Phylogenetic and biogeographical history of the Afro-Madagascan genera *Delonix*, *Colvillea* and *Lemuropisum* (Fabaceae: Caesalpinioideae)

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Our understanding of the taxonomic limits and biogeographical evolution of the Afro-Madagascan genus *Delonix* and the closely related monospecific Madagascan endemic genera *Colvillea* and *Lemuropisum* have been hampered by unresolved intergeneric and interspecific relationships. Here we study the phylogenetics of the group using nucleotide sequences from ribosomal ITS, four low-copy nuclear genes and four plastid regions. We find poorly resolved gene trees typical of closely related species, but most species are resolved as monophyletic, and we report conflicting phylogenetic placements only for *D. brachycarpa* and *C. racemosa*. A time-calibrated biogeographical analysis of the concatenated matrix of all nine loci suggests that the ancestral area of the *Delonix*, *Lemuropisum* and *Colvillea* clade was in south-western Madagascar *c.* 24–18 Ma. An out-of-Madagascar dispersal to Africa took place during the Miocene, most likely by vegetative rafting. *Delonix* is not monophyletic due to the robustly supported placement of *L. edule* in *Delonix*, warranting treatment of *Lemuropisum* as congeneric with *Delonix* and establishment of the new name combination *Delonix edule*, in line with the close morphological similarities between these two genera. In contrast, the phylogenetic position of *C. racemosa* is unstable occurring in the *Delonix–Lemuropisum* clade in the nuclear gene trees, but sister to this clade in the plastid gene tree. The status of *Colvillea* as a distinct genus, therefore, remains questionable, but it is here maintained based on a suite of morphological distinctions, pending further insights into the relationships of this elusive genus.

ADDITIONAL KEYWORDS: Afro-Madagascar disjunction – Caesalpinioideae – divergence time analysis – dry forest species – hybridization – Leguminosae – low-copy nuclear genes.

INTRODUCTION

Over 80% of all vascular plant species and 19% of genera in Madagascar are endemic (Callmander *et al.*, 2011; Buerki *et al.*, 2013). Fabaceae (Leguminosae), suggested as a proxy for the study of global patterns of angiosperm diversity (Nic Lughadha *et al.*, 2005; LPWG, 2013; Yahara *et al.*, 2013), have the most endemic Madagascan arborescent genera of any family (Schatz, 2001; Du Puy *et al.*, 2002). However, the status, origins, evolution and biogeography of most Madagascan plant genera and species remain elusive because of a lack of well-sampled and -dated species-level phylogenetic analyses (Du Puy *et al.*,

2002; Buerki *et al.*, 2013). One such group, resolved as monophyletic in phylogenetic studies by Haston, Lewis & Hawkins (2005) and Bruneau *et al.* (2008), is the trigeneric clade formed by the near-endemic genus *Delonix* Raf., and the closely related, monospecific, endemic genera *Colvillea* Bojer ex Hook. and *Lemuropisum* H.Perrier. *Delonix* includes 11 species, two of which are found in Africa [*D. baccal* (Chiov.) Baker f. is restricted to eastern Somalia, northern Kenya and Ethiopia; *D. elata* (L.) Gamble is widespread, occurring in East Africa, Arabia and possibly extending to India] and the other nine are endemic to Madagascar [*D. brachycarpa* (R.Vig.) Capuron, *D. boiviniana* (Baill.) Capuron, *D. decaryi* (Baill.) Capuron, *D. floribunda* (Baill.) Capuron, *D. leucantha* Du Puy, Phillipson & R.Rabev., *D. regia* (Hook.) Raf., *D. pumila* Du Puy, Phillipson & R.Rabev., *D. tomentosa* (R.Vig.)

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Capuron and *D. velutina* Capuron]. The Madagascan species are distributed along the west coast in the northern dry deciduous forest and the southern spiny thicket, coinciding with White's (1983) circumscription of the West Malagasy dry deciduous forest which corresponds to the dry deciduous forest and sub-arid scrubland zones of Cornet (1974) and Du Puy & Moat (1998) (Fig. 1). *Colvillea racemosa* Bojer ex Hook., found in western, southern and extreme northern Madagascar, and *Lemuropisum edule* H.Perrier, restricted to the south-west, occur sympatrically with *Delonix* spp.

The morphological variation in the *Delonix/Colvillea/Lemuropisum* clade is extensive and has complicated the taxonomic classification. Differences in flower morphology led Baillon (1884) and Capuron (1968),
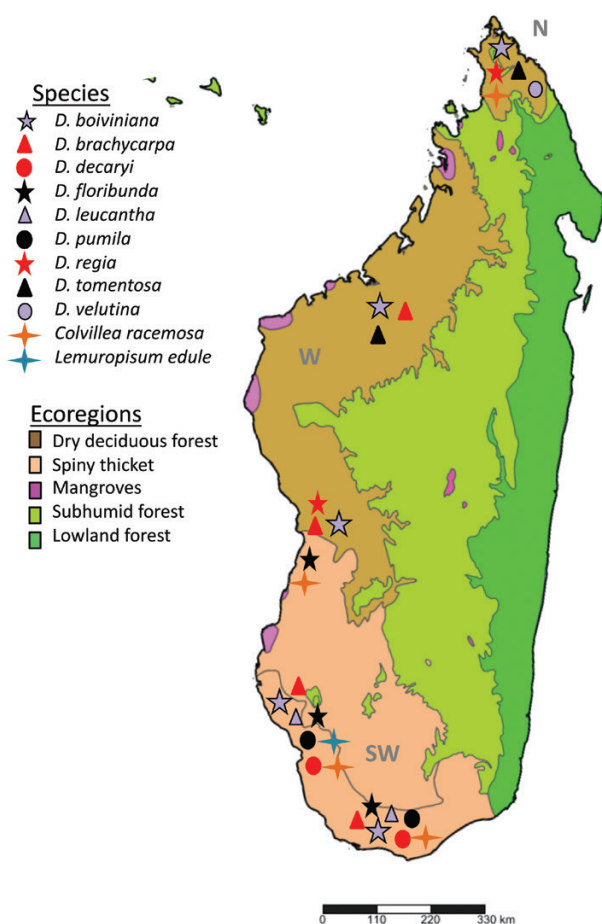

Figure 1. Distribution of *Delonix* spp. and the monospecific genera *Colvillea* and *Lemuropisum* in Madagascar. Each point represents an observation or sampling point taken from Du Puy *et al.* (1995), Rivers *et al.* (2011), from field work in Madagascar by Anne Bruneau, and from maps provided by Anahita Aebli (University of Zurich). The three geographical regions designated for the ancestral biogeographic range reconstruction are indicated in grey (N, North; W, West; SW, South-west).

to divide *Delonix* into two genera, *Poinciana* L., which included the majority of species, and *Aprevalia* Baill., including only *D. floribunda* and *D. velutina*. Since then, species in the *Delonix/Colvillea/Lemuropisum* clade have been grouped into multiple, inconsistent and incongruent subdivisions based on different vegetative and reproductive characters (Polhill & Vidal, 1981; Polhill, 1994; Du Puy, Phillipson & Rabevohitra, 1995; Banks, 1997).

Despite this morphological diversity, molecular phylogenetic analyses have yielded poorly resolved topologies because of low levels of DNA sequence variation among species and between the three genera (Simpson, Larkin & Weeks, 2003; Haston *et al.*, 2005; Hawkins *et al.*, 2007; Manzanilla & Bruneau, 2012). Previous analyses have suggested that *Delonix* is not monophyletic, because *Lemuropisum* is nested in it (Haston *et al.*, 2005; Hawkins *et al.*, 2007; Rivers *et al.*, 2011) and *Colvillea* is placed either in *Delonix* in a polytomy (Simpson *et al.*, 2003; Haston *et al.*, 2005; Hawkins *et al.*, 2007) or weakly supported as sister to a *Delonix/Lemuropisum* clade (Bruneau *et al.*, 2008). Species-level relationships have also never been resolved. Although this could be a consequence of reliance on slowly evolving plastid loci for phylogeny reconstruction, the observed morphological diversity coupled with low interspecific and intergeneric sequence divergence could also be evidence for hybridization between sympatric *Delonix* spp. Rivers *et al.* (2011) found lower genetic diversity between *Delonix* spp. than within species, also suggesting the possibility of intergeneric and/or interspecific hybridization. However, the retention of ancestral polymorphism resulting in incompletely sorted lineages in the *Delonix/Colvillea/Lemuropisum* clade could provide an alternative explanation for the lack of resolution noted in recent phylogenetic analyses. The evidence suggests that this clade is formed by a group of closely related taxa in which phylogenetic reconstruction is particularly problematic due to possible reticulation and/or incomplete lineage sorting, which can be challenging to disentangle (Doyle, 1992; Maddison & Knowles, 2006; Naciri & Linder, 2015). Here we aim to resolve phylogenetic relationships amongst the 13 species in the *Delonix/Colvillea/Lemuropisum* clade, via independent and combined analyses based on plastid loci, ribosomal ITS and four low-copy nuclear gene sequences, sampling all species as well as intraspecific diversity via multiple accessions of species across their ranges, whenever possible.

