2, ITS4	95°C for 2 min; 95°C for 30 s, 50°C for 1 min, 72°C for 1 min, for 35 cycles; 72°C for 7 min	White et al 1990, Douzery et al 1999
Leafy	sxlDEL	txrGUI, Leafyb : 5’-GAG CCT GGG GAA GTT GCA CGT GGC A	94°C for 4 min; 94°C for 30 s, 60°C for 1 min, 72°C for 110 s, for 45 cycles; 72°C for 7 min	Archambault & Bruneau 2004, Sinou et al, unpublished
AIGP	AIGP-F	AIGP-R	94°C for 3 min; 94°C for 30 s, 53-55°C for 30 s, 72°C for 2 min, for 35 cycles; 72°C for 5 min	Choi et al 2006
tRALs	tRALs-F, tRALsFi 5’- GTW AGA GTT GTA GCA ATT GGA C	tRALs-R, tRALsRi 5’- GCA AAA GCT TTA GCC TCT CGT GTG	See *tRALs*	Choi et al 2006, This study
CTP	CTP-F, CTPFi 5’- CCG GAC CMC CAT AAT AGA AGT AG	CTP-R CTPRi 5’-CCA TGT TCT AGG ATA CTG GCC WGS CCC	See *tRALs*	Choi et al 2006, This study
EIF3E	EIF3E-F	EIF3E-R	95°C for 3 min; 94°C for 40 s, 56°C for 30 s, 72°C for 110 s, for 35 cycles; 72°C for 5 min	Li et al 2008

Phylogenetic analyses

Sequences were aligned with MUSCLE (Edgar 2004) as implemented in Geneious Pro 4.8.5, and alignments were subsequently checked by eye with BioEdit 7.0.8.0 (Hall 1999). Nexus files were generated with SeqState 1.4.1 (Müller 2006). Three concatenated matrices were created each containing 49 sequences representing the 13 species of *Delonix*, *Colvillea* and *Lemuropisum*, as well as 20 samples representing the outgroup genera: all the plastid loci (ALLcp), all the nuclear loci (ALLnc), and loci from both genome (ALLcpnc). Each of the 49 sequences, for each matrix, was assembled by combining the individual sequences for the selected loci of a particular taxa. These concatenated matrices represented a reduced matrix that was analyzed (indicated with * in Table I) in order to minimize the number of missing sequences when concatenating multiple loci. Missing sequences were included as missing data. In the construction of the concatenated matrices, sequences were chosen on the basis of minimizing the number of samples used for a species. When multiple clones per species were available, if these were grouped into monophyletic entities in the individual analysis, one randomly chosen clone sequence was included in the concatenated matrices. For ougroup taxa, when more than one sequence was available for a particular locus, one sequence was chosen randomly (indicated by * in Table I). In this process, sequences from different species of the same genus were sometimes concatenated together. Six outgroup genera were represented by a chimera sequence of two or more sequences from different species of the same genus: *Bussea* (*B. perrieri* and *B. sakalava*), *Peltophorum* (*P. pterocarpum* and *P. dubium*), *Tachigali* (*T. vasquezii, T. glauca, T. paniculata*, and *T. amplifolia*) in both the ALLcp and ALLnc, *Jacqueshuberia* (*J. brevipes* and *J. loretensis*) and *Calliandra* (*C. inaequilatera* and *C. trinerva*) in the ALLcp, and *Erythrophleum* (*E. suavolense* and *E. ivorense*) in the ALLnc. Although not an ideal process, previous studies have showed these genera to be monophyletic (Bruneau et al

2008, Wojciechowski et al 2004) and because these outgroup taxa were not the focus of this study but were only present to calibrate the phylogeny, we considered this approach to be appropriate in this case.

The ten individual matrices and the three concatenated matrices were analyzed under parsimony and Bayesian phylogenetic methods. The genus *Diptychandra* was used to root the trees in most analyses while *Tachigali* was used for rooting the *Leafy* topologies. The parsimony analysis was implemented in PAUP* version 4.0b (Swofford 2002). Indels were coded according to the simple indel coding of Simmon & Ochoterena (2000). A first heuristic search was performed with 1000 replicates of random addition sequence, tree bisection–reconnection (TBR) branch swapping, retaining only five 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 a limit of 10 000 trees saved. Because this second analysis uses the topologies obtained initially, it allows for the investigation of more optimal topologies than a ‘‘one-step’’ analysis (Davis et al 2004). Strict consensus trees were 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.

For the Bayesian analysis, the concatenated matrices were partitioned by loci in order to attribute to each the appropriate nucleotide substitution model as estimated by MrModelTest 2.3 (Nylander 2004) and according to the Akaike criterion (Akaike 1974). Coded indels were analyzed as restriction sites following the instructions in the MrBayes 3.1 manual instructions. Bayesian analyses were performed using MrBayes 3.1.2 Manual (Ronquist & Huelsenbeck 2003) with 50,000,000 generations, two parallel runs of eight Markov Chain Monte Carlo each, and four swaps per swapping cycles. Trees were sampled every 1000 generations, and the first 25% generations were discarded as burn-in (Ronquist et al 2009).

Divergence time estimates

Divergence time estimates for each node were obtained based on the position of three fossils. No fossil is known for the Peltophorum clade and the closest occur within subfamily Mimosoideae and the Dimorphandra Group of subfamily Caesalpinioideae. The first calibration point was identified as *Eomimosoideae plumosa* and was placed at the stem node of the genus *Dinizia*, estimated at 45 Ma (Crepet & Dilcher 1977, Herendeen & Dilcher 1990); the second fossil was *Albizia*, and was placed at the Ingeae node and was estimated at 45 Ma (Guinet et al 1987); the third fossil was placed at the stem node of the genus *Acacia* and was estimated at 46 Ma (Herendeen & Jacobs 2000). These three fossils were previously used in Bruneau et al (2008).

Two methods for estimating the divergence times were used: the penalized likelihood (Sanderson 1997, 2002) and Bayesian MCMC (Drummond et al 2012) methods. The penalized likelihood method with rate smoothing, as implemented in r8s 1.70, allows for stringency relaxation of the molecular clock by setting values of smoothing that specify how much the rate of evolution is allowed to change along branches of the phylogeny (Sanderson 2002). The appropriate smoothing value was estimated by performing a cross validation step where smoothing values from 0.001 to 10 000 were covered by increments of $10^{0.5}$. The root of the phylogeny was fixed at 55 Ma following Bruneau et al (2008), while the calibration points were constrained to a minimum age corresponding to their respective estimated ages. A truncated Newton algorithm with five initial starts and five perturbed restarts was used on a fully resolved majority rule consensus tree, obtained from the Bayesian analysis of the combined data (ALLcpnc). The analysis was performed several times with the exclusion of one to two different calibration points in order to evaluate the impact of each calibration point on the age estimates.

The presence of a molecular clock was assessed in the combined dataset (ALLcpnc) using a likelihood ratio test to compare clock- and nonclock-like models (Felsenstein 1981). This test was performed in PAUP* 4.0 (Swofford 2002) where the likelihood of the data with, and without, the molecular clock was calculated. The difference between these two values was multiplied by two and taken as the test value for a chi-square test with df = 48 and p = 0.05. Divergence times were also estimated using a Bayesian MCMC method as implemented in BEAST 1.7.2 (Drummond et al 2012) using the Bayesian concatenated ALLcpnc partitioned matrix as input in BEAUTi v.1.7.2. The substitution models were unlinked and set according to each partition, while tree topology and clock model parameter remained linked. Taxon groups were created to represent the position of each fossil, and also to respect the monophyly of major clades recovered in the parsimony and Bayesian analyses, this seemed appropriate since this analysis was not performed to evaluate phylogeny reconstruction but divergence times. The Yule speciation process (Yule 1925, Gernhard 2008) with a random starting tree was selected as the tree prior. Prior distributions were set to a normal distribution (stdev=0.5) for each calibration point with the age of the fossil as the mean (Heled & Drummond 2012). Strict and relaxed lognormal molecular clock models were analyzed. For the strict clock analysis, the clock.rate parameter was set to a normal distribution (mean=0, stdev=1) prior distribution and the analysis performed for 100 000 000 generations with sampling every 2 000 generations. The relaxed clock analysis was performed for 150 000 000 generations, sampling every 2 500 generations and the prior distribution of the ucld.mean and ucld.stdev parameters set to a normal distribution (mean=0, stdev=1). Trees for both analyses were summarized with burn-in values set to the first 25% of trees sampled (Ronquist et al 2009) using TreeAnotator v.1.7.2 (Drummond et al 2012) and were resumed in a maximum clade credibility tree (MCCT).

Ancestral range reconstruction

Ancestral geographic ranges were reconstructed according to a dispersal-extinction-cladogenesis model in a maximum likelihood framework as implemented in Lagrange 20120508 (Ree 2005, Ree & Smith 2008). The online Lagrange configurator 20120508 was used to configure the matrices (www.reelab.net/lagrange/configurator). The analyses were performed using the topology of a subtree from the Bayesian analysis (from Mr.Bayes) of the concatenated ALLcpnc matrix containing only the 13 species of *Delonix, Colvillea*, *Lemuropisum*, and *Conzattia multiflora* as outgroup taxon. Tree root age was set to 33 Ma based on inference of divergence times from Bruneau et al (2008). Species were attributed one or several of the following seven geographic ranges based on their distribution: Mexico, Africa, Northern Madagascar (Diego Suarez), Northern inland Ankara Plateau, Western coastal Bemaraha Plateau (Belo), South-West Toliara region, and southern region from Autovombe to Itampolo. Dispersal constraints were modified to test for three hypotheses. First, we tested a null hypothesis (H0) of no dispersal constraint in time. The second hypothesis (H1) postulated a constant presence in time of a strong dispersal barrier (representing the Mozambique channel) between Africa and Madagascar (dispersal constraint was set to 0.4) along with a mild dispersal barrier between northern and southern Madagascar (0.7). This second scenario (H2) represents a long distance dispersal event. The third hypothesis had a time dependent dispersal constraint with two time matrices (from 33-25 Ma and from 24-0 Ma) to account for the hypothesized presence of a land bridge from 45 to 26 Ma between Africa and Madagascar (McCall 1997). For this scenario, the oldest time matrix was set to a milder dispersal barrier between Africa and Madagascar (0.6), whereas the recent time matrix was set to a strong dispersal barrier between the two continents (0.4).

Results

Cloning

The sequences for two (*Leafy* and *EIF3E*) of the six nuclear loci required cloning resulting in a total of 136 and 155 clones respectively. For *EIF3E,* sequences for *Acacia caven* and *Diptychandra aurantiaca* were not cloned because the direct sequencing resulted in clean, polymorphism-free sequences. The 14 clones of *ITS* sequences for *Colvillea racemosa* showed no polymorphism and thus no sign of unhomogenized paralogous copies. In addition, because direct sequencing of *Delonix* and *Lemuropisum* samples showed no polymorphism, no other *ITS* sequences were cloned.

Sequence characteristics

A total of 742 sequences were used in this study of which 672 are new. The sequences obtained were approximately the same length for each locus in all taxa studied, except for the *Leafy* sequence of *Parkinsonia aculeata*, which has a large insertion (174 bp) at the 5' end of the intron. In addition, the sequences of *D. tomentosa* are shorter for *AIGP* (by 148 bp), *rps16* (by 263 bp), *trnD-trnT* (missing the *trnY-trnT* intron), and *trnL-trnF* (missing the *trnL* exon). For *D. decaryi,* sequences are shorter for *rps16* (by 125 bp) and *trnD-trnT* (missing the *trnY-trnT* intron). The *trnD-trnT* sequence for *D. leucantha* is also missing the *trnY-trnT* intron. Repeated attempts at amplifying the missing part of each locus for these three species failed. Sequences amplification for the longest locus *matk/3'trnk* failed for *D. tomentosa*, *D. brachyarpa*, *D. leucantha* and *D. decaryi*. The difficulty in DNA amplification, and consequently the shorter or absent sequences, for these species could be due to the low yield and poor quality of DNA obtained from poorly conserved brownish-black leaf material (Rogers & Bendich 1985). The *EIF3E* region had a 33 bp insertion in the sequences of *D. regia* and, in addition, the intron alignment for the Mimosoideae species was ambiguous for this locus.

Table III. Sequence characteristics, parsimony and Bayesian analyses statistics for the ten individual loci and the three concatenated matrices in the phylogenetic analyses of genera *Delonix, Colvillea* and *Lemuropisum*, and outgroup taxa. CI, consistency index; RI, retention index; L, length; MPT, most parsimonious trees; AIC, akaike criterion.