Understanding the timing and directionality of the Afro-Madagascan disjunction in *Delonix* could provide insights into the geographical origins of Madagascan legumes and Madagascan biogeography more generally. Biogeographic affinities with Africa are prevalent (Yoder & Nowak, 2006), with 22% of endemic

Madagascan plant genera having sister lineages in Africa (Buerki *et al.*, 2013). Although both vicariance and dispersal hypotheses have been proposed for the origin of the Madagascan flora, divergence time phylogenetic analyses in a number of plant groups indicate times of origin for the Madagascan taxa usually postdating the separation of Africa and Madagascar (80 Ma; Masters, de Wit & Asher, 2006) pointing to dispersal as the primary mechanism for the origin of many taxa (cf. Buerki *et al.*, 2013). Strong winds connect Africa and Madagascar (Muñoz et al., 2004) and animals can easily transport seeds externally or internally (Yoder & Nowak, 2006; Nathan *et al.*, 2008). Although the dispersal mode of species in the *Delonix/Colvillea/Lemuropisum* clade is not known, long-distance dispersal via animals and wind has been proposed for many Fabaceae (Hurr *et al.*, 1999; Lavin *et al.*, 2004; Lavin, Herendeen & Wojciechowski, 2005; Bessega, Vilardi & Saidman, 2006). Studies have also suggested the dispersal of organisms across large bodies of water on floating vegetative rafts (sweepstake hypothesis: Simpson, 1940; Thiel & Haye, 2006), an explanation that has recently received support as sea currents in the Mozambique Channel have been shown to have flowed from Africa to Madagascar during the Cenozoic–Oligocene and to have reverted to the present Madagascar-to-Africa direction during the Miocene (Ali & Huber, 2010). The presence of uplifted land forming an interrupted bridge in the Mozambique Channel from 45 to 26 Ma linking Madagascar and Africa (McCall, 1997) is another explanation proposed for the colonization of a number of Madagascan mammals and this is also plausible for plants.

The objectives of this study are to determine the intergeneric and interspecific phylogenetic relationships for the 11 *Delonix* spp., *C. racemosa* and *L. edule*, to assess the status of the Madagascan endemic genera *Colvillea* and *Lemuropisum* and to use the resulting phylogenetic pattern to estimate the timing and direction of the Madagascar–Africa disjunction in *Delonix*.

MATERIAL AND METHODS

TAXON SAMPLING

Ninety-seven samples, 49 representing the 13 species of *Delonix*, *Colvillea* and *Lemuropisum*, and 48 samples from related taxa, were sequenced (Appendix), with 28 ingroup terminals included in the final concatenated matrix (see the following). The 49 samples of related taxa are part of a large subset of outgroup taxa necessary to include three fossil calibration points for the divergence time estimates (Bruneau *et al.*, 2008). Following phylogenetic analyses by Bruneau *et al.* (2008), the outgroup includes 15 samples from seven genera (ten species) of the

Dimorphandra Group (*Mora* Benth., *Moldenhawera* Schrad., *Dimorphandra* Schott, *Diptychandra* Tul., *Dinizia* Ducke, *Erythrophleum* Afzel. ex R.Br., *Campsiandra* Benth.), eight samples from two genera (eight species) of the *Tachigali* Group (*Tachigali* Aubl., *Jacqueshuberia* Ducke), 15 samples from five genera (seven species) of the *Peltophorum* Group [*Conzattia* Rose, *Parkinsonia* L., *Peltophorum* (Vogel) Benth., *Bussea* Harms, *Schizolobium* Vogel] and ten samples from six genera (seven species) of the mimosoid clade (*Inga* Mill., *Parkia* R.Br., *Pentaclethra* Benth., *Mimosa* R.Br., *Calliandra* Benth., *Vachellia* Wight & Arn.) (Appendix). For all ingroup species except *D. tomentosa*, we included several individuals per species: *Colvillea racemosa*, three; *Lemuropisum edule*, three; *D. baccal*, two; *D. boiviniana*, eight; *D. brachycarpa*, four; *D. decaryi*, four; *D. elata*, four; *D. floribunda*, four; *D. leucantha*, three; *D. pumila*, three; *D. regia*, eight; and *D. velutina*, two.

SELECTION OF MOLECULAR MARKERS

Nine molecular markers were sequenced, four from the plastid genome (*trnL-trnF*, *matK*, *rps16* and *trnD-trnT*), four low-copy nuclear genes (*Leafy*, *AIGP*, *tRALs* and *EIF3E*) and the 5.8S subunit and flanking internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal DNA. The plastid *matK*, *rps16* and *trnL-F* loci were included because they had been previously sequenced for representatives of *Delonix*, *Colvillea* and *Lemuropisum*; here we improve upon the sampling for each of these three loci (Appendix). Plastid *trnD-T*, containing three introns (Shaw *et al.*, 2005), the nuclear ribosomal ITS region and the second intron and partial flanking exons 2 and 3 of the low-copy nuclear *Leafy* gene were chosen because previous studies have found them to be effective in resolving relationships among closely related legumes (Archambault & Bruneau, 2004; Scherson, Vidal & Sanderson, 2008; Simon *et al.*, 2011; Turchetto-Zolet *et al.*, 2012). The three other low-copy nuclear loci were selected from the studies of Choi *et al.* (2006) and Li *et al.* (2008) based on preliminary investigations by Babineau, Gagnon & Bruneau (2013), which demonstrated the utility of *AIGP* and *EIF3E* in resolving interspecific relationships in *Delonix* and of *tRALs* in resolving intergeneric relationships in caesalpinioid legumes.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total DNA from 30 mg of dried leaf material was extracted using a modified cetyltrimethylammonium bromide protocol from Joly *et al.* (2006). Internal primers were designed for *rps16*, *trnD-T*, *Leafy* and *tRALs* to amplify and sequence recalcitrant samples (Table 1).

We followed Babineau *et al.* (2013) for the polymerase chain reaction (PCR) recipe. PCR cycle settings for *trnL-F*, *matK* and *rps16* are followed as described in Bruneau *et al.* (2001, 2008) and Oxelman, Lidén & Berglund (1997), respectively, and those for *trnD-T*, ITS, *Leafy*, *AIGP*, *tRALs* and *EIF3E* are described in Table 1. Nested PCRs were performed for *trnL-F*, *matK*, *rps16*, *trnD-T*, ITS, *Leafy* and *tRALs* when the first PCR failed to produce sufficient amounts of product using the conditions described above. PCR products were purified following Joly *et al.* (2006). Sequencing was performed on an Applied Biosystems 3730XL DNA Analyser (McGill University Genome Quebec) using BigDye 1.1 reagents and following the manufacturer's protocol.

Sequences were assembled and edited with Sequencher 4.7 (GeneCodes Corporation, Ann Arbor, MI, USA). After direct sequencing, nuclear loci sequences that were observed to be polymorphic were cloned. These samples were amplified in

Table 1. Primers used to amplify each of the nine molecular regions in the phylogenetic study of *Delonix*, *Colvillea* and *Lemuropisum*

Locus	Forward 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 *et al.* (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 & Bruneau (unpublished)
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	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), this study
AIGP	AIGP-F	AIGP-R	Same as tRALs	Choi *et al.* (2006)
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)

Full oligonucleotide sequences are given for newly developed primers, and PCR cycle protocols are provided if modified from the original source.

triplicate reactions to reduce PCR recombinants and *Taq* induced errors (Cronn *et al.*, 2002). Cloning was performed using the CloneJET PCR cloning kit (Thermo Fisher Scientific, Ottawa, ON, Canada), following the manufacturer's instructions. All sequenced transformed colonies were visually inspected upon alignment: sequences were submitted to a BLASTn search (www.ncbi.nlm.nih.gov/blast) and eliminated if they did not correspond to published sequences. At least three clones per sequence were included in the alignment. In addition, because ITS is often reported as having paralogous copies that escape concerted evolution (Alvarez & Wendel, 2003; Feliner & Roselló, 2007), we cloned the ITS sequences of *C. racemosa* as an added precaution to verify the nucleotide sequence homogeneity, the presence of pseudogenes and the copy number in one member of our ingroup.

SEQUENCE ALIGNMENT, MATRIX CONCATENATION AND 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). Phylogenetic analyses were performed on the ITS and each of the low-copy nuclear gene matrices individually, and on two concatenated matrices, one containing the plastid loci (ALLcp) and one with all loci from both genomes (ALLcpnc). The concatenated matrices include reduced sampling (samples underlined in Appendix) in order to minimize missing data when concatenating sequences across multiple loci, but they both include at least one sample from each of the 13 species of *Delonix*, *Colvillea* and *Lemuropisum* and 20 samples representative of the outgroup taxa. When multiple clones per sample were available, because these formed monophyletic groups in the individual analyses, one randomly chosen clone per sequence was included to represent the sample in the concatenated matrices. In order to minimize missing data (loci), we combined two samples of *D. decaryi* in the concatenated matrices (Appendix). For outgroup taxa, sequences from different species of the same genus were sometimes concatenated. Six outgroup genera were represented by a chimaeric sequence of two or more sequences from different species of the same genus: *Bussea* (*B. perrieri* R.Vig. and *B. sakalava* Du Puy & R.Rabev.), *Peltophorum* [*P. pterocarpum* (DC.) Bayer ex K.Heyne and *P. dubium* (Spreng.) Taub.], *Tachigali* [*T. vasquezii* Pipoly, *T. glauca* Tul., *T. paniculata* Aubl. and *T. amplifolia* (Ducke) Barneby] in ALLcp and ALLcpnc, *Jacqueshuberia* (*J. brevipes* Barneby and *J. loretensis* R.S.Cowan) and *Calliandra C. inaequilatera* Rusby and *C. trinervia* Benth. var. *carbonaria* (Benth.) Barneby] in ALLcp and *Erythrophleum*

[*E. suavolense* (Guill. & Perr.) Brenan and *E. ivorense* A.Chev.] in ALLcpnc. Chimaeric sequences created for the ALLcp are also represented in the ALLcpnc matrix. Although this is not an ideal approach, it is justified, given that previous studies have shown these genera as sampled to be monophyletic (Wojciechowski, Lavin & Sanderson, 2004; Bruneau *et al.*, 2008) and because these outgroup taxa are not the primary focus of this study but were included only to facilitate time-calibration. The concatenated ALLcp matrix contained 49 *trnL-F*, 45 *matK*, 48 *rps16* and 49 *trnD-T* sequences and the ALLcpnc matrix contained, in addition to the ALLcp sequences, 46 ITS, 48 *AIGP*, 44 *tRALs*, 41 *EIF3E* and 34 *Leafy* sequences.

All matrices were analysed using a Bayesian phylogenetic approach. *Diptychandra* was used to root the trees in most analyses and *Tachigali* was used for the *Leafy* analysis. The concatenated matrices were partitioned by locus in order to attribute the appropriate nucleotide substitution model estimated by MrModelTest 2.3 (Nylander *et al.*, 2004) under the Akaike information criterion (AIC). Coded indels were analysed under the F-81 model. Bayesian inference was performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with two parallel runs of eight Monte Carlo Markov chains of 50 000 000 generations each and four swaps per swapping cycle. We used Tracer v.1.6 (Rambaut *et al.*, 2014) to monitor convergence and effective sample size (ESS) values for all parameters. When the analyses reached convergence (ESS > 200), trees were sampled every 1000 generations and the first 25% generations were discarded as burn-in (Ronquist, Van Der Mark & Huelsenbeck, 2009). Matrices are available in TreeBase (project ID 19392; https://treebase.org/treebase-web/search/study/summary.html?id=19392).

ESTIMATION OF DIVERGENCE TIMES

Divergence times were estimated using the Bayesian MCMC method implemented in BEAST 1.7.2 (Drummond *et al.*, 2012) on the concatenated ALLcpnc matrix, partitioned by locus as described for the Bayesian analysis described above and calibrated with three fossils. The closest fossils known are from the mimosoid clade and the Dimorphandra group (subfamily Caesalpinioideae) and were previously used by Bruneau *et al.* (2008) and Simon *et al.* (2009). We included *Eomimosoidea plumosa* Crepet & Dilcher, 1977, a fossil described from flowers, leaves and fruits from North America that has an estimated age at 45 Ma (Herendeen & Dilcher, 1990), which we placed at the stem node of *Dinizia* following Bruneau *et al.* (2008); an *Albizia* Durazz. pollen polyad fossil from Egypt with an estimated age of 45 Ma (Guinet *et al.*, 1987), which we placed at the Ingeae crown node and a

leaf fossil from Tanzania that has been attributed to the *Vachellia* clade of *Acacia* Mill. *s.l.* with an estimated age at 46 Ma (Herendeen & Jacobs, 2000), which we placed at the stem node of the mimosoid clade following arguments by Simon *et al.* (2009) that this fossil could not be unequivocally assigned to any particular genus in this clade.

The presence of a molecular clock could not be rejected based on a likelihood ratio test [critical value of 65.17 for the χ^2 distribution (d.f. = 48, *P* = 0.05)], but a relaxed uncorrelated lognormal clock model was selected rather than a strict clock model because we have evidence from the phylogram that the evolutionary rates differ along branches. The substitution models, clock model and tree topologies were unlinked and set according to each gene, except for the plastid tree topology, which remained linked. The analysis was performed for 150 000 000 generations, sampling every 2500 generations and the prior distribution of the ucld. mean and ucld.stdev parameters set to normal distribution (SD = 1). Taxon groups were created to represent the position of each calibration point. The Yule speciation process (Gernhard, 2008) with a random starting tree was selected. Prior distributions were set to a normal distribution (SD = 2.0) for each calibration point with the age of the fossil as the mean (Drummond *et al.*, 2012). Maximum credibility trees for this analysis were summarized with burn-in values set to the first 25% of trees sampled (Ronquist *et al.*, 2009) using TreeAnnotator v.1.7.2 (Drummond *et al.*, 2012).

Ancestral geographical range reconstruction

Ancestral geographical ranges were reconstructed for all nodes of the trigeneric clade using the dispersal-extinction-cladogenesis (DEC) model implemented in Lagrange 20120508 (Ree *et al.*, 2005; Ree & Smith, 2008). The online Lagrange configurator 20120508 was used to prepare the matrices (www.reelab.net/lagrange/configurator). The analyses were performed using the topology from the BEAST divergence time analysis, but excluding all outgroup taxa except *Conzattia multiflora* Robinson. The results of the divergence time analysis were used to set the root age of the tree. Species were assigned to one or several of the following five geographical ranges using data from herbarium specimens and geographical descriptions in Du Puy *et al.* (1995) and Brenan (1967): Mexico (for *Conzattia multiflora*); East Africa and Arabia; northern Madagascar (Diego Suarez) [N in Fig. 1]; northwestern inland Ankara Plateau and western coastal Bemaraha Plateau (Belo) (W); south-western Toliara region and the southern region from Autovombe to Itampolo (SW). The south-western region corresponds

to the spiny thicket vegetation and the north and west to the dry deciduous forest ecoregions (Fig. 1). Dispersal constraints were modified to test for three hypotheses on the Africa–Madagascar species disjunction. First, we tested a null hypothesis (H0) of no dispersal constraint in time. The second hypothesis (H1) tested for a long-distance dispersal event and postulates a constant presence in time of a strong dispersal barrier (i.e. by the Mozambique Channel) between Africa and Madagascar (dispersal constraint was set to 0.4 instead of the default 1.0 for no dispersal constraint) along with a mild dispersal barrier between the non-adjacent northern and southern Madagascar regions (dispersal constraint of 0.7). The third hypothesis (H2) had a time-dependent dispersal constraint with two time matrices (from 33 to 25 Ma and from 24 to 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 stronger dispersal constraint between the two continents (0.4). No directionality was imposed on any of the dispersal constraints in the DEC model. The AIC was calculated for each model to assess the significance of results among the three hypotheses. The maximum number of ancestral areas allowed at each node was set to three.

RESULTS

Cloning

The sequences for two (*Leafy* and *EIF3E*) of the four low-copy nuclear loci required cloning resulting in a total of 136 and 155 clones, respectively. For *EIF3E*, sequences for *Vachellia caven* (Molina) Seigler & Ebinger and *Diptychandra aurantiaca* Tul. were not cloned because the direct sequencing resulted in clean, polymorphism-free sequences. No polymorphism was observed in the direct sequencing of ITS in the ingroup samples and we found no sign of unhomogenized paralogous copies in 14 clones of *Colvillea racemosa* sequenced.

Phylogenetic analyses

In this study, 621 sequences were newly generated. Sequence characteristics and statistics for the Bayesian phylogenetic analysis of the nine individual loci and two concatenated datasets are shown in Table 2. In all the analyses (individual and concatenated), multiple clones for an individual always grouped together. In addition, when enough resolution was present (i.e. not a basal polytomy), multiple individuals of the same species mostly coalesced into monophyletic groups, except in *EIF3*, *AIGP* and *Leafy* where *D. floribunda*

Table 2. Sequence characteristics, Bayesian analysis statistics, including for outgroup taxa, for the nine individual locus matrices and two concatenated matrices (four plastid loci, ALLcp; plastid + nuclear loci, ALLcpnc) in the phylogenetic analyses of *Delonix*, *Colvillea* and *Lemuropisum*

	Number of sequences (ingroup species – outgroup species)	Aligned length (bp)	Missing data (%)	Number of indels	Evolutionary model
trnL-trnF	61 (13–20)	1191	5.6	75	GTR + G
matK	53 (9–19)	1798	0.3	41	GTR + G
rps16	60 (12–19)	974	1.6	76	GTR + G
trnD-trnT	59 (13–18)	1620	5.0	151	GTR + G
ITS	55 (13–17)	904	1.3	166	GTR + G
AIGP	57 (12–20)	465	0.6	9	SYM + G
tRALs	52 (10–17)	355	0.5	16	HKY + G
EIF3E	156 (8–16)	893	0.4	105	HKY + G
LEAFY	136 (11–7)	1021	0.0	170	K80 + I + G
ALLcp	49 (13–20)	5549	3	296	–
ALLcpnc	49 (13–20)	9118	8	596	–

individuals are paraphyletic to *D. pumila*, and in *AIGP*, where *D. boiviniana* individuals are polyphyletic in a poorly resolved topology (Fig. 2).

The Bayesian majority rule consensus trees for the four individual plastid loci (not shown), for three of the low-copy nuclear loci (*EIF3E*, *Leafy*, *AIGP*) and for the concatenated plastid matrix are all poorly resolved with a polytomy at the base of the *Delonix/Colvillea/Lemuropisum* clade (*EIF3E*, *AIGP*; Fig. 2) or of the *Delonix/Lemuropisum* clade (plastid, *Leafy*; Fig. 2). The consensus trees for ITS and *tRALs* (Fig. 2) are better resolved and support two sister clades: one containing the African species, *D. baccal* and *D. elata*, along with *D. boiviniana*, *D. brachycarpa* and *D. regia* (named the Widespread clade) and the other (Spiny Thicket) clade containing *Lemuropisum edule*, *D. velutina*, *D. leucantha*, *D. decaryi*, *D. tomentosa*, *D. pumila* and *D. floribunda*. The *tRALs* gene tree lacks *D. brachycarpa* and *D. decaryi*. Although poorly resolved, the *EIF3* and *AIGP* gene trees are not incongruent with respect to these two clades. However, the *tRALs* gene tree is also incongruent. However, in the *Leafy* and combined plastid gene trees these two clades are not resolved. The position of *D. brachycarpa* appears to be particularly unstable. In the *Leafy* and plastid gene trees, this widespread species occurs with Spiny Thicket clade species, whereas it groups with *D. boiviniana* of the Widespread clade in the ITS tree. The position of *C. racemosa* is also ambiguous. The ALLcp analysis (Fig. 2) resolves *Colvillea racemosa* as a strongly supported sister species to a *Delonix/Lemuropisum* clade, whereas in *Leafy* and *tRALs*, *C. racemosa* occurs nested in a *Delonix/Colvillea/Lemuropisum* clade, and in ITS, *AIGP* and *EIF3E* the three genera form a basal polytomy. The fully resolved ALLcpnc Bayesian consensus tree places *C. racemosa* as sister to a *Delonix/Lemuropisum*

clade (Fig. 3). In contrast, *L. edule* is clearly nested in *Delonix* in all analyses and strongly supported as such in the *tRALs* and *EIF3E* gene trees (Fig. 2).