	Number of sequences (ingroup species – outgroup)	Aligned length (bp)	Missing data (%)	Number of parsimony informative characters	CI	RI	L	Number of indels	Number MPT	Evolutionary model (AIC)
trnL-trnF	61 (13-20)	1191	5.6	177 (14%)	0.79	0.83	597	75	10000	GTR +G
matK	55 (9-19)	1798	0.3	280 (15%)	0.78	0.81	1044	41	10000	GTR +G
Rps16	60 (12-19)	974	1.6	241 (23%)	0.68	0.72	856	76	10000	GTR +G
trnD-trnT	59 (13-18)	1620	5.0	405 (23%)	0.70	0.66	1575	151	10000	GTR +G
ITS	55 (13-17)	904	1.3	694 (65%)	0.45	0.58	3733	166	18	GTR +G
AIGP	57 (12-20)	465	0.6	123 (26%)	0.68	0.75	500	9	10000	SYM +G
tRALs	52 (10-17)	355	0.5	126 (34%)	0.66	0.78	438	16	1283	HKY +G
CTP	51 (11-17)	336	0.0	152 (45%)	0.48	0.67	681	3	2266	K80 +G
EIF3E	156 (8-16)	893	0.4	485 (49%)	0.57	0.90	1372	105	10000	HKY +G
Leafy	136 (11-7)	1021	0.0	399 (34%)	0.69	0.90	1129	170	10000	K80 +I +G
ALLcp	49 (13–20)	5585	3	1033 (17%)	0.73	0.72	3926	326	6	-
ALLnc[1]	49 (13–20)	3609	14	1324 (33%)	0.55	0.62	5987	344	48	-
ALLcpnc[1]	49 (13–20)	9192	8	2354 (24%)	0.61	0.65	9996	667	15	-

[1] excluding the locus *CTP*

Phylogenetic analysis

Sequence characteristics and statistics for parsimony and Bayesian phylogenetic analysis of the ten individual loci and the three concatenated matrices are shown in Table III. In all analyses, individual and concatenated, with parsimony and Bayesian methods, multiple samples of the same species of all three genera *Delonix, Colvillea*, and *Lemuropisum*, always formed monophyletic groups (see Appendix). In both parsimony and Bayesian analyses of *EIF3E* and *Leafy*, clones from the same sample were monophyletic and clones from different samples of the same species also formed monophyletic groups.

The parsimony and Bayesian consensus trees for the individual markers were poorly resolved. The majority of these loci (*trnL-trnF, matK, rps16, trnT-trnD, EIF3E, Leafy* and *AIGP*) yielded a large polytomy at the base of the *Delonix/Colvillea/Lemuropisum* clade or *Delonix/Lemuropisum* clade (Appendix A1 to A10). The consensus trees for *ITS* and *tRALs* are the two best resolved and both showed similar species relationships where *Colvillea* and *Lemuropisum* are nested within *Delonix*. The consensus tree for *CTP* showed highly incongruent relationships where species, genera, tribe and subfamily did not form monophyletic groups (Appendix A10). Alignment was unambiguous and the BLASTn results for the sequences, including the ones from Choi et al (2006), were identified to a predicted mRNA Leguminosae sequence consistent with *CTP* as an exon. Because we could not identify the incongruence as being caused by the presence of a duplication, pseudogenes, allelic polymorphism, incompleate lineage sorting or non-specific primers, and because each phenomenon requires a specific treatment in phylogenetic reconstruction, the locus *CTP* was eliminated from the concatenated matrices and further analyses.

The concatenated matrices representing 49 samples created from the individual loci sequences contained the following sequences: the all plastid loci (ALLcp) matrix contained 49 *trnL-trnF*, 45 *matk*, 48 *rps16*, and 49 *trnD-trnT* sequences, the all nuclear loci (ALLnc) matrix contained 46 *ITS*, 48 *AIGP*, 44 *tRALs*, 41 *EIF3E*, and 34 *Leafy* sequences, and the ALLcpnc matrix included the same sequences for the nine loci. The consensus topology for the concatenated plastid sequences (Appendix A11) lacks resolution within the *Delonix/Colvillea/Lemuropisum* clade and the positions of *Colvillea racemosa* and *D. regia* are unstable between the parsimony and Bayesian analyses. The concatenated nuclear loci tree (Appendix A12) shows *Colvillea racemosa* nested within *Delonix*. The parsimony and bayesian consensus trees of the concatenated ALLcpnc sequences are both fully resolved and yield the same topology with each other and with the bayesian divergence time maximum clade credibility tree (MCCT), whereby *Colvillea racemosa* is supported as sister species to the *Delonix/Lemuropisum* group (Fig 3). This topology represents a credible species tree following the supermatrix method (sensu Gadagkar et al 2005) and represents the consensus species relationships with the genetic information of nine loci from both genomes.

The consensus species tree (Fig 3) shows topological congruence with the best resolved individual tree topologies, suggesting that *Delonix* is paraphyletic due to the nested position of *Lemuropisum edule*. However, the position of *Colvillea racemosa* is ambiguous. This species most likely occurs as sister to the *Delonix/Lemuropisum* clade (Fig 3), a position supported in the *matk* individual topology, but not by some nuclear loci, not in the concatenated nuclear topology where it is supported as nested within *Delonix* (*ITS* and *tRALs*), nor in the concatenated nuclear tree topology (ALLnc). Thus appears to be a topological incongruence between some nuclear loci and one plastid locus on the position of the genus *Colvillea*. When internal, the position for *Colvillea* is fixed as sister to *Lemuropisum edule*. However, the majority of loci, both nuclear and plastid (*AIGP, EIF3E, trnL-trnF, trnD-trnT, rps16*), simply lack resolution. The low support values for the exclusion of *Colvillea* are most likely due to this conflicting position. Based on prior biological knowledge of this species, such as genetic uniqueness (Bruneau et al 2008, Rivers et al 2011), exclusive pollen morphology (Banks 1997), and particular flower morphology (DuPuy et al 1995), we chose the hypothesis whereby *Colvillea racemosa* occurs as external to the *Delonix/Lemuropisum* clade for the subsequent analyses.

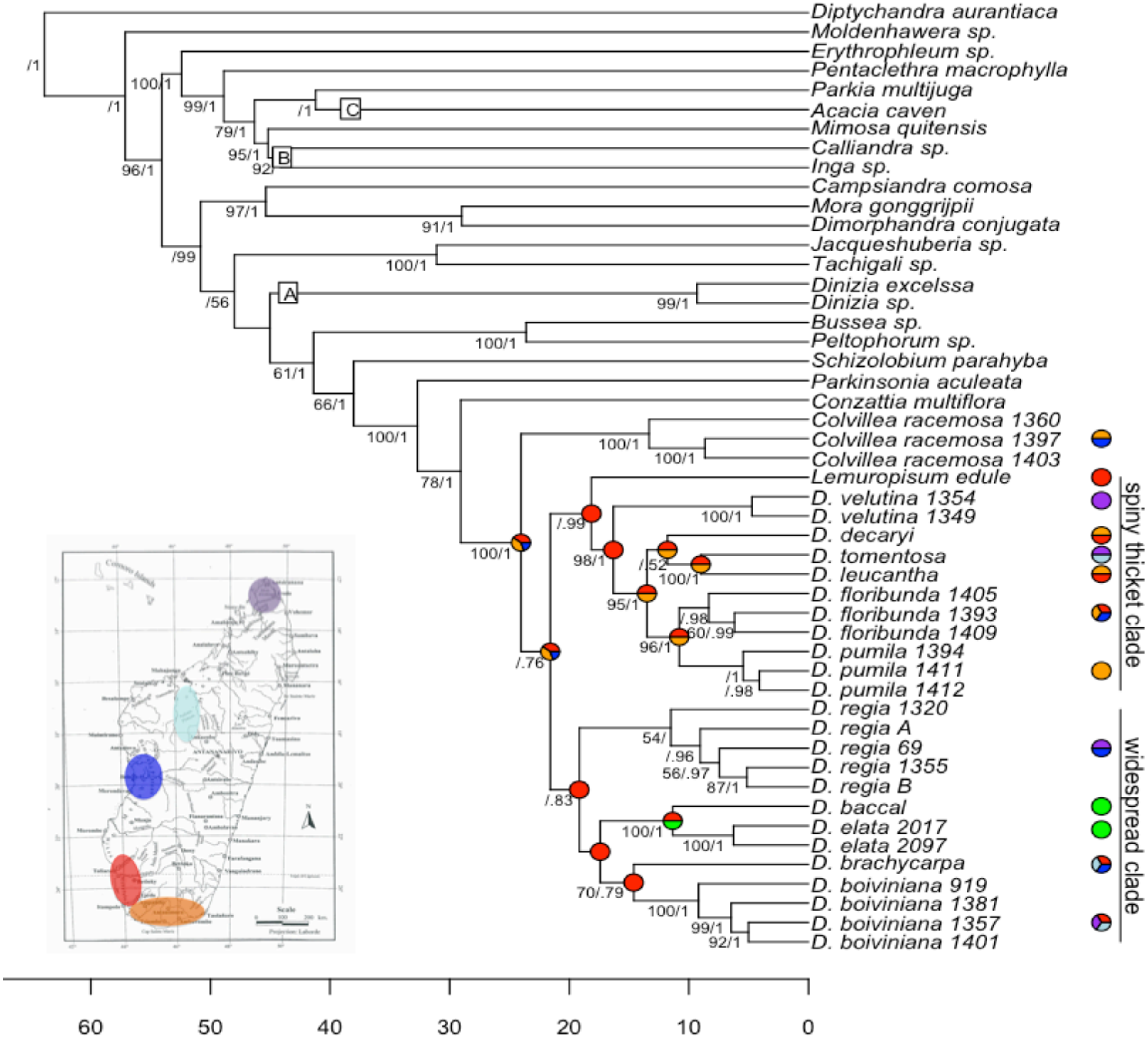


Figure 3. Chronogram of the Bayesian maximum clade credibility tree (MCCT) of the nuclear + plastid concatenated supermatrix with results from the ancestral geographic range reconstruction indicated by the colored circles in the phylogeny. Geographical range colors as follows : purple for northern Madagascar (Diego Suarez), light blue for Northwestern inland Ankara plateau, dark blue for Western coastal Bemaraha plateau (Belo), red for the Southwestern Toliara region, orange for the southern region from Autovombe to Itampolo, and green for Africa. Support values indicated as bootstrap/posterior probability below branches. Letters A (Dinizia 45 Ma), B (Ingeae 45 Ma) and C (Acacia 46 Ma) represent the position of fossil calibration points. Time is in million years.

Divergence time estimate

The likelihood ratio test, performed on the combined plastid and nuclear concatenated matrix, with a critical value of 65.17 (df = 48 and p = 0.05) for the chi-square distribution, found significant rate heterogeneity among lineages and justified the use of the smoothing rate method in the penalized likelihood estimation and the use of the relaxed clock in the Bayesian age estimation method. A smoothing value of $\lambda = 10$ produced the lowest error in the cross-validation step. The fossil cross-validation step suggested that none of the three individual fossil calibration points (or combination of two) impacted significantly the age estimates of the nodes of interest as all the permutated analyses revealed ages similar to the optimal three fossil-calibrated analyses. The Bayesian MCMC method revealed ages slightly younger, by about 4 to 5 Ma, than those estimated by the penalized likelihood method (Table IV). Ages from the likelihood analysis were within the 95% high and low Highest Posterior Density (HPD) intervals of the Bayesian MCMC age estimates. This difference is minor, does not affect the interpretation of results, and can be attributed to the greater amount of information (whole nucleotide sequences versus branch lengths) and prior distribution specification in the Bayesian analysis. The *Delonix/Colvillea/Lemuropisum* clade is estimated to have diverged from *Conzattia* around 24 Ma (95% HPD 19-29 Ma). *Colvillea racemosa* diverged around 21 Ma (95% HPD 17-26 Ma) while the remaining species from the genera *Delonix* and *Lemuropisum* separated between 18 and 11 Ma (Table IV).

Table IV. Divergence time estimates under the penalized likelihood with rate smoothing (λ=10) and mean age under the partitioned Bayesian relaxed molecular clock model, with 95% lower and higher Highest Posterior Density (HPD), and ancestral geographic range estimates under the divergence-extinction-cladogenesis model for the H1 hypothesis for the most common recent ancestor of the major clades in the fossil calibrated phylogeny of *Colvillea, Delonix* and *Lemuropisum*. Ages in million years.

Clade	**Penalized likelihood**	**Bayesian relaxed clock (low-high 95% HPD)**	**Ancestral geographic range***
Colvillea/Delonix /Lemuropisum	27.80	24.04 (19-29)	W, SW, S
Delonix/Lemuropisum	25.99	21.59 (17-26)	W, SW, S
Spiny thicket clade	23.46	18.14 (13-23)	S
D. velutina/D. pumila	22.57	16.31 (11-21)	S
D. decaryi/D. pumila	21.01	13.50 (9-18)	S, SW
D. tomentosa/D. leucantha/D. decaryi	20.35	11.79 (8-16)	S, SW
D. floribunda/D. pumila	14.60	10.79 (7-15)	S, SW
Widespread clade	24.24	19.16 (14-24)	S
African *Delonix/D. boiviniana*	23.42	17.41 (13-22)	S
African *Delonix* species	16.35	11.35 (6-16)	S, A
D. brachycarpa/D. boiviniana	21.76	14.62 (10-19)	S

* Geographical range acronyms as follows : N for northern Madagascar (Diego Suarez), NW for Northwestern inland Ankara plateau, W for Western coastal Bemaraha plateau (Belo), SW for the Southwestern Toliara region, S for the southern region from Autovombe to Itampolo, and A for Africa.

Ancestral geographical range estimates

The hypothesis of long distance dispersal (H1) was recovered as the most likely to represent our data based on the lower global likelihood (ln= 49.01) compared to the null hypothesis (H0: ln = 53.08) and the land bridge hypothesis (H2: ln= 49.86). The dispersal rate was estimated at 0.01829, while the extinction rate was estimated at 0.0166. Deeper nodes were found to have multiple possible ancestral geographic ranges (Table IV). The ancestral geographic range at each node of the phylogeny were clear: the majority of species originated from ancestors found in the South and South-West of Madagascar.

Discussion

Multilocus, multigenome, supermatrix approach untangles the phylogenetic relationships of closely related genera and species

The inclusion of multiple plastid and nuclear loci was beneficial in our study because it allowed for the identification of phylogenetic incongruence between the two genomes, for examining species relationships and for obtaining more phylogenetic data for taxa that have been showed to have extremely low levels of genetic variability. Many loci, individually, lacked resolution for the genera *Delonix, Colvillea* and *Lemuropisum*, but combined, were able to resolve the polytomy that plagued previous phylogenetic studies (Simpson et al 2003, Haston et al 2003, 2005, Hawkins et al 2007, Manzanilla & Bruneau 2012). The lack of resolution in many plastid regions (*trnL-trnF, matk* and *rps16*) is unsurprising, nevertheless, plastid loci are useful phylogenetic tools that can help to resolve closely related species relationships when used along with nuclear loci (Shaw et al 2005, 2007, Moore et al 2010). The better resolution of some nuclear loci (individually, as in *ITS* and *tRALs*, and in combination in ALLnc) is an indication of the phylogenetic utility of nuclear genome loci in untangling relationships of closely related plant species (Sang 2002, Small et al 2004, Hughes et al 2006, Steele et al 2008, Duarte et al 2010). The fact that other nuclear regions (*AIGP, EIF3E, Leafy*) lack interspecific, and sometimes even intergeneric, resolution is consistent with the results of Manzanilla & Bruneau (2012) and implies that these loci could simply be uninformative at that specific taxonomic level (Satta et al 2000). The unresolved nuclear loci, coupled with the unresolved relationships of the combined plastid sequences analysis, is evidence of the genetic proximity, despite much morphological diversity, for *Delonix, Colvillea*, and *Lemuropisum,* which could be the consequence of a rapid radiation (DuPuy et al 1995, Simpson et al 2003, Haston et al 2003, 2005, Hawkins et al 2007, Rivers et al 2011). Our study illustrates the utility of combining multiple independent sources of variation (loci and genomes) to better understand aspects of species evolutionary history (Rokas et al 2003, Felsenstein 2004, Gagdakar et al 2005, de Queiroz & Gatesy 2006, Baker et al 2009).

Generic relationships: one fewer endemic genus in Madagascar

Important assessments can be made about the generic relationships of *Delonix, Colvillea*, and *Lemuropisum*. The genus *Delonix* is not supported as monophyletic in any of our analyses due to the nested, strongly supported, position of *Lemuropisum edule*. The genera *Delonix* and *Lemuropisum* share a similar flower morphology, as species from both genera possess generally white petals, and subequal, valvate calyx segments, which are leathery and thickened (Du Puy et al 1995). The pollen structure of *Lemuropisum edule* is identical to that found for the majority of *Delonix* species, and they are thought to share the same moth pollinator (DuPuy et al 1995, Banks 1997). Based on nuclear AFLP data, *Lemuropisum* was shown to be genetically close to *D pumila* and to be strongly associated with other sympatric southern Madagascar species such as *D. floribunda* and *D. decaryi* (Rivers et al 2011), an assessment that our study corroborates.

The generic relationships are ambiguous when it comes to *Colvillea racemosa.* We consider its placement as external to the *Delonix/Lemuropisum* clade as a valid hypothesis. Based on the recovery of this external position for *Colvillea* by the species tree, obtained from two independent methods of phylogenetic analysis with nine, sometimes incongruent, individual loci sampled from two distinct genomes, we consider it to be the most likely position for this genus. This suggests that a phylogenetic signal can be recovered even among multiple unresolved loci (Rokas et al 2003, Maddison & Knowles 2006, Wiens et al 2008, Baker et al 2009). In addition, the distinct phyletic nature of *Colvillea racemosa* is congruent with the uniqueness of its floral morphology, the species having resupinate inflorescence with orange petals, large terminal panicles, with four fused calyx segments and clustered stamens, in addition to its unusual pollen structure and the possession of 15 private alleles (DuPuy et al 1995, Banks 1997, Rivers et al 2011).

Regardless, doubt persists as to the definitive phylogenetic placement of *Colvillea racemosa*. Haston et al (2005) also recorded an unstable position for this taxon. The alternative internal supported position seen in some nuclear loci (*ITS* and *tRALs*) for *Colvillea* could help to better understand the biology of this species. Incongruence between multiple individual loci trees is common as each one has its own evolutionary history (de Queiroz et al 1995, Huelsenbeck et al 1996, Doyle 1997, Maddison 1997, Wendel & Doyle 1998, Degnan & Rosenberg 2006, Meng & Kubatko 2009). The incongruence between nuclear and plastid genome loci in terms of the position of *Colvillea racemosa* could be attributed to incomplete lineage sorting or hybridization followed by introgression (Sang et al 1997, Rieseberg et al 2000, Sang & Zhong 2000). Future studies should aim to test and differentiate between incomplete lineage sorting and hybridization by using recently developed methods (Joly et al 2009, Joly 2012). When placed within *Delonix*, *Colvillea racemosa* is always sister to the spiny thicket clade containing *Lemuropisum edule, D. velutina, D. tomentosa, D. decaryi, D. pumila,* and *D. floribunda,* among others. *Colvillea* is sympatric with many of these species and therefore hybridization via cross pollination is geographically plausible, especially considering that *Colvillea* is reported to share the same sunbird pollinator as *D. floribunda* and *D. velutina* (DuPuy et al 1995). Hybridization is also plausible on a genetic level as there is no significant genetic distance between populations of *Colvillea* and *Delonix* (Rivers et al 2011). The possibility of hybridization is consistent with findings that Leguminosae species occurring in dry spiny thicket-like habitat, worldwide, have low genetic barriers and possibly more gene flow (Lavin et al 2004) than plants found in other biomes as illustrated by Rivers et al (2011).

In light of previous studies and the data shown here, we suggest a taxonomic revision for the genera *Delonix* and *Lemuropisum*. A previous proposal for a taxonomic revision of the genus *Delonix* argued for the inclusion of both *Lemuropisum* and *Colvillea* (Haston et al 2005). Our results strongly support the inclusion of *Lemuropisum* but are equivocal as to the inclusion of *Colvillea* within *Delonix*. Two alternatives are possible: divide the genus *Delonix* into two genera, or include *Lemuropisum* as a species of the genus *Delonix*. The latter is more parsimonious and congruent with molecular, morphological and ecological data (DuPuy et al 1995, Banks et al 1997, Simpson et al 2003, Haston et al 2003, 2005, Hawkins et al 2007, Bruneau et al 2008, Rivers et al 2011). We would therefore suggest the name *Delonix edule* to replace *Lemuropisum edule* H. Perrier.

Interspecific relationships associated with geographic distribution patterns give insight on morphological evolution

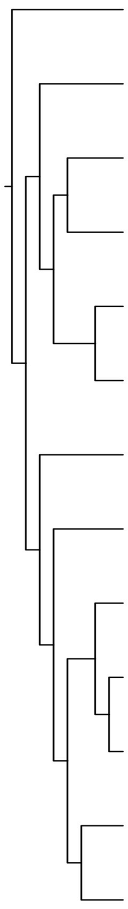
The genera *Delonix, Colvillea* and *Lemuropisum* appear to have evolved from a Madagascan ancestor, found in the South and West of the island, that lived during the late Eocene from 33 to 24 Ma. Similar geographic conditions gave rise to the *Delonix/Lemuropisum* ancestor between 24 to 21 Ma in the early Miocene. This is evidence that Madagascar is an independent evolutionary source (Raxworthy 2002, Yuan et al 2005, Krüger et al 2012), contrary to the view that Madagascar species might be direct descendants of African ancestors (Schatz 2001, de Wit et al 2003, Vences et al 2003, Yoder & Nowak 2006, Tsy et al 2009, Anthony et al 2010). This implies that the diversification of *Delonix* species was not triggered by the colonization of Madagascar.

The two clades recovered for *Delonix* are associated with the geographic distribution of species: one contains the most widespread species occurring throughout western Madagascar and also in Africa, Arabia and India, while the other clade contains rare species with small ranges mostly restricted to the southern spiny thicket. Some morphological characters, such as number and size of leaflets, as well as pod type, seem to be linked to the species habitat rather than to evolutionary relationships (Fig 4). Coversely, other morphological characters, such as pollen type, tend to be linked to phylogenetic relationships rather than to habitat. Red-orange flower color appears to be an ancestral character that rapidly changed to white and yellow in the two clades but was retained in *D. regia*. The famous cigar or bottle-shaped trunk characteristic of many *Delonix* species seems to have evolved independently twice given that cylindrical trunks are not found in the first diverging species of the two clades and a transition to swollen/cigar-shaped trunks is observed in recent species of both clades. Interestingly, *D. boiviniana* possibly is an intermediate in the widespread clade as it exhibits both types of trunk shape. The geographical structure observed in the phylogeny of *Delonix* and *Lemuropisum* is another example of this tendancy noted for Leguminosae occurring in dry succulent habitats (Lavin et al 2004, Schrire et al 2009). Species from the succulent biome tend to conserve the same niche after continental dispersal, as seems to be the case for the African *Delonix* species that both retained the same habitat as their Madagascan ancestor and Madagascan sister species.

The widespread clade diverged around 19 Ma (± 5 Ma) from a southern Madagascar ancestor. The species are characterized by having bigger leaflets and a type I and II pollen morphology (Fig 4: DuPuy et al 1995, Banks 1997). Type I pollen morphology differs from II and III in having denser granules at the base of the lumina, rounded muri, larger aperture lengths and absence of layering on the microperforation (Banks 1997). The close relationship between the two African species supports previous molecular work and is consistent with the possession of unique pods for these two species (Du Puy et al 1995, Haston et al 2005, Hawkins et al 2007). As in our analyses, Haston et al (2005) also recovered a close relationship between *D. regia* and the African species. There are many morphological similarities between *D. boiviniana* and *D. brachycarpa,* which lead DuPuy et al (1995) to suggest these taxa to be conspecific. Our data strongly support them as closely related yet distinct species. Indeed, with multiple samples of *D. brachycarpa* for some loci, we observe that the two species are found together in a group where *D. brachycarpa* is the first diverging taxon (e.g *ITS*). However, *D. brachycarpa* is also sometimes placed with other *Delonix* species in a few individual loci analyses while still remaining monophyletic (e.g *Leafy*, *trnL-trnF*).

The spiny thicket clade diverged from a southern Madagascar ancestor during the Miocene around 18 Ma (± 5 Ma). This clade is characterized by type II and III pollen morphology, white and yellow flowers generally pollinated by moths, a tendency to be shrubby and dense with smaller organs (flowers, petals, small and lesser number of leaflets) (Fig 4: DuPuy et al 1995, Banks 1997). Pollen morphology of types II and III are very similar in having sparse granules at the base of the lumina, reduced aperture lengths and bi-layered microperforations (Banks 1997). Other molecular studies also recovered a group formed by *Lemuropisum edule, D. pumila* and *D. floribunda* (Haston et al 2005, Rivers et al 2011), while morphological studies grouped *D. decaryi, D. leucantha, D. floribunda* and *D. pumila* together (Du Puy et al 1995). Placement of the northern rare species *D. velutina* and *D. tomentosa* in the spiny thicket clade can seem counterintuitive because they both possess characters more typical of the widespread clade (elongated pods, higher number and bigger leaflets). However, we suggest that these characters are driven by ecological conditions of the dry deciduous forest and were acquired by these two species following their recent expansion into northwestern Madagascar. These two species are well supported within the phylogeny and both are derived from a southern Madagascar ancestor, suggesting the southern populations of *D. velutina* and *D.*

tomentosa are extinct and that the two species were introduced (naturally or not) recently in the north. This would explain their rarity and small range but also the retention of southern spiny thicket morphological characteristics such as cigar-shaped trunks. These species also retained ecological characteristics of the spiny thicket habitat such as growth at lower altitude contrary to the other Madagascan dry deciduous forest species that grow at higher elevation (DuPuy et al 1995).



	Distribution	Habitat	Pollen type	Pollinator	Number leaflets	Leaflet size	Flower color	Trunk shape	Pods	Altitude (m)
***Colvillea racemosa*[a]**	W, SW	DDF/ST	IV	sunbird	high	++	orange	normal	long	low
***D. regia*[b]**	N, W	DDF	I	moth	high	++	red	normal	short	300-1000
***D. boiviniana*[a]**	N, NW, S	DDF/ST	II	moth		+	white	Cigar/ normal	short	400-700
***D. brachycarpa*[a]**	NW, W, S	DDF/ST	II	moth		+	white	normal	short	600-900
***D. elata*[?]**	Africa-India	ST	I	sunbird	high	++	white	Cigar	Type F	600-900
***D. baccal*[a]**	Kenya	ST/ riverine	I	sunbird	high	++	white	Cigar?	Type F	?
***Lemuropisum edule*[c]**	S	ST	II	moth	few		white	normal	unique	?
***D. velutina*[d]**	A	DDF	II/III	sunbird	high	++	white	Cigar	long	250
***D. decaryi*[a]**	S, SW	ST	II	moth	few		white	Cigar	Type A	100
***D. tomentosa*[c]**	N, NW	DDF	II	moth	high	++	white	swollen	long	100-500
***D. leucantha*[b]**	S, SW	ST	II	moth	few		white	swollen	Type A	150
***D. floribunda*[a]**	W, SW, S	DDF/ST	III	sunbird	few		white	Cigar	Type A	100
***D. pumila*[c]**	SW	ST	II	moth	few		white	swollen	Type A	100

Figure 4. Morphological and ecological characters mapped onto the phylogeny of *Delonix, Colvillea* and *Lemuropisum*. Information retrieved from Du Puy et al (1995), Banks (1997), Orwa et al (2009), Rivers et al (2011), IUCN (2012). Conservation status is indicated by the species name: [a] near threatened, [b] vulnerable, [c] endagered, [d] critically endangered.DDF, deciduous dry forest; ST, spiny thicket. Pollen type from Banks (1997) see text for details.