ESTIMATION OF DIVERGENCE TIMES

The concatenated plastid and nuclear loci Bayesian consensus tree (Fig. 3) was used for the biogeographical analyses. Although we report differences in the phylogenetic positions of *Colvillea* and *D. brachycarpa*, the major biogeographical disjunctions discussed here are observed in all individual gene trees having sufficient resolution and in the concatenated matrix topology. The ancestor to the *Delonix/Colvillea/Lemuropisum* clade is estimated to have diverged from *Conzattia c.* 29 Ma (95% HPD 34.78–23.30 Ma). The crown node age of the *Delonix/Colvillea/Lemuropisum* clade was estimated at 24 Ma (96% HPD 29.55–18.02 Ma). The two *Delonix/Lemuropisum* clades, Widespread and Spiny Thicket, diverged from each other *c.* 19 and 18 Ma, respectively (Table 3). African *Delonix* spp. diverged *c.* 17 Ma (22.34–12.69 Ma) from their Madagascan ancestors (Table 3).

ANCESTRAL GEOGRAPHICAL RANGE RECONSTRUCTION

The hypothesis of long-distance dispersal (H1) was estimated to be more likely to represent our data based on its lower global likelihood and AIC values (ln = 49.01, AIC= –3.78) compared to the null hypothesis (H0: ln = 53.08, ΔAIC= +0.16) and the land bridge hypothesis (H2: ln = 49.86, ΔAIC = +0.03). Most *Delonix* spp. originated from ancestors found in south-western Madagascar, including the ancestor of the two African *Delonix* spp. (Fig. 3).
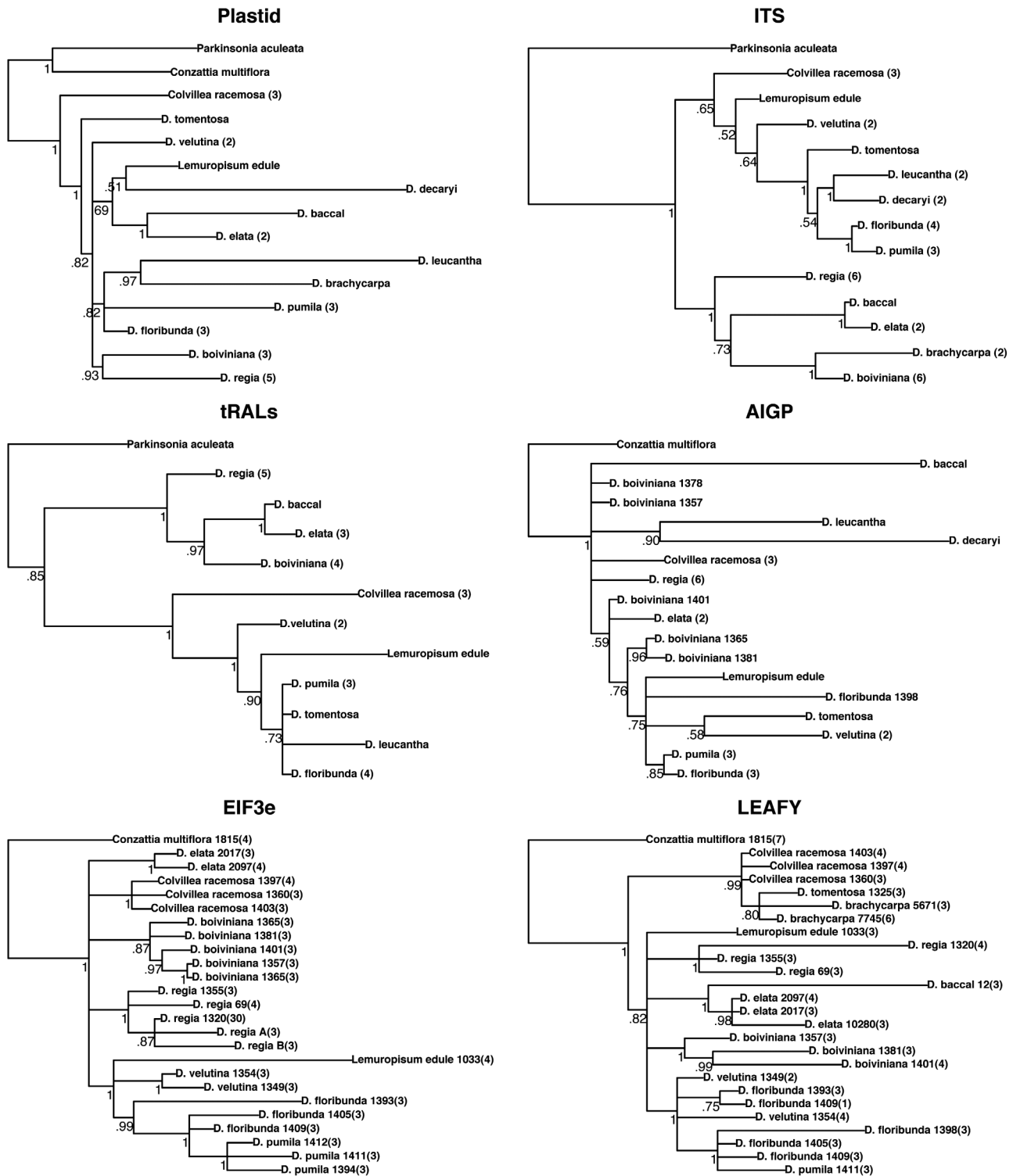
Figure 2. Bayesian majority rule consensus trees for the concatenated plastid loci, nuclear ribosomal ITS and four low-copy nuclear loci used in a phylogenetic analysis of species of *Delonix*, *Colvillea* and *Lemuropisum*. Posterior probability indicated below the branches. Numbers in parentheses indicate the number of individuals sampled per species, except in the *Leafy* and *EIF3E* topologies, where numbers in parentheses after the collection number represent the number of clones per individual.
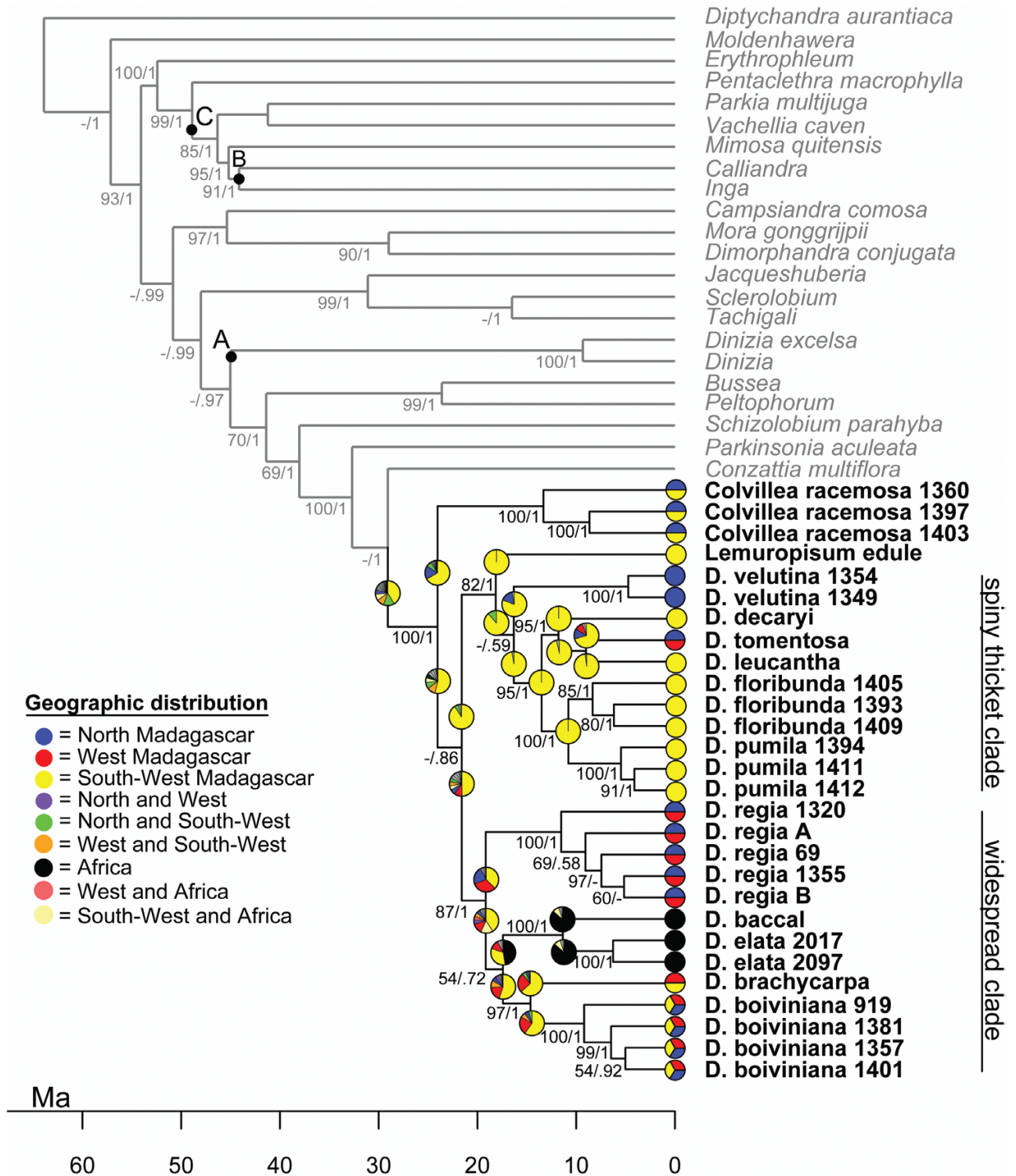
Figure 3. Divergence times and ancestral geographical range reconstruction for the *Delonix/Colvillea/Lemuropisum* clade. Chronogram of the Bayesian majority rule consensus tree analysis of the nuclear and plastid concatenated matrix (ALLcpnc). Coloured pie charts represent the proportional likelihood (>5%) of ancestral distribution for the taxa reconstructed by Lagrange. Colours correspond to different ancestral area combinations from the reconstruction analysis. Posterior probability indicated below the branches. Letters represent the position of the three fossil calibration points described in the text: A (*Eomimosoidea plumosa*), 45 Ma; B (*Albizia* pollen polyad), 45 Ma; C (*Vachellia* clade leaf fossil), 46 Ma.