Out of Madagascar dispersal during the Miocene

The long distance dispersal for the African *Delonix* ancestor implies migration from southern Madagascar to eastern Africa across the Mozambique Channel between 17 (± 5 Ma) and 11 Ma (± 5 Ma) during the Miocene. Miocene dispersal around Madagascar has been recorded in many groups (Vences et al 2003, Li et al 2009, Master et al 2006, Clayton et al 2009, Strjik et al 2012, Zhou et al 2012). Although there is an accumulation of evidence for unidirectional long distance dispersal from Africa to Madagascar (Schatz 1996, Renner 2004a, Yoder & Nowak 2006, Schaefer et al 2009, Schrire et al 2009, Tsy et al 2009, Anthony et al 2010, Wikström et al 2010), our study supports recent findings of unidirectional dispersal in the opposite direction (Raxworthy 2002, Yuan et al 2005, Wikström et al 2010, Krüger et al 2012). Our results support the observation that "Out of Madagascar" events are fairly recent and that dispersal from Madagascar to Africa, at least in vertebrate taxa, all date from late Eocene to middle Miocene (Raxworthy 2002, Crottini et al 2012) and up to the Pliocene in some plant groups (Yuan et al 2005). In addition, it is thought that open terrestrial landscape, such as the spiny thicket of Madagascar, where the African *Delonix* ancestor is estimated to have lived, may have facilitated long distance dispersal because of its open and sparse vegetation (Nathan 2008).

The Davie fracture zone landbridge hypothesis is eliminated as the dispersal took place well after the disappearance of the land bridge (45-26 Ma: McCall 1997). Even if it had persisted during the Miocene, our results rejects it as an axplanation, as geographic facilitation (H2 hypothesis rejected on lower likelihood) most likely does not explain the distribution of *Delonix*. Dispersal by migratory birds across the Mozambique Channel during the Miocene has been invoked in some plant taxa (e.g., Les et al 2003, Renner 2004a). The long distance dispersal of seeds is thought to be mediated by large, migratory animals, especially birds that visit many different plant species. Birds also have larger home range, larger gut capacity, and often travel long distances at high speed on a yearly basis (Nathan 2008, Gillespie et al 2012). The sunbird *Nectarinia souimanga* has been observed to visit the flowers of many *Delonix* species, including the African ones, and is assumed to be their pollinator based on personal observations (DuPuy et al 1995). This bird easily travels across large ocean barriers (Warren 2003). However, a pollinator is not necessarily the seed disperser, and the animals responsible for seed dispersal of *Delonix, Colvillea* and *Lemuropisum* remain unknown. *Delonix* seeds are edible and possess no physiological adaptation for attachement to animals, reinforcing the idea that they are dispersed by endozoochory (DuPuy et al 1995, Grant et al 1995). Several migratory birds, such as the squacco heron, cuckoo, pratincole and broad-billed roller (Moreau 1966), travel yearly between Madagascar and Africa and have been identified as potential seed dispersal vectors in Afro-Madagascan *Melastoma* (Renner 2004a). However, all of these birds are insectivores and are not known to eat seeds (Langrand 1990, Morris & Hawkins 1998, Kushlan & Hancock 2005). This hypothesis is therefore less likely as an explanation for the dispersal of *Delonix* or of any plant dispersed by endozoochory.

Our result supports dispersal without geographic facilitation, and along with other sources of evidence such as biological characteristics of *Delonix* species, past oceanic and climatic models, strongly suggest that the dispersal from Madagascar to Africa occurred by rafting as suggested by the sweepstake hypothesis (Simpson 1940). Long distance dispersal by floating on vegetative rafts has been shown to be a likely scenario for many organisms (Morley & Dick 2003, Thiel & Gutow 2005, Gillespie et al 2012). Usually invoked to explain dispersal from Africa to Madagascar, there is no reason to believe that it did not occur in the opposite direction when conditions were favorable such as during the Miocene. Indeed, oceanic currents and surface winds returned to their present-day direction flowing from Madagascar to Eastern Africa during the early-mid Miocene (von der Heydt & Dijkstra 2006, Ali & Huber 2010). The pods of *Delonix* species can most likely float, especially if entwined in vegetation, as they are long, flat and woody (DuPuy et al 1995). Also, seeds of Leguminosae, including some Caesalpinioideae species, have a germination rate of 50 % or more after 30 days immerged in water and even up to 60 days for some species (Lopez 2001).

Past range movement and implications for the evolution and conservation of Madagascar spiny thicket plant species

Our results imply a correlation between the evolutionary history, geographic distribution, and conservation status of plant species in Madagascar. The three biodiversity centers identified for *Delonix* by DuPuy et al (1995) can be chronologically ordered: the southern center (Toliara) is the oldest center, and was the primary location for *Delonix* species until recently. Species most likely colonized the western center (Belo) following the coastline and eventually arrived in the northern center (Diego Suarez). *Delonix* species from the widespread clade show a less precarious conservation status as is expected of species with wide distributions crossing different habitats. The widespread species also demonstrated considerable genetic diversity (Rivers et al 2011). Our results show that species from this clade evolved from a southern ancestor, implying they expanded their distribution in the past to colonize northern and northwestern dry deciduous forest. In contrast, the spiny thicket clade shows a higher number of species with urgent conservation status. This cannot be solely linked to the spiny thicket vegetation type as the two dry deciduous forest species (*D. velutina* and *D. tomentosa*) are among the most endangered within the whole genus. Except for these two species, the remaining species from the spiny thicket clade have maintained ranges relatively similar to that of their ancestors. Therefore the correlation between spiny thicket and precarious conservation status lies in past and present ecological embrittlement of this habitat. The recent migration of *Delonix* species from the two clades within

Madagascar could be attributed to the aridification of Madagascar during the Pliocene, which led to extensive landscape fragmentation in southern Madagascar, but might have been caused by human activity, which intensified around 2000 to 500 years ago (Burney et al 1997, de Wit 2003, Harper et al 2007, Strijk et al 2012).

However, another explanation to *Delonix* species range expansion and loss within Madagascar is transport by recently extinct animals. Today, lemurs are known to visit *Delonix* species and are important seed dispersers in the dry deciduous forest (Bollen et al 2004, Voigt 2004, Sato 2012). Extinct lemurs from the spiny thicket were inferred as potential seed dispersers for *Delonix* as well as for many other endemic spiny thicket plant taxa (Godfrey 2008, Crowley et al 2011). Also, the reduction in organ size and the shrubbiness of Madagascar spiny thicket species, as seen in many *Delonix* and *Lemuropisum* species, are most likely ancestral physical traits for defense against the endemic, extinct, and herbivore elephant-bird (Grubb 2003). Our study corroborates this explanation, as these traits seem to be associated with ecology rather than phylogeny in our data. The extinction of these large endemic animals, around 2000 Ma, was most probably triggered by human activity and climate changes (Muldoon 2010). Therefore, large lemurs and elephant-birds most likely had strong interactions with *Delonix* species (and possibly *Colvillea* and *Lemuropisum*) plausibly as seed dispersers. If the smaller, extant, lemurs have taken over the role of seed disperser after the extinction of the bigger animals in the spiny thicket remains to be answered. The absence of information concerning the mechanisms and vectors of seed dispersal for *Delonix, Lemuropisum* and *Colvillea* is an important gap that needs to be addressed, for genetic, biogeography and conservation issues. Dispersal patterns have important implications in population dynamics, such as range expansion, recruitment pattern, and genetic structure (Levin et al 2003, Nathan 2006). The extinction of seed dispersers for these three genera would support observation of low regeneration growth in natural habitat as well as the restricted distribution of endangered species (Rivers et al 2011). This is the case for many other plant species in Madagascar as trees in the spiny thicket show more limited dispersal than in other ecoregions on the island (Voigt 2009). *Delonix* might be a past example of present day impact on plant extinction level due to the extinction of lemur populations (Wright et al 2011). A better understanding of dispersal vectors will help to estimate the consequences of forest fragmentation and of climate change on dispersal patterns (Levey et al 2005, Trakhtenbrot et al 2005, Nathan 2008). Deeper understanding of past ecological interactions of endemic plant species in Madagascar with extinct and extant animal species is important in order to assess their present and future dynamics (Bleher & Bohning-Gaese 2001).

We argue for an increased protection of the southern Madagascar spiny thicket due to its actual and historical evolutionary, and geographic, importance for *Delonix, Colvillea* and *Lemuropisum*. This habitat is critically endangered according to the World Wildlife Fund (WWF 2013). Only four of the 22 national parks in Madagascar cover spiny thicket thus 98% of its primary vegetation is not protected (DuPuy & Moat 1998, Madagascar National Parks Association 2013). Many Madagascar endemic legumes species, such as *Delonix*, are associated with sand and limestone soils but these formations are poorly protected and need urgent conservation efforts (DuPuy & Moat 1998, DuPuy et al 2002).

The dry deciduous forest is also a conservation concern since many *Delonix* species (*D. velutina*, *D. tomentosa* and naturally occurring *D. regia*) from different evolutionary lineages are restricted to this vegetation type. It is the third largest vegetation zone in Madagascar but less than 70% of its primary forest is protected (DuPuy & Moat 1998, Schatz 2001). High altitude species like *D. regia* and *D. brachycarpa* might become increasingly endangered in the future as temperatures in northern Madagascar mountains have increased dramatically recently and shifts in species distribution and vegetation zone have been recorded (Raxworthy et al 2008).

Conclusion

The protection of *Delonix, Colvillea* and *Lemuropisum* species populations, pollinators, dispersal vectors, biodiversity centers, vegetation type and sand/limestone soil is not mutually exclusive and can be achieved by identifying areas with maximum combination of these important conservation factors. This study highlights the importance of collaborative work between systematics, taxonomy, biogeography, and conservation biology (Callmander 2005). The establishment of new nationals parks in Madagascar following the current management method might not be the answer seeing the inefficiency of these parks to protect forest loss within their boundaries (Ingram & Dawson 2007). The inevitable prospect of climate change and forest fragmentation in Madagascar is an incentive to increase our taxonomic and phylogenetic knowledge of Madagascar plants as this information helps conservation efforts by providing accurate and up to date identification tools of rare taxa, and estimation of actual and historical species distributions (Schatz 2002).

Delonix edule (Perrier) Babineau & Bruneau comb. nov.
basionym: Lemuropisum edule H. Perrier, Bull. Soc. Bot. France 85: 494. 1938.
Type specimen: H. Perrier de la Bâthie 19183 (P, TAN). 1933.

Chapitre 3 : perspectives futures

Le chapitre 3 explore les perspectives futures de ce projet et propose des pistes d'analyse concernant les espèces des genres *Delonix, Colvillea* et *Lemuropisum*. Ce chapitre vient répondre à la question : Que faire avec l'information récoltée durant ce projet?

Quoi faire d'autre avec les données moléculaires?

Il existe plusieurs façons de combiner l'information de plusieurs loci pour la reconstruction d'un arbre phylogénétique. L'une d'elles est la méthode de type supermatrice telle que décrite au chapitre 2. Une alternative est la méthode superarbre qui, contrairement à la méthode supermatrice, consiste à construire un arbre-consensus à partir des topologies des arbres provenant de l'analyse individuelle de loci (Degnan & Salter 2005, Degnan & Rosenberg 2009). Une reconstruction préliminaire a été générée avec nos données chloroplastiques et nucléaires en utilisant le paramètre de regroupement de la plus petite divergence de distance entre espèces (shallowest divergence: Maddison & Knowles 2006) telle qu'implantée dans le programme ape 3.0-4 du logiciel R 2.15.1 (R Core Team 2012). Ce superarbre (Annexe A14) est moins résolu que l'arbre de type supermatrice ce qui est congruent avec les observations de Gadagkar et al (2005) pour ce type de méthode, mais représente néanmoins plusieurs relations phylogénétiques similaires à la topologie supermatrice. Cette congruence entre les deux types de reconstruction d'arbre d'espèces inspire confiance concernant la validité de nos résultats et des relations intergénériques et interspécifiques retrouvées entre *Delonix, Colvillea* et *Lemuropisum*. Une autre méthode de reconstruction d'arbre à partir de plusieurs loci est la méthode Bayésienne de coalescence telle qu'implantée dans *BEAST (Drummond et al 2012). Cette dernière utilise une méthode Bayésienne avec chaines de Markov Monte Carlo pour estimer la coalescence de multiples espèces en se basant sur plusieurs loci et l'information moléculaire de multiples individus. Cette méthode coestime la topologie des arbres de gènes se trouvant à l'intérieur d'un même arbre d'espèce et estime à la fois l'arbre d'espèce, les temps de divergences, taille des populations et les arbres de gènes (Heled & Drummond 2010). Cette méthode n'a pas été utilisée lors de ce projet pour les espèces des genres *Colvillea, Delonix* et *Lemuropisum* mais est recommendandée pour de études futures de ce groupe.

Une autre étude possible grâce aux données moléculaires récoltées lors de cette recherche, serait de vérifier l'efficacité des loci sélectionnés pour le barcodage des plantes (CBOL Plant Working Group 2009). Ces données permettraient de vérifier l'utilité de la méthode d'identification par code à barre à un niveau intergénérique et interspécifique pour des espèces proches parentes, endémiques à Madagascar et menacées d'extinction.

Que faire avec les hypothèses émises?

Hybridation

Afin de vérifier la capacité d'hybridation entre *Colvillea* et certaines espèces des genres *Delonix* et *Lemuropisum*, et également être en mesure de distinguer entre un phénomène d'hybridation ou d'assortiment incomplet de cette lignée, des études empiriques et expérimentales, en laboratoire et en nature, devraient être entreprises. D'une part, celles-ci permettraient de tester l'impact de l'hybridation sur les modèles de reconstruction phylogénétique et, d'autre part, de tester la capacité des espèces à former des hybrides (Rieseberg et al 2000, Joly et al 2009, Joly 2012). De plus, il serait intéressant d'étudier le flux génique, et la capacité d'hybridation, entre les espèces, et également les populations d'une même espèce, vivant dans la forêt décidue sèche et celles vivant dans le fourré aride.

Changement taxonomique

Une révision taxonomique formelle de *Lemuropisum* devrait être entreprise nécessitant la collaboration de plusieurs herbiers. De plus, comme la famille des Légumineuses possède huit genres monospécifiques endémiques à Madagascar, incluant *Colvillea* et *Lemuropisum* (DuPuy 2002) et que les résultats démontrent que l'un d'entre eux est artificiel, une vérification exhaustive des affinités évolutives et du statut taxonomique pour les six autres serait de mise afin de s'assurer de leur véracité en tant que genres distincts. Cette vérification est pertinente considérant les récents changements taxonomiques effectués au niveau générique dans la famille des Légumineuses (Kirkbride 2005, Lewis et al 2005, Schrire 2008).

Conclusion

Ce projet de recherche montre l'avantage d'une approche multiloci dans la reconstruction phylogénétique d'espèces proches, et ce, malgré la présence d'incongruence topologique et l'absence de résolution de certains loci. L'obtention d'un patron phylogénétique résolu pour les espèces des genres *Delonix*, *Colvillea* et *Lemuropisum*, ainsi que l'apport d'information temporelle et spatiale concernant leur histoire biogéographique, suggère plusieurs conclusions concernant leur évolution. D'abord, le genre *Delonix*, tel que décrit présentement, est paraphylétique due à la position interne de *Lemuropisum edule*. Le genre *Colvillea* semble être un genre distinct bien que sa position soit ambiguë et cette incongruence est possiblement due à l'hybridation ou à un assortiment incomplet. La majorité des espèces proviennent d'ancêtres restreints au fourré aride du sud de Madagascar, ce qui suggère d'importants changements dans l'aire de répartition des espèces. La dispersion de l'ancêtre des espèces de *Delonix* africaines a eu lieu au Miocène à partir du sud de Madagascar vers l'Afrique, vraisemblablement par flottaison sur un radeau végétatif. Nos résultats supportent des évidences récentes de la dispersion unidirectionnelle d'organismes à partir de Madagascar, indiquant que cette île n'est pas seulement un réceptacle, mais également une source indépendante de biodiversité. Le fourré aride de Madagascar a eu, et continu d'avoir, un rôle important dans l'histoire évolutive des genres *Delonix, Colvillea* et *Lemuropisum*, ainsi que dans l'histoire évolutive d'une multitude d'autres espèces végétales qui y sont endémiques. Ceci suggère le besoin pour une conservation accrue de ce milieu vulnérable en misant sur les interactions écologiques plante-pollinisateur-disperseur et sur certains facteurs abiotiques qui sont déterminant dans l'évolution et le maintient de la diversité végétale.

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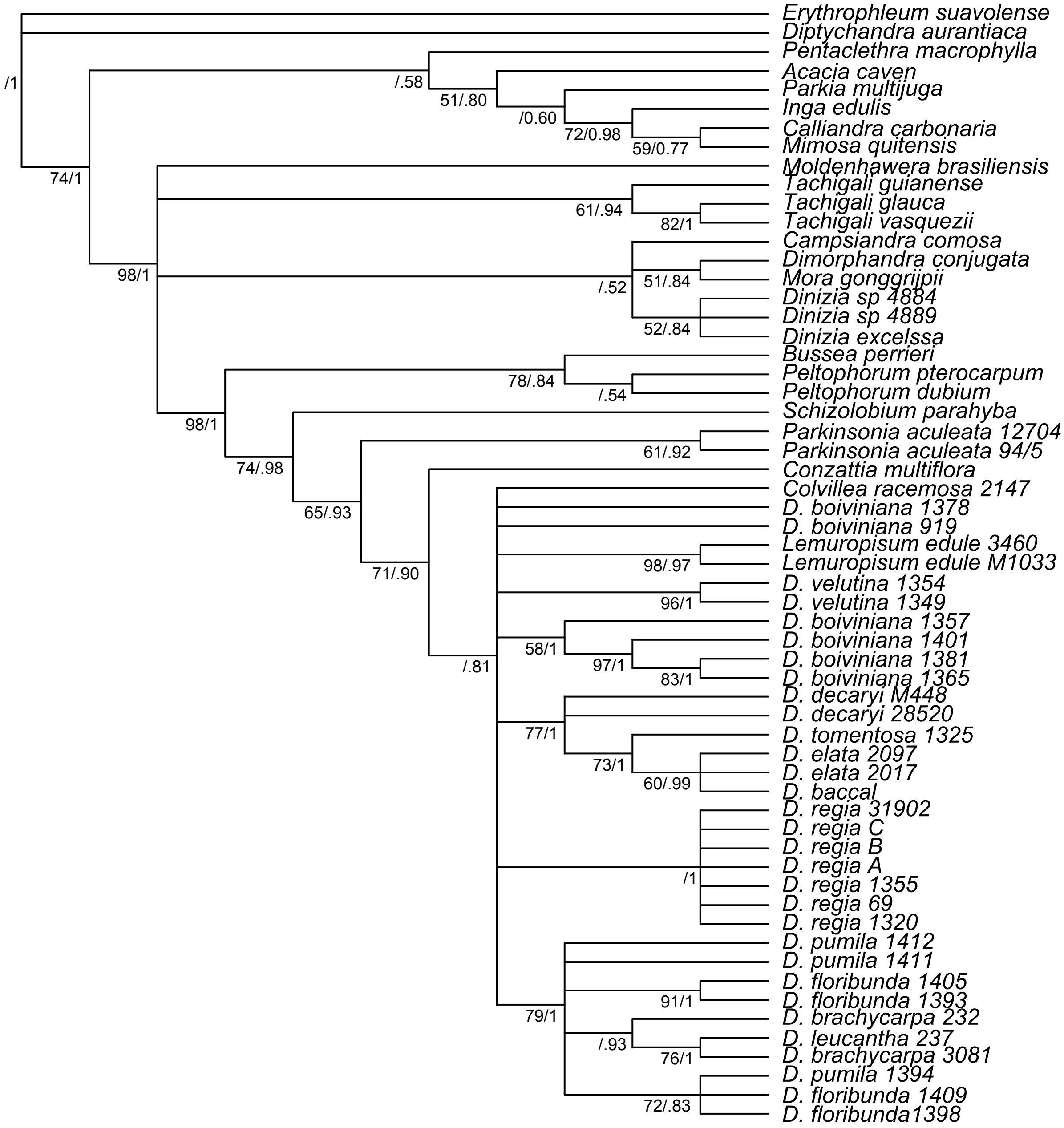
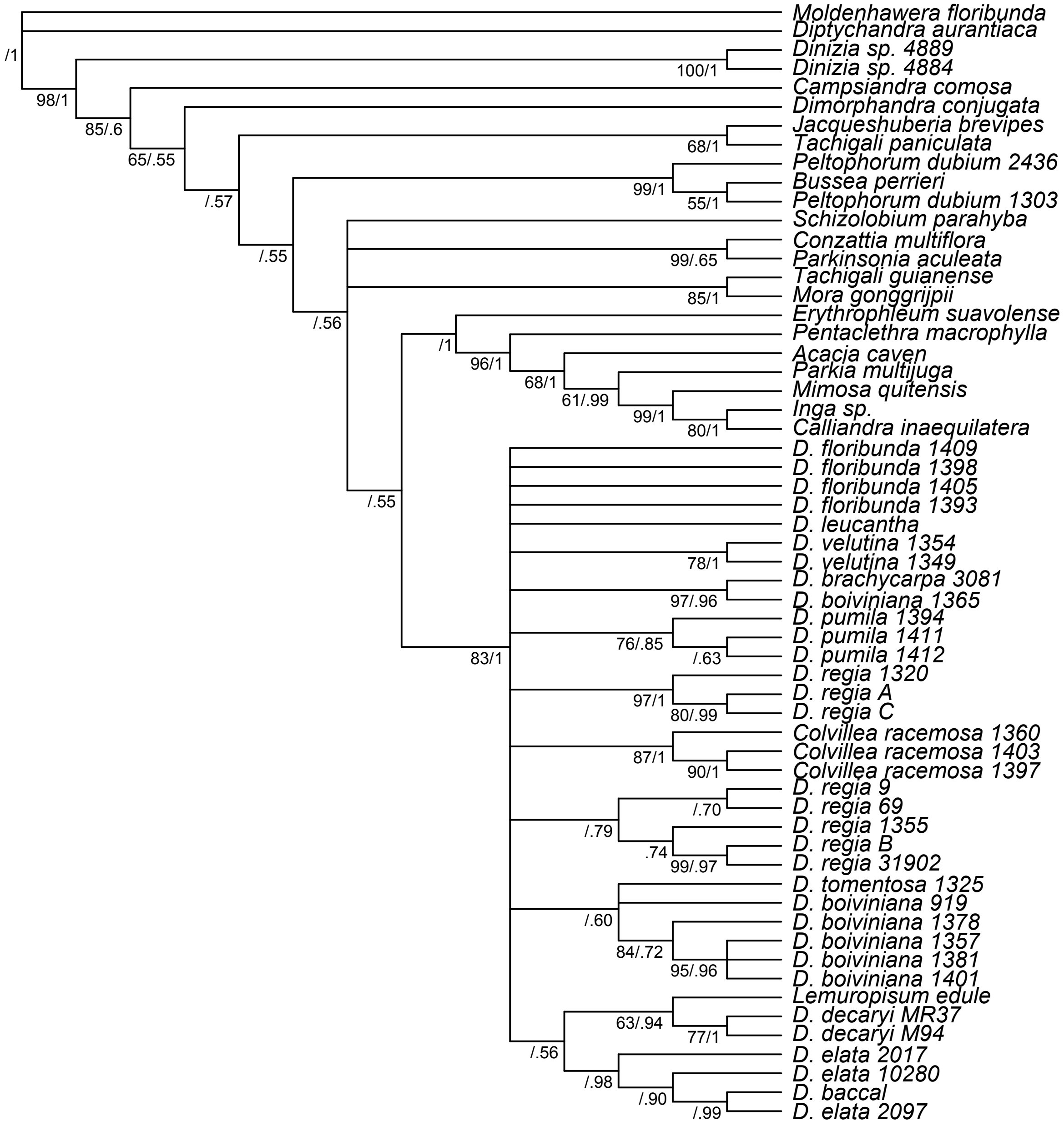
Annexe

A1-A12 : Topologies consensus des analyses phylogénétiques de parcimonie et bayésienne pour les dix loci individuels et pour les deux matrices concaténées des loci chloroplastiques (ALLcp) et des loci nucléaires (ALLnc) dans l'étude des genres *Delonix*, *Colvillea* et *Lemuropisum*.