Table 3. Divergence time estimates under partitioned Bayesian uncorrelated log-normal relaxed molecular clock model, with 95% low and high highest posterior density (HPD), and most probable reconstructed ancestral geographical range for the H1 hypothesis for the most common recent ancestor of the major clades in the calibrated phylogenetic analysis of *Colvillea*, *Delonix* and *Lemuropisum*

Clade	Bayesian relaxed clock (low–high 95% HPD)	Ancestral geographical range
Delonix/Colvillea/Lemuropisum	24.03 (18.02–29.55)	SW
Spiny thicket clade	18.14 (13.24–23.17)	SW
Delonix/Lemuropisum	21.59 (16.79–26.83)	SW
D. velutina/D. pumila	16.31 (11.63–21.24)	SW, N
D. decaryi/D. pumila	13.50 (9.32–18.01)	SW
D. tomentosa/D. leucantha/D. decaryi	11.79 (8.45–16.20)	SW
D. floribunda/D. pumila	10.79 (6.66–15.03)	SW
Widespread clade	19.16 (14.48–24.15)	SW
African *Delonix/D. boiviniana*	17.41 (12.69–22.34)	SW
African *Delonix* spp.	11.35 (6.22–16.73)	A
D. brachycarpa/D. boiviniana	14.62 (9.58–19.96)	SW

Ages are in millions of years. Geographical range as follows: N, northern Madagascar (Diego Suarez); W, north-western inland Ankara plateau and western coastal Bemaraha plateau (Belo); SW, south-western Toliara and southern region from Autovombe to Itampolo; A, Africa.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS, PARAPHYLETIC SPECIES, LINEAGE SORTING AND HYBRIDIZATION IN THE *DELONIX*, *COLVILLEA* AND *LEMUROPISUM* CLADE

Delonix is not supported as monophyletic in any of our analyses. Although the phylogenetic position of the mono-specific *Colvillea* is ambiguous, occurring either as sister to or grouped with *Delonix* spp., *L. edule* clearly is nested in *Delonix*, warranting a taxonomic change (see the following). As reported for numerous phylogenetic studies of closely related species (e.g. Naciri & Linder, 2015), we recover a mixture of divergent and potentially reticulate relationships in the *Delonix/Colvillea/Lemuropisum* clade, suggestive of a fundamentally divergent species history complicated by specific reticulation events, conflicting gene histories and genes that lack phylogenetic signal. However, despite low genetic variation among species, most species are resolved as monophyletic, and only *C. racemosa* and *D. brachycarpa* show conflicting phylogenetic positions among gene trees.

Several processes including hybridization, incomplete lineage sorting, introgression, gene paralogy issues, parallel evolution, poor species delimitation, differences in coalescence time of the loci studied and differences in effective population size of the species studied can result in gene tree heterogeneity among closely related species (Joly, McLenachan & Lockhart, 2009; Naciri & Linder, 2015). In this clade of closely related tropical tree species with low levels of variation in all loci studied here and in previous studies, including the Amplified fragment length polymorphism (AFLP) analyses of Rivers *et al*. (2011), the observed polytomies appear to be the result of lack

of phylogenetic signal. This could be due to an early burst of rapid diversification resulting in short branch lengths along the backbone of the trees. In the following, we highlight a few examples where we can more clearly suggest causes of observed gene tree heterogeneity or absence of reciprocal monophyly.

Interspecific hybridization between sympatric species may account for the conflicting positions of *D. brachycarpa* in the plastid, *Leafy* and ITS gene trees. For example, the grouping of *D. brachycarpa* and *C. racemosa* in the *Leafy* gene tree could be a sign of a reticulate history for these two species, which are found together in the south and west of Madagascar. *Delonix brachycarpa* is also sympatric with *D. leucantha*, with which it groups as a sister species in the plastid topology presented here, and in a similar clade in the analyses of Haston *et al.* (2005) who resolved this species with *D. floribunda* and *D. pumila*, two other southern Madagascar species (they did not sample *D. leucantha*). However, Du Puy *et al.* (1995) considered that the morphologically similar *D. brachycarpa* and *D. boiviniana*, which differ principally in the density of the indumentum, could be conspecific, supporting their strongly supported sister relationship in the ITS gene tree. *Delonix boiviniana* is a locally common, widespread and morphologically variable species (Du Puy *et al.*, 1995) with a high level of intraspecific polymorphism (Rivers *et al.*, 2011), the distribution of which to a large degree overlaps with that of the less common *D. brachycarpa*, scattered in deciduous woodland over limestone. Although for most of the nuclear loci studied, the gene lineages have coalesced in *D. boiviniana*, in the *AIGP* gene tree, accessions are scattered across the phylogenetic tree suggesting a high degree of polymorphism and insufficient time

for coalescence, which can be relatively long in a widespread species. For *D. brachycarpa* greater sampling across the range and sequencing of more nuclear loci (e.g. *AIGP*) would be of interest to understand the population and gene dynamics and affinities of this species better.

In contrast, the unstable phylogenetic position for *Colvillea* noted here and in the plastid study of Haston *et al.* (2005) is most likely caused by incomplete lineage sorting. The plastid data place *Colvillea* as external to the *Delonix*/*Lemuropisum* clade on its own branch, with no clear signal of sharing plastid sequences with an extant species in the *Delonix*/*Lemuropisum* clade. The nuclear data resolve *Colvillea* as sister to either the Spiny Thicket clade (Fig. 2: ITS and *tRALs*) or with *D. brachycarpa* and *D. tomentosa* (Fig. 2: *Leafy*), but never nested in either of the two *Delonix*/*Lemuropisum* clades. This phylogenetic pattern of incongruence suggests a situation in which the plastid sequences, which have smaller effective population size, have coalesced and clearly differentiate *Colvillea* from the *Delonix*/*Lemuropisum* clade, but that the nuclear data still require time before reaching coalescence. This scenario of incomplete lineage sorting – rather than hybridization, for example – is supported by the morphological uniqueness of *Colvillea* relative to *Delonix* and *Lemuropisum*.

In all gene trees, *D. floribunda* groups with *D. pumila*, either in a polytomy (plastid, *tRALs*) or as reciprocally monophyletic sister species (ITS, *AIGP*), or *D. pumila* accessions are nested in a paraphyletic grade of *D. floribunda* accessions. This phylogenetic pattern is expected of an ancestor–descendant species relationship (Davis & Nixon, 1992; Rieseberg & Brouillet, 1994; Naciri & Linder, 2015; Pennington & Lavin, 2016), which appears likely for this species pair given their overlapping geographical distribution and contrasting effective population sizes. The pattern could also simply be indicative of a lack of time for coalescence to reciprocal monophyly. *Delonix floribunda* is a common species in south-western Madagascar, occurring sympatrically with the more restricted *D. pumila*. *Delonix pumila* is a small shrub-like tree known from only one or two populations in the area of La Table near Toliara and is considered endangered. Rivers *et al.* (2011) found *D. pumila* to have a low genetic diversity and noted no genetic differentiation between sample sites, as might be expected for a small segregate offshoot of more widespread species. In contrast, for *D. floribunda*, Rivers *et al.* (2011) reported levels of genetic diversity around the mean of that observed for the genus as a whole and noted no geographical structuring of genetic diversity across sampled sites. Morphologically the two species are different, *D. pumila* having the more typical broad white-petaled flowers for the genus and *D. floribunda* having flowers with reduced, narrow yellow petals, considered to be pollinated by sunbirds (Du Puy *et al.*,

1995). However, flower morphology associated with pollination syndromes appears to be homoplasious in the *Delonix*/*Colvillea*/*Lemuropisum* clade (see the following) and does not accurately reflect phylogenetic relationships.