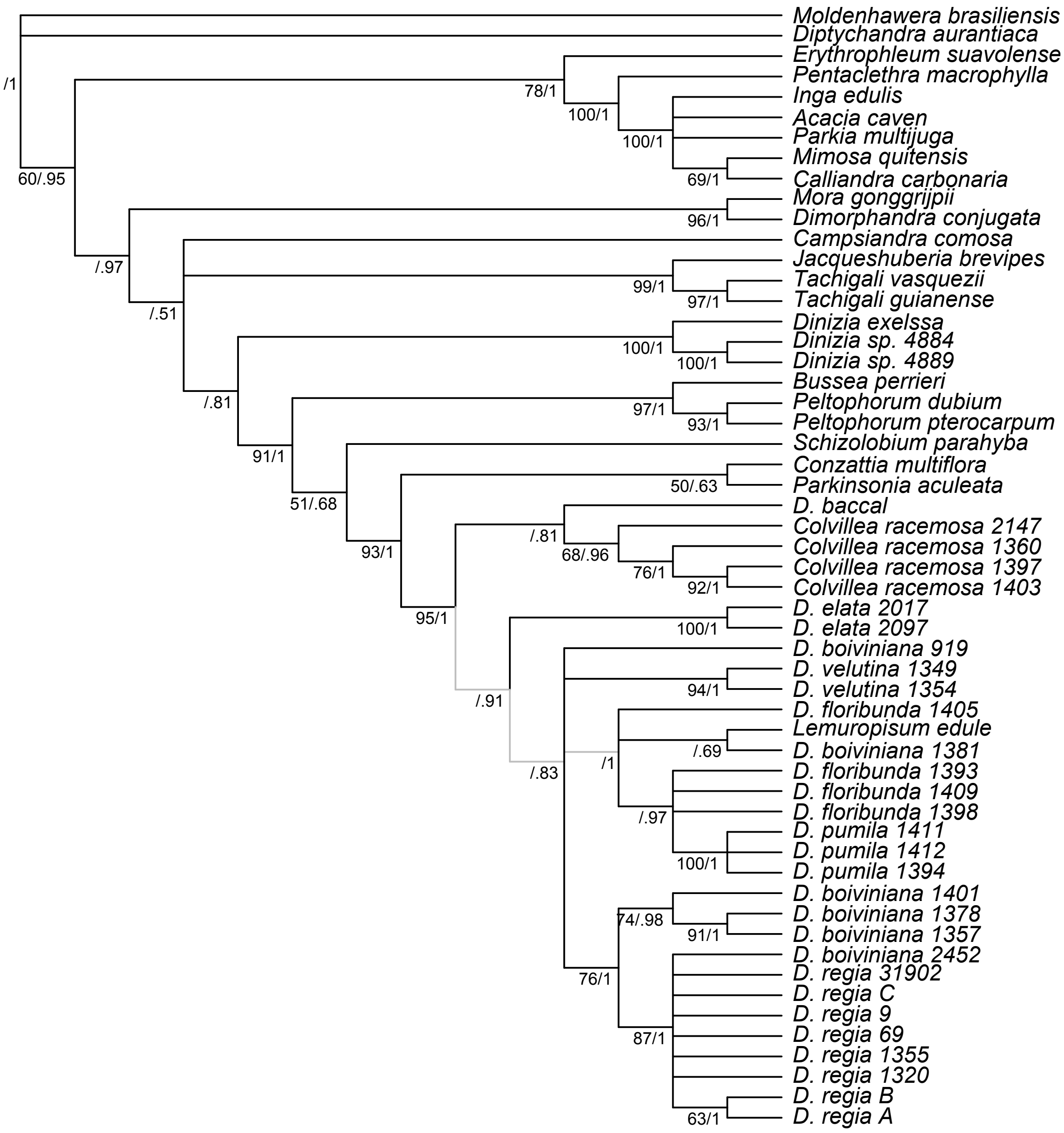
A13 : Topologie de l'arbre d'espèces estimé à partir de l'information topologique des dix loci selon le paramètre de regroupement de la plus petite divergence de distance entre espèces.



A1. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *trnL-trnF*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches.

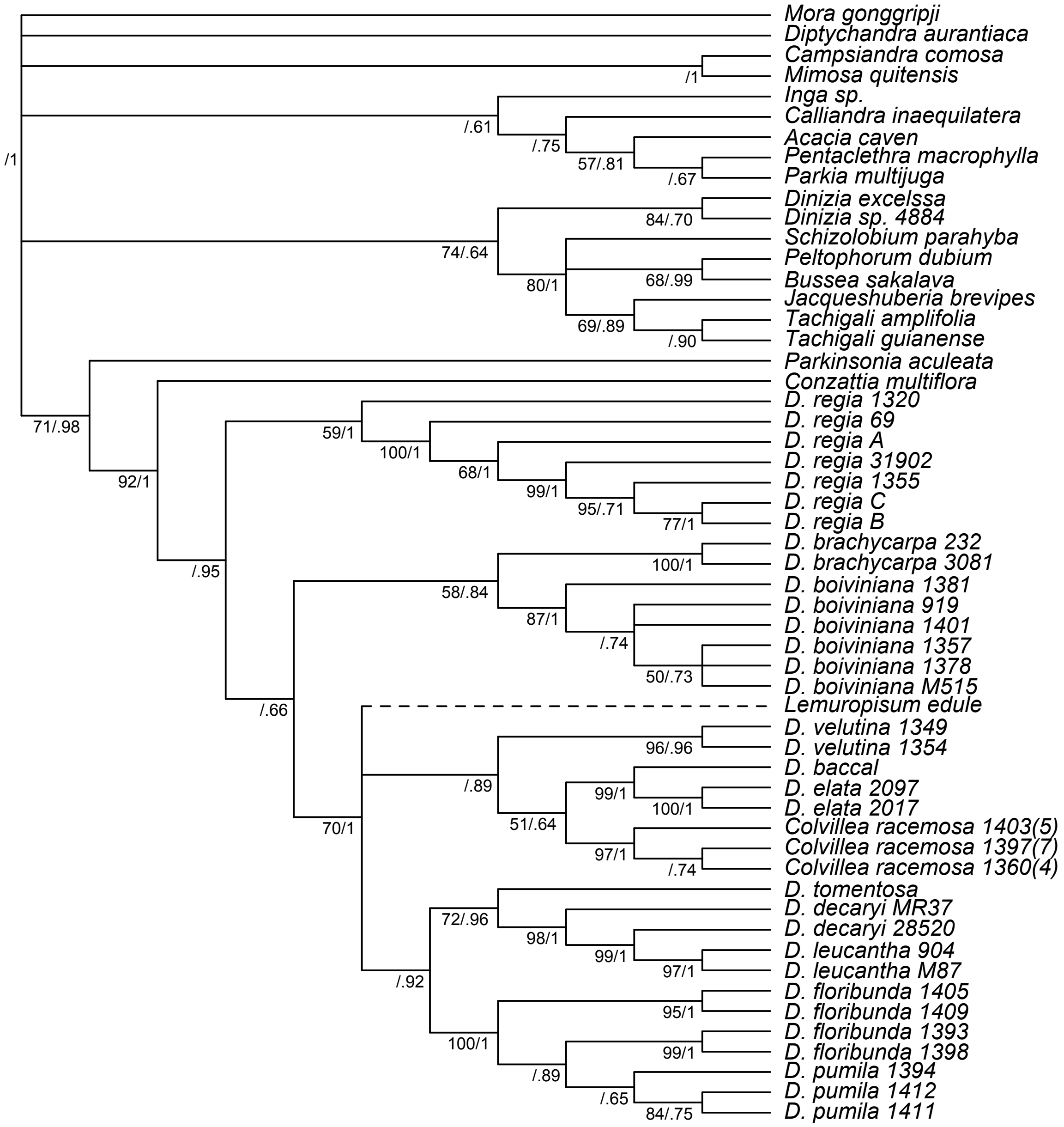


A2. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *trnD-trnT*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches.

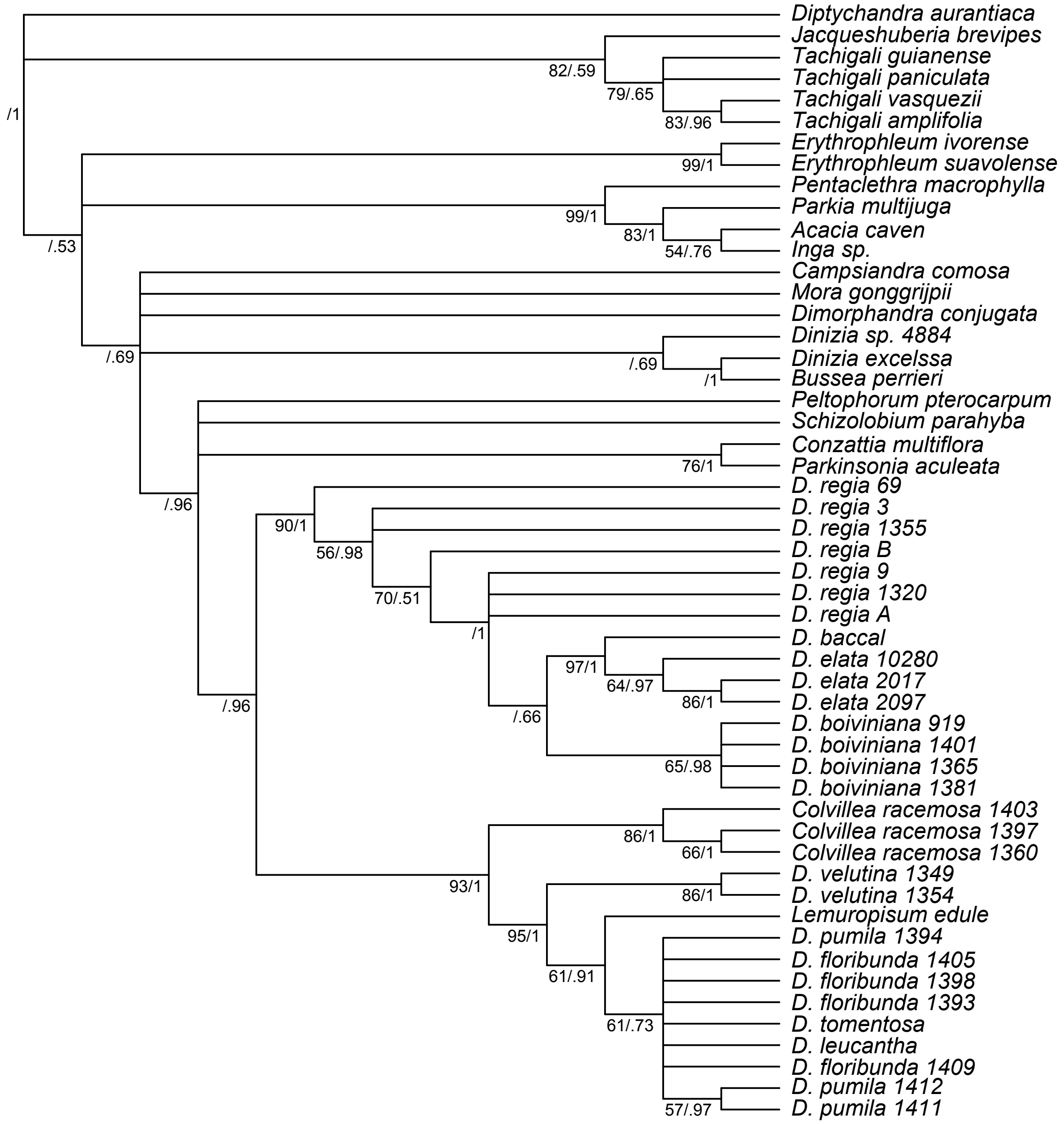


A3. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *matk*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. Les branches grises représentent les branches qui ne sont pas résolues dans le consensus strict de l'analyse de parcimonie.

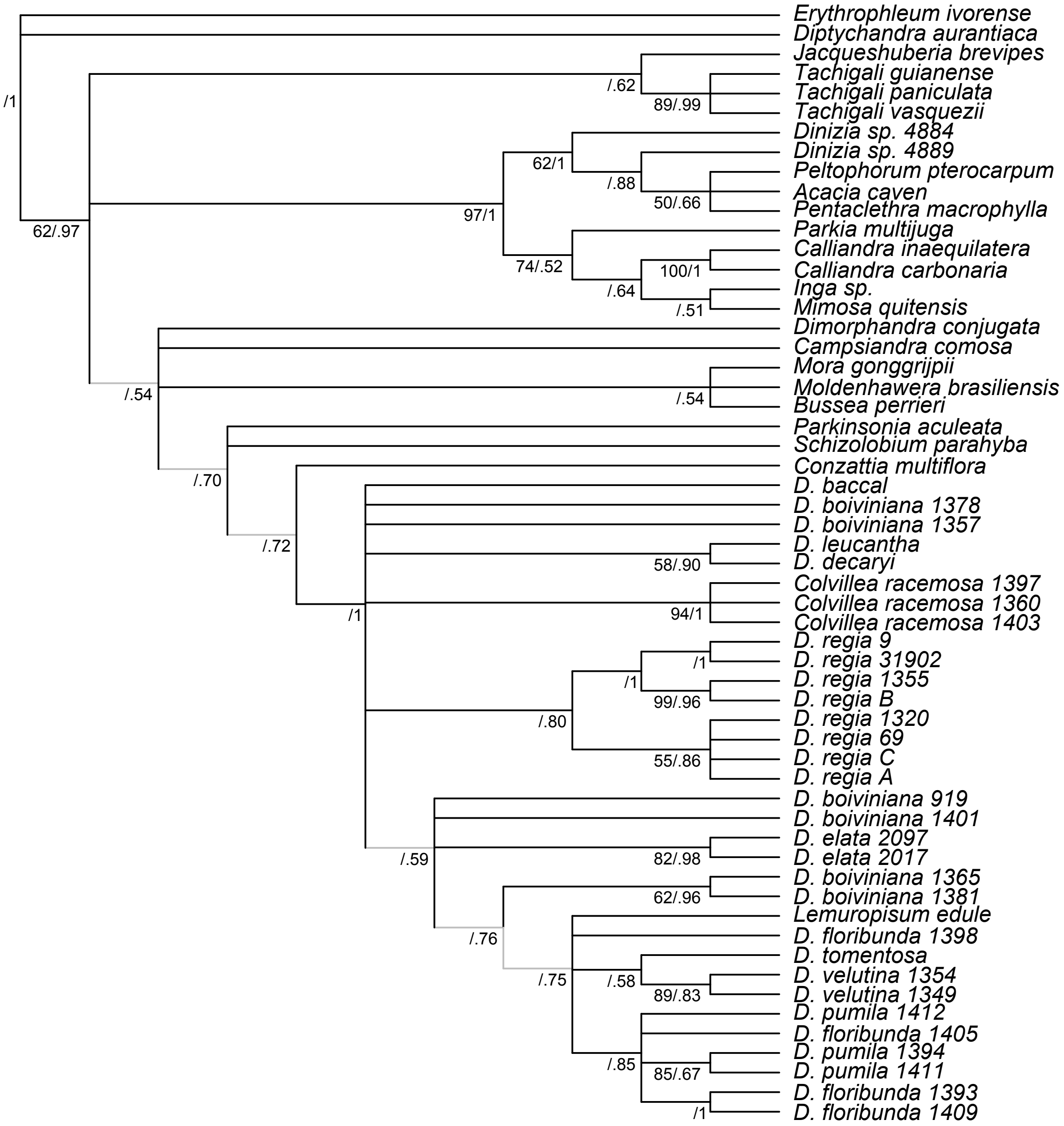
A4. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *rps16*. Les valeurs de support de bootstrap ≥50% de l’analyse de parcimonie / probabilités postérieures de l’analyse bayésienne sont indiquées sous les branches.