GEOGRAPHICALLY ASSOCIATED CLADES IN *DELONIX*

Some geographical structure is apparent in the two *Delonix* clades recovered. The Widespread clade contains species that occur in the north, west and south of Madagascar and the two African species, one of which has a distribution that extends to Arabia and India (possibly as an introduced ornamental; *D. elata*). The Madagascan species of this clade occur in the dry deciduous woodlands, generally on limestone outcrops or sand, and have broader distributions than the species in the Spiny Thicket clade. The Spiny Thicket clade comprises species with small ranges mostly restricted to the southern arid bioclimatic zone (Fig. 3). However, *D. tomentosa* occurs in the west and north and *D. velutina* occurs in northern Madagascar, both in deciduous woodlands, but both presently with restricted distributions. The four xerophytic scrubland species, *D. floribunda*, *D. decaryi*, *D. leucantha* and *D. pumila*, are part of this clade (monophyletic in the ITS gene tree), often on sand or limestone outcrops, *D. leucantha* in particular occurring on the peculiar and extreme 'tsingy' habitats of western Madagascar. Our analyses suggest that the southern xerophytic scrubland species are derived in the *Delonix* clade and may be of more recent origin than some of the dry forest lineages in the north and west. Of the seven species in the *Delonix* clade currently considered to be threatened by International Union for the Conservation of Nature (ICUN, 2015; *D. baccal*, *D. decaryi*, *D. leucantha*, *D. pumila*, *D. tomentosa*, *D. velutina*, *L. edule*), all occur in the Spiny Thicket clade except the east African *D. baccal*.

In contrast to the observed, albeit weak, geographical structure, the three morphological groups identified by Du Puy *et al.* (1995) based on leaf and fruit characters are only partially supported by our analyses. Species in the Widespread clade, which diverged *c*. 19 Ma (±5 Ma) from a southern Madagascar ancestor, are morphologically diverse. *Delonix regia* and the two African species have bi-pinnate leaves with many pinnae, each pinna with numerous small leaflets. These three species also have a similar pollen type with dense granules at the base of the lumina, rounded muri, large aperture lengths and absence of layering on the microperforation (Banks, 1997). The close relationship between the two African species was postulated by Du Puy *et al.* (1995) based on the presence of a flat, coriaceous pod, rather than the typical lignified pod seen in the rest of the genus; this is also supported

by previous molecular studies (Haston *et al.*, 2005). In contrast to the other three species in this clade, *D. boiviniana* and *D. brachycarpa* have leaves with fewer pinnae and generally larger leaflets, but leaflet size varies in *D. boiviniana* (Du Puy *et al.*, 1995). These two sister species have pods that are short and curved (Du Puy *et al.*, 1995), a unique feature in the genus, but their pollen is typical of that found in most other *Delonix* spp. (see the following; Banks, 1997).

Species in the Spiny Thicket clade, which diverged from a southern Madagascar ancestor during the Miocene *c.* 18 Ma (±5Ma), are mostly characterized by having flowers with white petals, the median petal also having a yellow patch, and they are considered to be moth pollinated (Du Puy *et al.*, 1995). The white-petaled moth-pollinated species also share a similar pollen type with sparse granules at the base of the lumina, reduced aperture lengths and bi-layered microperforations (Banks, 1997). The two species, which had been segregated as *Aprevallia* by Baillon (1884) and Capuron (1968), *D. velutina* and *D. floribunda*, belong to this clade. These two species have reduced petals that are yellow, are pollinated by sunbirds (Du Puy *et al.*, 1995) and have pollen of the same type as found in *D. regia* and the two African species of the Widespread clade. *Delonix regia* has red petals and is thought to also be sunbird-pollinated, further highlighting homoplasy of characters associated with pollination. The four scrubland species have similar linear-oblong pods and leaves with few pinnae and leaflets, differentiating them from *D. tomentosa* and *D. velutina*, which have long flattened strap-shaped pods and leaves with many pinnae and numeours leaflets (as in *D. regia*). The close relationship of these four species was noted by Du Puy *et al.* (1995). However, Du Puy *et al.* (1995) also considered *D. velutina*, *D. tomentosa* and *D. regia* to be close relatives based upon their similar fruit and leaf characters, but our analyses tend to suggest that strap-shaped pods and leaves with numerous pinnae and small leaflets are plesiomorphic in the clade. It appears that the swollen cigar-shaped trunk may be a synapomorphy for the Spiny Thicket clade because it is generally absent from the Widespread clade, although *D. boiviniana* and *D. elata* can have either slender or swollen trunks.

OUT OF MADAGASCAR DISPERSAL DURING THE MIOCENE

Delonix, *Lemuropisum* and *Colvillea* evolved from a Madagascan ancestor. More broadly, however, this trigeneric clade typical of dry forest regions is sister to *Conzattia* and *Heteroflorum* [the latter not sampled here, but sampled by Haston *et al.* (2005)], two dry forest Mexican endemic genera. This documents yet another example of niche conservatism with a notable pattern of sister groups spanning seasonally dry tropical forests of Mesoamerica, the Caribbean, the Horn of Africa and Madagascar (Lavin & Luckow, 1993; Lavin *et al.*, 2004). The ancestral area of the *Delonix/Lemuropisum* clade in Madagascar was most likely in the south and west of the island, during the mid-Oligocene to early Miocene (29 to 21 Ma; Fig. 3). Our study suggests significantly older ages than those of Buerki *et al.* (2013), who estimated late Miocene ages for *Lemuropisum* and *Colvillea*, but our dates are not dissimilar to their age estimates for the origin of the monospecific spiny thicket Madagascan legume genus *Brenierea* Humbert (early Oligocene to mid-Miocene) and generally agree with their assessment that most endemic Madagascan genera originated after the Eocene.

At the time of diversification of the *Delonix* clade, the major biomes of Madagascar were already established (Yoder & Nowak, 2006; Buerki *et al.*, 2013). During the Eocene the once more widespread arid biome contracted to the southern part of Madagascar where conditions became drier and the spiny thicket biome is thought to have evolved (Yoder & Nowak, 2006). The Eocene also coincides with the development of the more recent humid, subhumid and dry bioclimatic regions, the latter in western and northern Madagascar (Yoder & Nowak, 2006). The reduction in the arid bioclimatic zone probably resulted in extinction, as speculated by Buerki *et al.* (2013) and it is possible that taxa, such as the *Delonix* clade, were once more widespread across Madagascar. Federman *et al.* (2015) suggested that climatic stochasticity in Madagascar may have caused lineage turnover in forests, periodically re-setting the ecological stage, and could provide an explanation for speciation of post-Eocene Madagascan taxa.

It is thus from this dry forest bioclimatic zone in south-west Madagascar that the African *Delonix* ancestor was transported to eastern Africa by long-distance dispersal across the Mozambique Channel during the Miocene between 17 and 11 Ma. This close relationship between the Masai-Somalia Horn of Africa spiny succulent-rich seasonally dry tropical forests and those of south-western Madagascar, also suggestive of niche conservatism, has been noted in other legume taxa (Lavin *et al.*, 2000; Thulin *et al.*, 2001). Our analysis supports recent studies that suggest unidirectional dispersal from Madagascar to Africa in plants (Yuan *et al.*, 2005; Krüger, Razafimandimbison & Bremer, 2012) and that these out-of-Madagascar events seem to be fairly recent, dating from the late Eocene to the mid-Miocene (Raxworthy *et al.*, 2008; Crottini *et al.*, 2012) and up to the Pliocene in some plant species (Yuan *et al.*, 2005). Of course, numerous counter examples of Africa-to-Madagascar dispersals have also been reported (e.g. Renner, 2004a; Janssen *et al.*, 2008; Schaefer, Heibl & Renner, 2009; Pock Tsy *et al.*, 2009; Anthony *et al.*, 2010; Wikstrom *et al.*, 2010;

Strjik *et al.*, 2012), including some for legumes (Schrire *et al.*, 2009; Thulin, Phillipson & Lavin, 2013), and there are some examples of Asia-to-Madagascar dispersals (Renner, 2004; Federman *et al.*, 2015).

The landbridge hypothesis can be discounted as an explanation for the Madagascar–Africa disjunction because the divergence took place well after the disappearance of the land bridge (45–26 Ma: McCall, 1997; see Table 3). Thus long-distance dispersal to Africa is the most likely explanation for this disjunction. Dispersal by migratory birds across the Mozambique Channel is unlikely because although *Delonix* seeds are edible, they possess no physiological adaptation for attachment to birds and no migratory birds feeding on the seeds of *Delonix*, *Colvillea* and *Lemuropisum* are known. For plants, long-distance dispersal on floating vegetative rafts has been suggested as a likely scenario (Morley & Dick, 2003; Gillespie *et al.*, 2012). During the mid-Miocene, oceanic currents and surface winds flowed from Madagascar to East Africa (von der Heydt & Dijkstra, 2006; Ali & Huber, 2010) and it has been estimated that dispersal from Madagascar to Africa could have been achieved in ten days (Stankiewicz *et al.*, 2006). The long and flat pods of *Delonix* spp. can most probably float. Also, seeds of some species of Caesalpinioideae have been shown to have a germination rate of >50% even after 30 days in water (Lopez, 2001).

TAXONOMIC STATUS OF *DELONIX*, *COLVILLEA* AND *LEMUROPISUM*

Delonix is not supported as monophyletic in any of our analyses due to the robustly supported placement of *L. edule* nested in *Delonix*. Species of *Delonix* and *Lemuropisum* have similar flower morphology, generally with white petals, and subequal, valvate calyx segments, which are leathery and thickened, and are thought to share the same moth pollinator (Du Puy *et al.*, 1995). The pollen structure of *L. edule* is identical to that found for the majority of *Delonix* spp. (Banks, 1997). AFLP data suggest that *L. edule* is genetically close to *D. pumila* and strongly associated with other sympatric southern Madagascan species such as *D. floribunda* and *D. decaryi* (Rivers *et al.*, 2011), an assessment corroborated by our study.