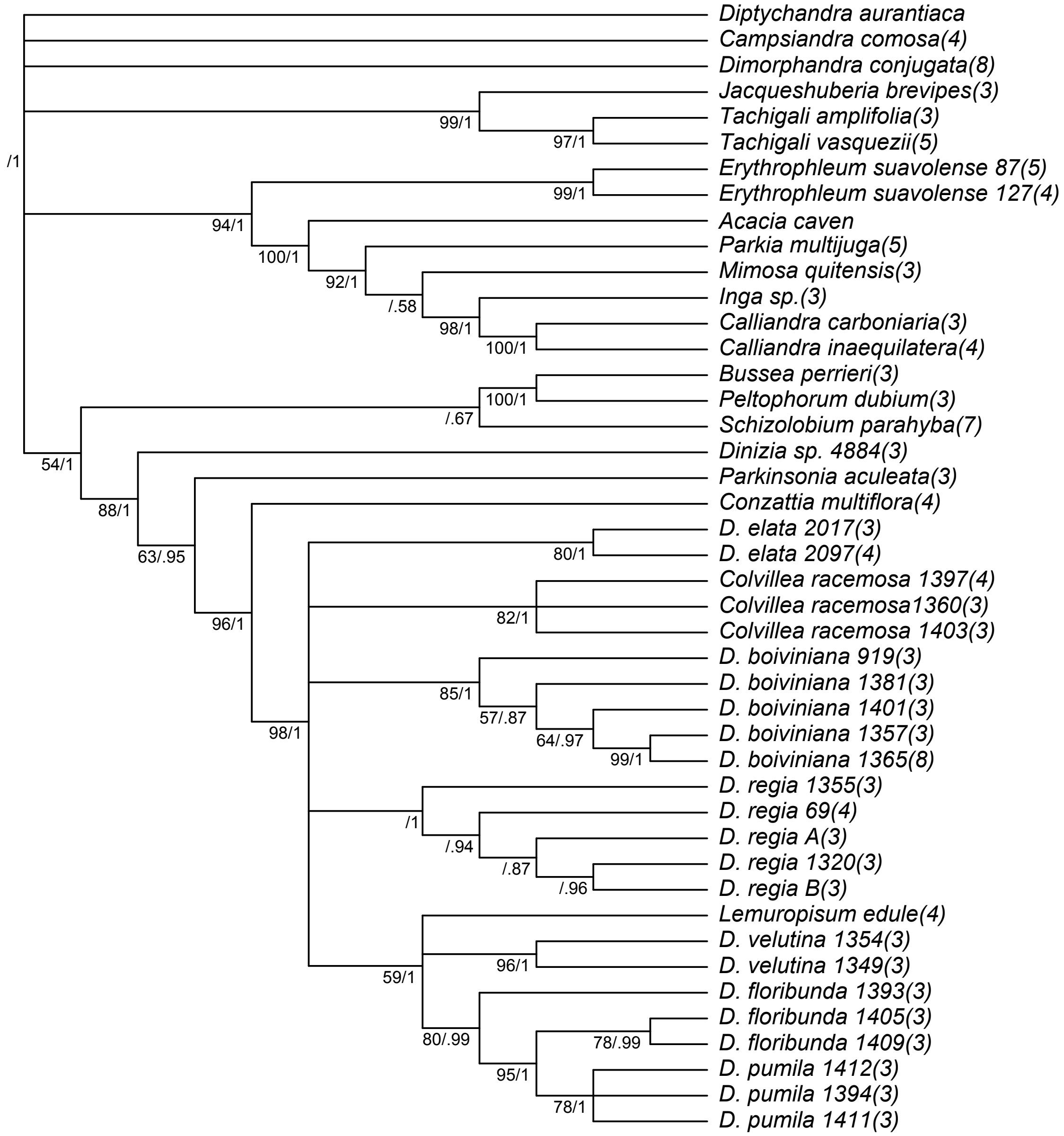
A5. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *ITS*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. La branche pointillée représente une position différente dans le consensus strict de l'analyse de parcimonie : *Lemuropisum* est rattaché à la branche commune de *D. velutina* et *Colvillea*.



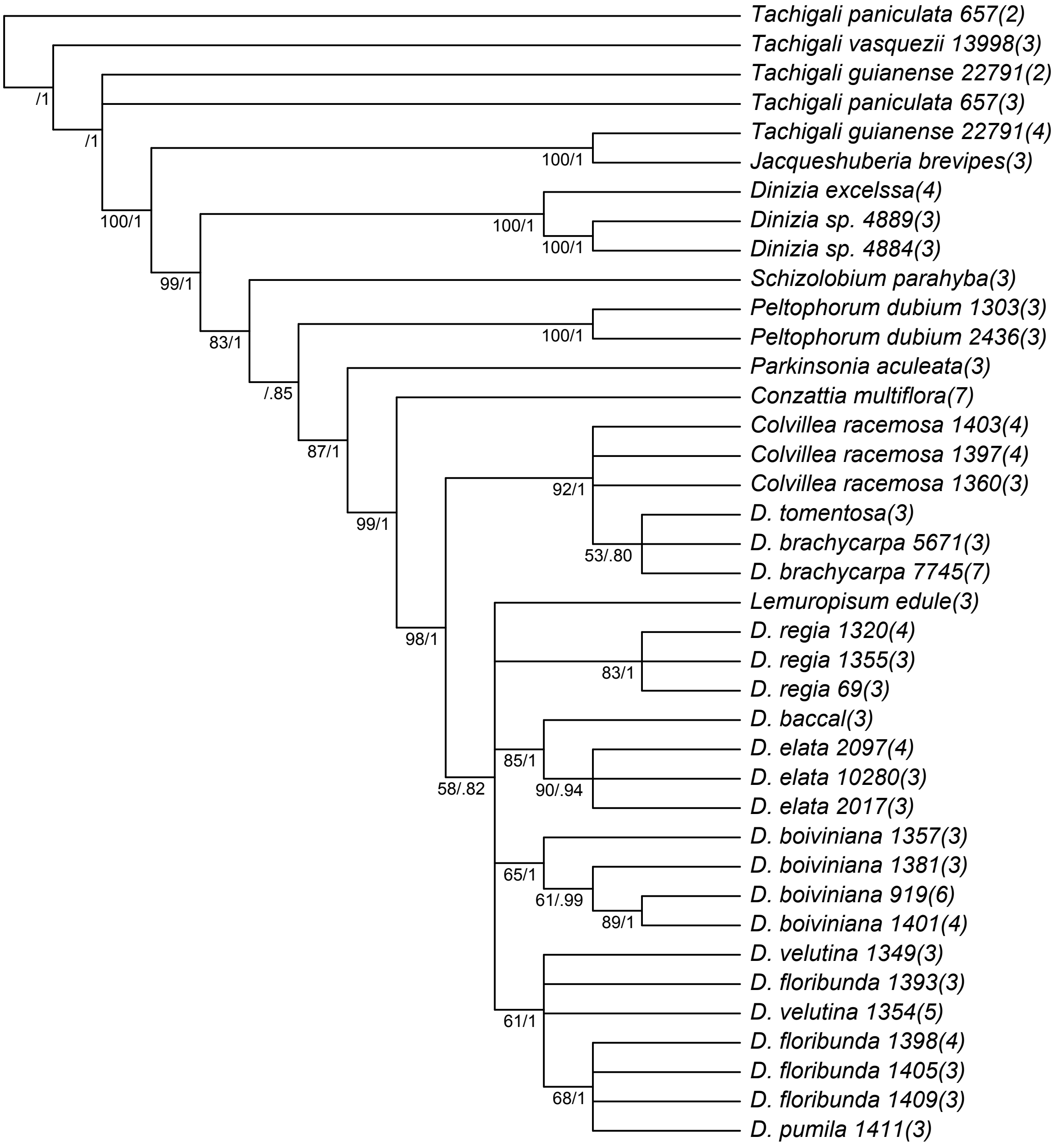
A6. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *tRALs*. Les valeurs de support de bootstrap ≥50% de l’analyse de parcimonie/ probabilités postérieures de l’analyse bayésienne sont indiquées sous les branches.



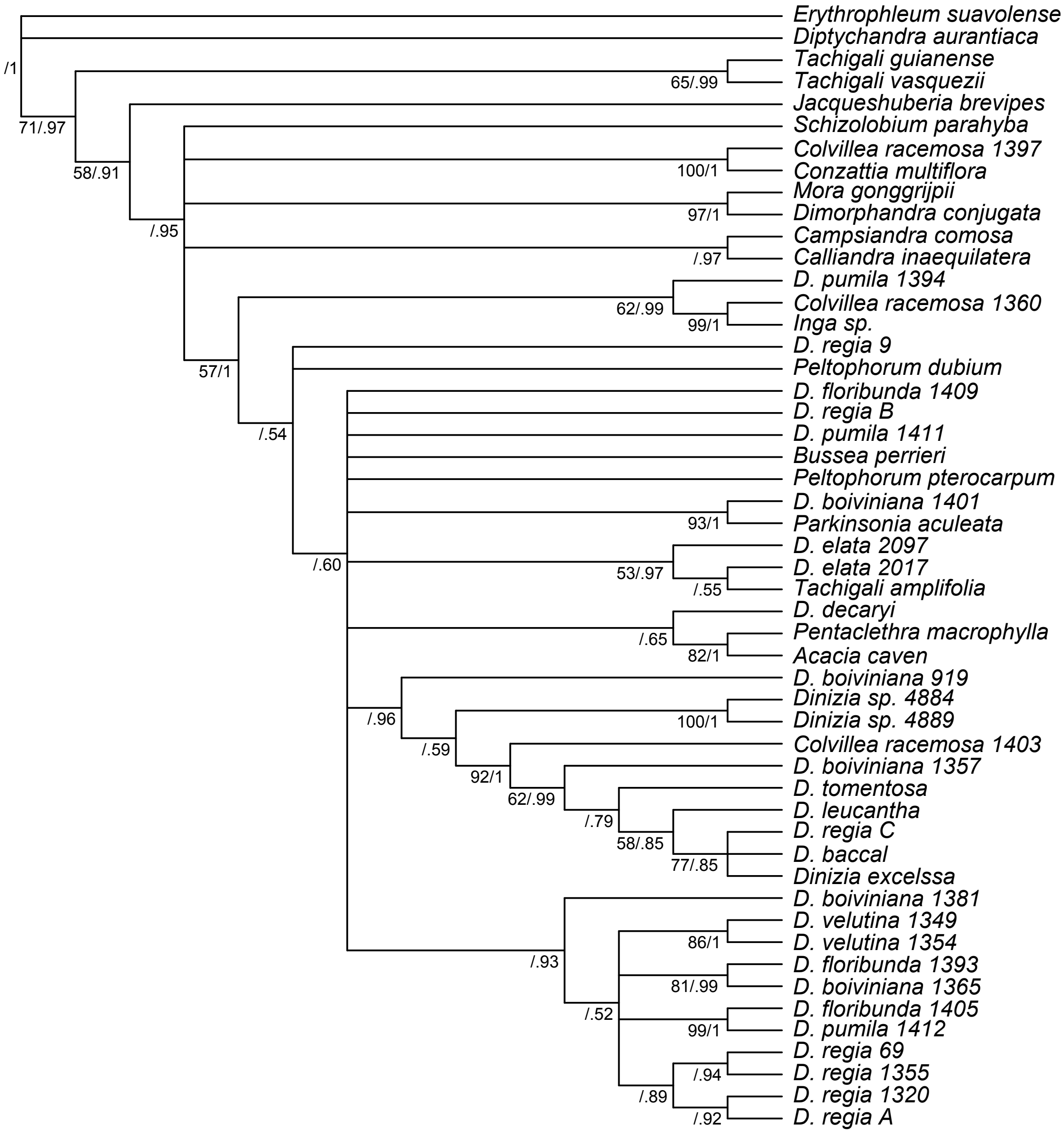
A7. Topologie consensus des analyses phylogénétique de parcimonie et bayésienne pour le locus *AIGP*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. Les branches grises représentent les branches non résolues dans le consensus strict de l'analyse de parcimonie.