In light of previous studies and the results found here, we propose including *Lemuropisum* in *Delonix*, as previously suggested by Haston *et al.* (2005). This taxonomic reduction is congruent with molecular, morphological and ecological data (Du Puy *et al.*, 1995; Banks, 1997; Simpson *et al.*, 2003; Haston *et al.*, 2005; Hawkins *et al.*, 2007; Bruneau *et al.*, 2008; Rivers *et al.*, 2011). In the following, we make the necessary new combination to replace *L. edule*.

Delonix edule (H.Perrier) Babineau & Bruneau comb. nov. Basionyn: *Lemuropisum edule* H.Perrier, Bull. Soc. Bot. France 85: 494. 1938. TYPE: MADAGASCAR. H. Perrier de la Bâthie 19183 (P, TAN). 1933–4. Locality: SW Madagascar, Manampetsa, calcareous (24°6′0″S; 43° 49′59.988″E). Phenology: flowers and fruits. Barcodes (P): MNHN-P-P00100456, MNHN-P-P00100457, MNHN-P-P00100458 (e.g. http://coldb.mnhn.fr/catalognumber/mnhn/p/p00100456).

Haston *et al.* (2005) also argued for the inclusion of *Colvillea* in *Delonix*, but because our phylogenetic analysis is inconclusive as to the position of *Covillea*, we refrain from synonymizing this genus at this time. The phylogenetic position of *C. racemosa* as sister to the *Delonix/Lemuropisum* clade is consistent with the uniqueness of its floral morphology. This species has large terminal panicles, resupinate flowers with orange petals, four fused calyx segments and clustered stamens (Du Puy *et al.*, 1995), an unusual pollen structure (Banks, 1997) and 15 unique alleles (Rivers *et al.*, 2011).

As for *Lemuropisum*, recent phylogenetic studies have cast doubt on the status of other endemic Madagascan legume genera, showing them to be nested in other genera [e.g. *Peltiera* Du Puy & Labat nested in *Ormocarpopsis* R.Vig. (Thulin *et al.*, 2013); *Alantsilodendron* Villiers nested in *Dichrostachys* Wight & Arn. (Hughes *et al.*, 2003)]. Determining the phylogenetic position of Madagascan genera (and species) remains essential for correct assessments of conservation status and developing conservation priorities. Only time will tell just how many Madagascan endemic legume genera will remain in the face of rigorous phylogenetic analysis.

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REFERENCES

Ali JR, Huber M. 2010. Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature* **463:** 653–657.

Alvarez I, Wendel JF. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29:** 417–434.

Anthony F, Diniz LEC, Combes M, Lashermes P. 2010. Adaptive radiation in *Coffea* subgenus *Coffea* L. (Rubiaceae) in Africa and Madagascar. *Plant Systematics and Evolution* **285:** 51–64.

Archambault A, Bruneau A. 2004. Phylogenetic utility of the *Leafy/Floricaula* gene in the Caesalpinioideae (Leguminosae): gene duplication and a novel insertion. *Systematic Botany* **29:** 609–626.

Babineau M, Gagnon E, Bruneau A. 2013. Phylogenetic utility of 19 low copy nuclear genes in closely related genera and species of caesalpinioid legumes. *South African Journal of Botany* **89:** 94–105.

Baillon H. 1884. Liste des plantes de Madagascar: un nouveau type de Caesalpiniées monopétales. *Bulletin Mensuel de la Société Linnéenne de Paris* **1:** 428–429.

Banks H. 1997. The pollen of *Delonix* (Leguminosae: Caesalpinioideae: Caesalpinieae). *Kew Bulletin* **52:** 417–434.

Bessega C, Vilardi JC, Saidman BO. 2006. Genetic relationship among American species of the genus *Prosopis* (Mimosoideae, Leguminosae) inferred from ITS sequences: evidence for long distance dispersal. *Journal of Biogeography* **33:** 1905–1915.

Brenan JPM. 1967. Leguminosae subfamily Caesalpinioideae. In: Milne-Redhead E, Polhill RM, eds. *Flora of tropical East Africa*. London: Crown Agents, 1–230.

Bruneau A, Forest F, Herendeen PS, Klitgaard BB, Lewis GP. 2001. Phylogenetic relationships in the Caesalpinioideae (Leguminosae) as inferred from chloroplast *trnL* intron sequences. *Systematic Botany* **26:** 487–514.

Bruneau A, Mercure M, Lewis GP, Herendeen PS. 2008. Phylogenetic patterns and diversification in the caesalpinioid legumes. *Botany* **86:** 697–718.

Buerki S, Devey DS, Callmander MW, Phillipson PB, Forest F. 2013. Spatio-temporal history of the endemic genera of Madagascar. *Botanical Journal of the Linnean Society* **171:** 304–329.

Callmander MW, Phillipson PB, Schatz GE, Andriambololonera S, Rabarimanarivo M, Rakotonirina N, Raharimampionona J, Chatelain C, Gautier L, Lowry II PP. 2011. The endemic and non-endemic vascular flora of Madagascar updated. *Plant Ecology and Evolution* **144:** 121–125.

Capuron R. 1968. Contribution à l'étude de la flore forestière de Madagascar, réduction du genre *Aprevalia* Baillon au rang de section du genre *Delonix* Raf. et description d'une espèce nouvelle (Leguminosae, Caesalp.). *Adansonia* **8:** 11–16.

Choi HK, Luckow MA, Doyle J, Cook DR. 2006. Development of nuclear gene-derived molecular markers linked to legume genetic maps. *Molecular Genetics and Genomics: MGG* **276:** 56–70.

Cornet A. 1974. *Essai de cartographie bioclimatique à Madagascar*. Paris: Laboratoire de Botanique 1–55, ORSTOM.

Cronn R, Cedroni M, Haselkorn T, Grover C, Wendel JF. 2002. PCR-mediated recombination in amplification products derived from polyploid cotton. *Theoretical and Applied Genetics* **104:** 482–489.

Crottini A, Madsen O, Poux C, Stauß A, Vieites DR, Vences M. 2012. Vertebrate time-tree elucidates the biogeographic pattern of a major biotic change around the K-T boundary in Madagascar. *Proceedings of the National Academy of Sciences of USA* **19:** 5358–5363.

Davis JI, Nixon KC. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Biology* **41:** 421–435.

Demesure B, Sodzi N, Petit RJ. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* **4:** 129–131.

Douzery EJ, Pridgeon AM, Kores P, Linder HP, Kurzweil H, Chase MW. 1999. Molecular phylogenetics of Diseae (Orchidaceae): a contribution from nuclear ribosomal ITS sequences. *American Journal of Botany* **86:** 887–899.

Doyle JJ. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany* **17:** 144–163.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Du Puy DJ, Labat J-N, Rabevohitra R, Villiers J-F, Bosser J, Moat J. 2002. *The Leguminosae of Madagascar*. Kew: Royal Botanical Gardens.

Du Puy DJ, Moat J. 1998. Vegetation mapping and classification in Madagascar (using GIS): implications and recommendations for the conservation of biodiversity. In: Huxley CR, Lock JM, Cutler DF, eds. *Chorology, taxonomy and ecology of the African and Madagascan floras*. Kew: Royal Botanic Gardens, 97–117.

Du Puy DJ, Phillipson PB, Rabevohitra R. 1995. The genus *Delonix* (Leguminosae: Caesalpinioideae) in Madagascar. *Kew Bulletin* **50:** 445–475.

Edgar RC. 2004. MUSCLE: A multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32:** 1792–1797.

Federman S, Dornburg A, Downie A, Richard AF, Daly DC, Donoghue MJ. 2015. The biogeographic origin of a radiation of trees in Madagascar: implications for the assembly of a tropical forest biome. *BMC Evolutionary Biology* **15:** 216.

Feliner GN, Roselló JA. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* **44:** 911–919.

Gernhard T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* **253:** 769–778.

Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK. 2012. Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology & Evolution* **27:** 47–56.

Givnish TJ. 1997. Adaptive radiations and molecular systematics: issues and approaches. In: Givnish TJ, Systma KJ, eds. *Molecular evolution and adaptive radiation*. Cambridge: Cambridge University Press, 1–54.

Glor RE. 2010. Phylogenetic insight on adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics* **41:** 251–270.

Godfrey LR, Jungers WL, Schwartz GT, Irwin MT. 2008. Ghosts and orphans: Madagascar's vanishing ecosystems. In: Fleagle JG, Gilbert CC, eds. *Elwyn Simons: a search for origins*. New York: Springer, 361–395.

Guinet P, Sabrouty E, Soliman HA, Omrah AM. 1987. Études des caractères du pollen des Légumineuses-Mimosoideae des sédiments Tertiaires du Nord Ouest de l'Égypte. *Mém. Travelodge, Ecole Pratique des Hautes Etudes, Institut Montpellier* **17:** 159–171.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows. *Nucleic Acids Symposium Series* **41:** 95–98.

Haston EM, Lewis GP, Hawkins JA. 2005. A phylogenetic reappraisal of the *Peltophorum* group (Caesalpinieae: Leguminosae) based on the chloroplast *trnL-F*, *rbcL* and *rps16* sequence data. *American Journal of Botany* **92:** 1359–1371.

Hawkins JA, Boutaoui N, Cheung KY, van Klinken RD, Hughes CE. 2007. Intercontinental dispersal prior to human translocation revealed in a cryptogenic invasive tree. *New Phytologist* **175:** 575–587.

Herendeen PS, Dilcher DL. 1990. Mimosoid legumes from the Eocene and Oligocene of southeastern North America. *Review of Palaeobotany and Palynology* **62:** 339–361.

Herendeen PS, Jacobs BF. 2000. Fossil legumes from the Middle Eocene (46.0 Ma) Mahenge Flora of Singida, Tanzania. *American Journal of Botany* **87:** 1358–1366.