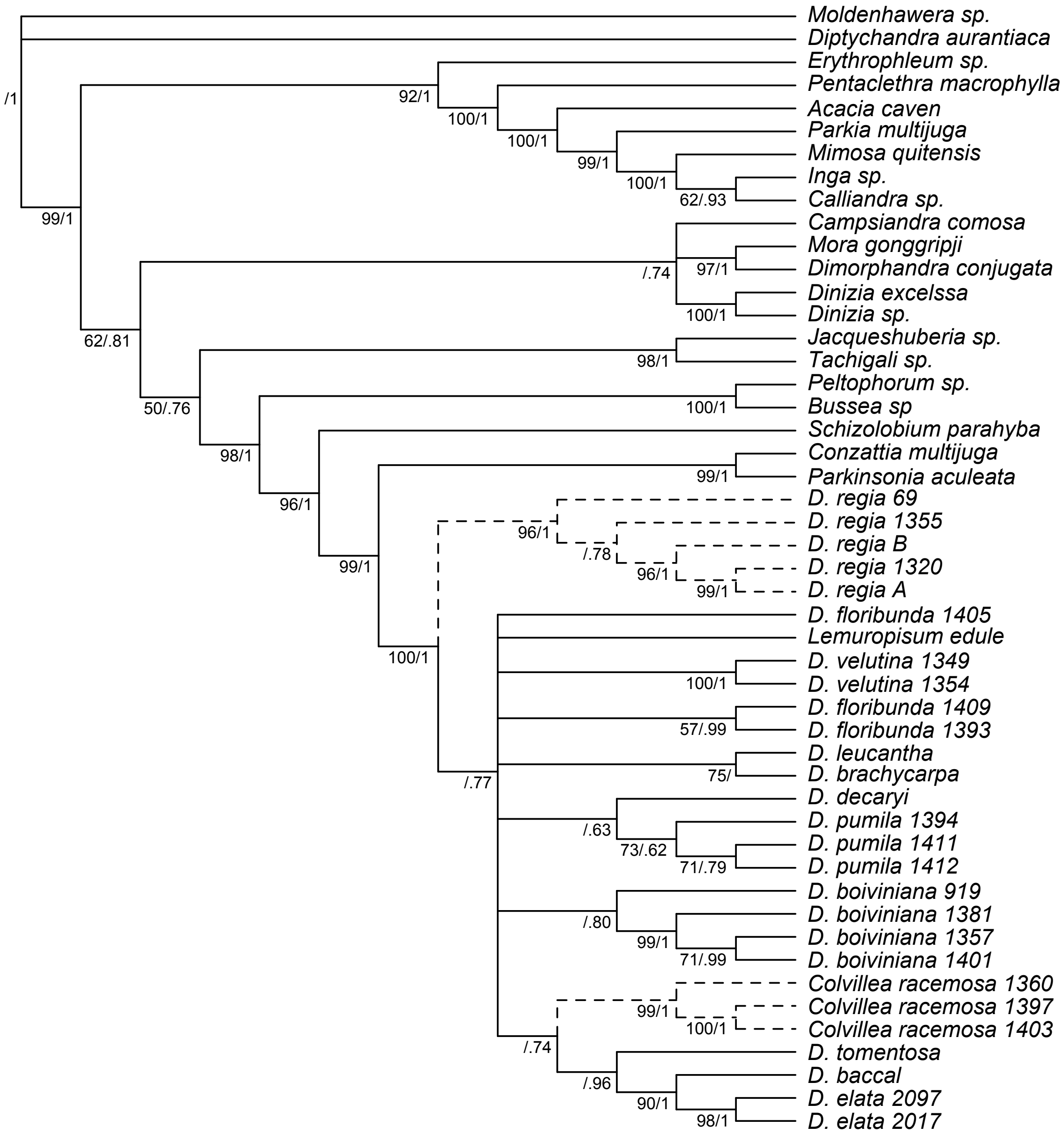
A8. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *EIF3E*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. Les chiffres entre parenthèses indiquent le nombre de clones occupant la même position.



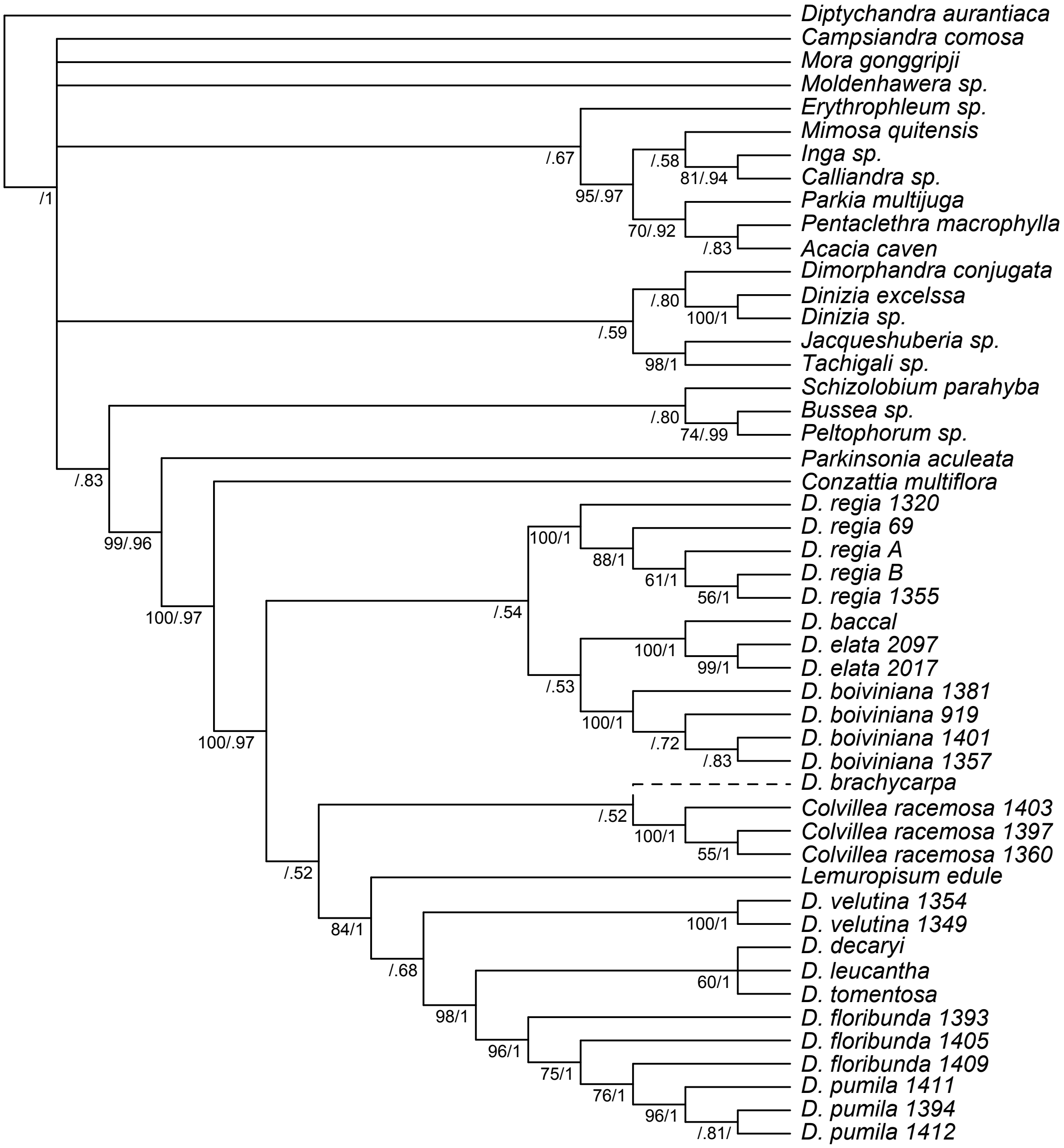
A9. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *Leafy*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. Les chiffres entre parenthèses indiquent le nombre de clones occupant la même position.



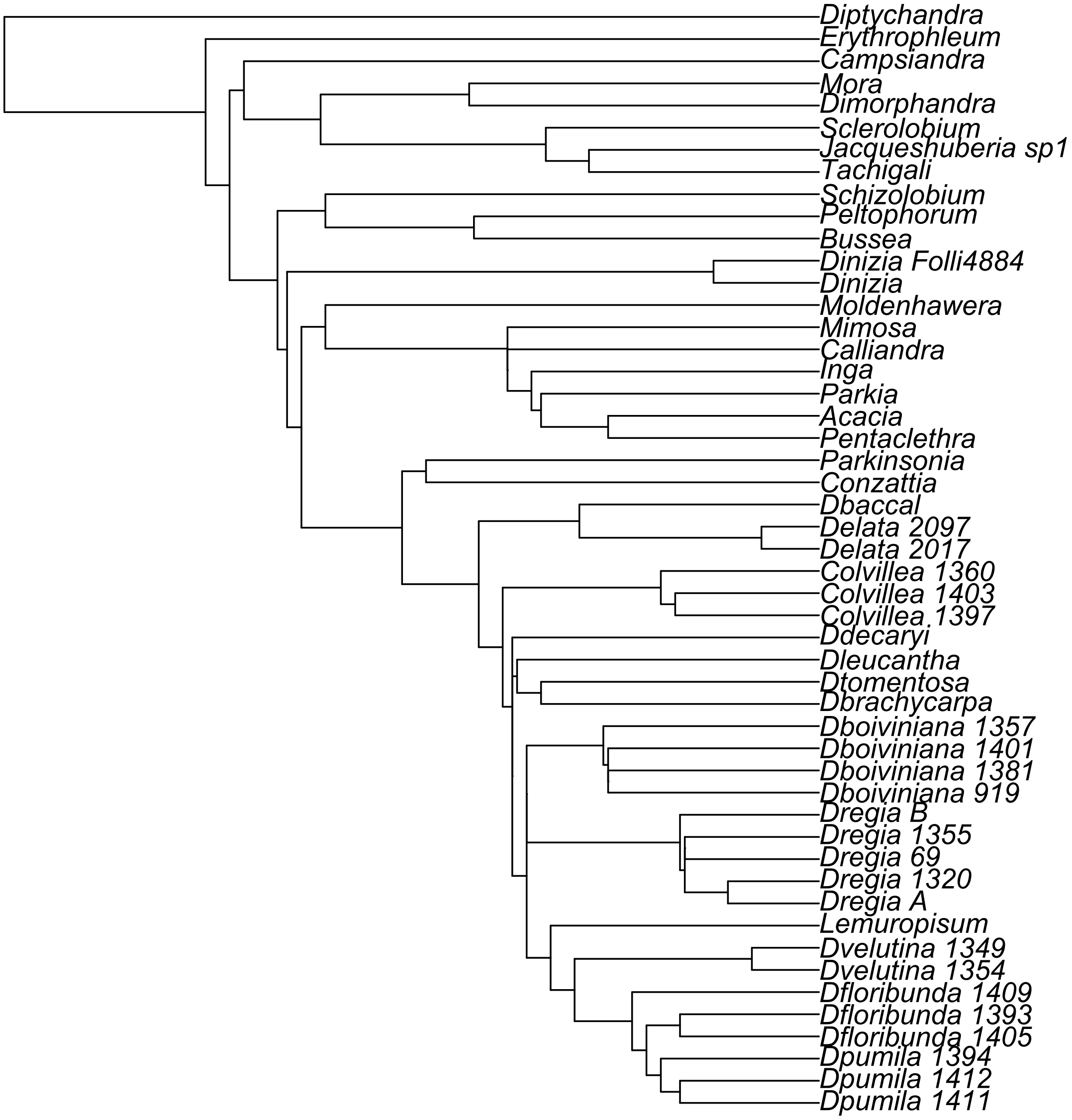
A10. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour le locus *CTP*. Les valeurs de support de bootstrap ≥50% de l’analyse de parcimonie/ probabilités postérieures de l’analyse bayésienne sont indiquées sous les branches.



A11. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour les loci chloroplastiques concaténés *trnL-trnF, trnD-trnT, matk* et *rps16*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. Les branches pointillées représentent les branches ayant une position différente dans le consensus strict de l'analyse de parcimonie : les positions des espèces *D. regia* et *Colvillea* sont interchangées.



A12. Topologie consensus des analyses phylogénétiques de parcimonie et bayésienne pour les loci nucléaires concaténés *ITS, AIGP, tRALs, EIF3E* et *Leafy*. Les valeurs de support de bootstrap ≥50% de l'analyse de parcimonie/ probabilités postérieures de l'analyse bayésienne sont indiquées sous les branches. La branche pointillée représente une position différente dans le consensus strict de l'analyse de parcimonie : *D. brachycarpa* est associé aux échantillons de *D. boiviniana*.



A13. Topologie de l’arbre d’espèce estimé à partir de l’information topologique des dix loci nucléaires et chloroplastiques selon le paramètre de regroupement de la plus petite divergence de distance entre espèces.