Hu JM, Lavin M, Wojciechowski MF, Sanderson MJ. 2000. Phylogenetic systematics of the tribe Millettieae (Fabaceae) based on chloroplast *trnK/matK* sequences and its implications for evolutionary patterns in Papilionoideae. *American Journal of Botany* **87:** 418–430.

Hughes CE, Bailey CD, Krosnick S, Luckow MA. 2003. Relationships among genera of the informal *Dichrostachys* and *Leucaena* groups (Mimosoideae) inferred from nuclear ribosomal ITS sequences. In: Klitgaard BB, Bruneau A, eds. *Advances in legume systematics, Part 10. Higher level systematics*. Kew: Royal Botanic Gardens, 221–238.

Hurr KA, Lockhart PJ, Heenan PB, Penny D. 1999. Evidence for the recent dispersal of *Sophora* (Leguminosae) around the southern oceans: molecular data. *Journal of Biogeography* **26:** 565–577.

International Union for the Conservation of Nature. 2015. *IUCN red list of threatened species, version 2015-4*. Available at: http://www.iucnredlist.org

Janssen T, Bystriakova N, Rakotondrainibe F, Coomes D, Labat JN, Schneider H. 2008. Neoendemism in Madagascan scaly tree ferns results from recent, coincident diversification bursts. *Evolution* **62:** 1876–1889.

Joly S. 2012. JML: testing hybridization from species trees. *Molecular Ecology Resources* **12:** 179–184.

Joly S, McLenachan PA, Lockhart PJ. 2009. A statistical approach for distinguishing hybridization and incomplete lineage sorting. *American Naturalist* **174:** E54–E70.

Joly S, Starr JR, Lewis WH, Bruneau A. 2006. Polyploid and hybrid evolution in roses east of the Rocky Mountains. *American Journal of Botany* **93:** 412–425.

Krüger Å, Razafimandimbison SG, Bremer B. 2012. Molecular phylogeny of the tribe Danaideae (Rubiaceae: Rubioideae): another example of out-of-Madagascar dispersal. *Taxon* **61:** 629–636.

Lavin M, Herendeen PS, Wojciechowski MF. 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Systematic Biology* **54:** 575–594.

Lavin M, Luckow M. 1993. Origins and relationships of tropical North America in the context of the boreotropics hypothesis. *American Journal of Botany* **80:** 1–14.

Lavin M, Schrire BP, Lewis G, Pennington RT, Delgado-Salinas A, Thulin M, Hughes CE, Matos AB, Wojciechowski MF. 2004. Metacommunity process rather than continental tectonic history better explains geographically structured phylogenies in legumes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **359:** 1509–1522.

Lavin M, Thulin M, Labat J-N, Pennington RT. 2000. Africa, the odd man out: molecular biogeographic studies of dalbergioid legumes (Fabaceae) suggest otherwise. *Systematic Botany* **25:** 449–467.

Legume Phylogeny Working Group (LPWG). 2013. Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. *Taxon* **62:** 217–248.

Li M, Wunder J, Bissoli G, Scarponi E, Gazzani S, Barbaro E, Saedler H, Varotto C. 2008. Development of COS genes as universally amplifiable markers for phylogenetics reconstructions of closely related plant species. *Cladistics* **24:** 727–745.

Lopez OR. 2001. Seed flotation and postflooding germination in tropical terra firme and seasonally flooded forest species. *Functional Ecology* **15:** 763–771.

Luckow M, Miller JT, Murphy DJ, Livschultz T. 2003. A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data. In: Klitgaard BB, Bruneau A, eds. *Advances in legume systematics, Part 10. Higher level systematics*. Kew: Royal Botanic Gardens, 197–220.

Luckow M, White PJ, Bruneau A. 2000. Relationships among the basal genera of Mimosoid legumes. In: Herendeen PS, Bruneau A, eds. *Advances in legume systematics, Part 9*. Kew: Royal Botanic Gardens, 165–180.

Maddison WP, Knowles LL. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* **55:** 21–30.

Manzanilla V, Bruneau A. 2012. Phylogeny reconstruction in the Caesalpinieae grade (Leguminosae) based on duplicated copies of the sucrose synthase gene and plastid markers. *Molecular Phylogenetics and Evolution* **65:** 149–162.

Masters JC, de Wit MJ, Asher RJ. 2006. Reconciling the origins of Africa, India and Madagascar with vertebrate

dispersal scenarios. *Folia primatologica; International Journal of Primatology* **77:** 399–418.

McCall RA. 1997. Implications of recent geological investigations of the Mozambique Channel for the mammalian colonisation of Madagascar. *Proceedings of the Royal Society B: Biological Sciences* **264:** 663–665.

Miller JT, Bayer RJ. 2001. Molecular phylogenetics of Acacia (Fabaceae: Mimosoideae) based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions. *American Journal of Botany* **88:** 697–705.

Morley RJ, Dick CW. 2003. Missing fossils, molecular clocks, and the origin of the Melastomataceae. *American Journal of Botany* **90:** 1638–1644.

Muñoz J, Felicísimo AM, Cabezas F, Burgaz AR, Martínez I. 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science (New York, N.Y.)* **304:** 1144–1147.

Naciri Y, Linder HP. 2015. Species delimitation and relationships: the dance of the seven veils. *Taxon* **64:** 3–16.

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A. 2008. Mechanisms of long-distance seed dispersal. *Trends in Ecology & Evolution* **23:** 638–647.

Nic Lughadha E, Baillie J, Barthlott W, Brummitt NA, Cheek MR, Farjon A, Govaerts R, Hardwick KA, Hilton-Taylor C, Meagher TR, et al. 2005. Measuring the fate of plant diversity: towards a foundation for future monitoring and opportunities for urgent action. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **360:** 359–372.

Nylander JAA, Ronquist F, Huelsenbeck JD, Nieves-Aldrey JL. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* **53:** 1–21.

Oxelman B, Lidén M, Berglund D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* **206:** 393–410.

Pennington RT, Lavin M. 2016. The contrasting nature of woody plant species in different Neotropical forest biomes reflects differences in ecological stability. *New Phytologist* **210:** 25–37.

Pock Tsy J-ML, Lumaret R, Mayne D, Vall AOM, Abutaba YIM, Sagna M, Raoseta SOR, Danthu P. 2009. Chloroplast DNA phylogeography suggests a West African centre of origin for the baobab, *Adansonia digitata* L. (Bombacoideae, Malvaceae). *Molecular Ecology* **18:** 1707–1715.

Polhill RM. 1994. Complete synopsis of legume genera. In Bisby FA, Buckingham J, Harborne JB, eds. *Phytochemical dictionary of the Leguminosae*. London: Chapman and Hall, 233–248.

Polhill RM, Vidal JE. 1981. Caesalpinieae. In: Polhill RM, Raven PH, eds. *Advances in legume systematics, Part 1*. Kew: Royal Botanic Gardens, 81–95.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer version 1.6. Available from http://beast.bio.ed.ac.uk/Tracer, accessed 29 May 2015.

Raxworthy CJ, Pearson RG, Rabibisoa N, Rakotondrazafy AM, Ramanamanjato JB, Raselimanana AP, Wu S, Nussbaum RA, Stone PA. 2008. Extinction vulnerability of tropical montane endemism from warming and upslope

displacement: a preliminary appraisal for the highest massif in Madagascar. *Global Change Biology* **14:** 1703–1720.

Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution; International Journal of Organic Evolution* **59:** 2299–2311.

Ree RH, Smith SA. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57:** 4–14.

Renner SS. 2004. Multiple Miocene Melastomataceae dispersal between Madagascar, Africa and India. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **359:** 1485–1494.

Rieseberg LH, Brouillet L. 1994. Are many plant species paraphyletic? *Taxon* **43:** 21–32.

Rivers M, Brummitt NA, Nic Lughadha E, Meagher TR. 2011. Genetic variation in *Delonix s.l.* (Leguminosae) in Madagascar revealed by AFLPs: fragmentation, conservation status and taxonomy. *Conservation Genetics* **12:** 1333–1344.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* **19:** 1572–1574.

Ronquist F, Van Der Mark P, Huelsenbeck PJ. 2009. Bayesian phylogenetic analysis using MrBayes. In: Lemey P, Salemi M, Vandamme AM, eds. *The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing, 2nd edn*. Cambridge: Cambridge University Press, 210–265.

Särkinen T, Marcelo Peñas JL, Daza Yomona A, Simon MF, Pennington RT, Hughes CE. 2011. Underestimated endemic species diversity in the dry inter-Andean valley of the Río Marañón, northern Peru: an example from Mimosa (Leguminosae, Mimosoideae). *Taxon* **60:** 139–150.

Schaefer H, Heibl C, Renner SS. 2009. Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society B* **276:** 843–851.

Schatz GE. 2001. *Flore générique des arbres de Madagascar*. Kew: Royal Botanical Gardens; St Louis: Missouri Botanical Gardens.

Schatz GE. 2002. Taxonomy and herbaria in service of plant conservation: lessons from Madagascar's endemic families. *Annals of the Missouri Botanical Garden* **89:** 145–152.

Scherson RA, Vidal R, Sanderson MJ. 2008. Phylogeny, biogeography, and rates of diversification of New World Astragalus (Leguminosae) with an emphasis on South American radiations. *American Journal of Botany* **95:** 1030–1039.

Schrire BD, Lavin M, Barker NP, Forest F. 2009. Phylogeny of the tribe Indigofereae (Leguminosae-Papilionoideae): geographically structured more in succulent-rich and temperate settings than in grass-rich environments. *American Journal of Botany* **96:** 816–852.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92:** 142–166.

Simon MF, Grether R, De Queiroz LP, Skema C, Pennington RT, Hughes CE. 2009. Recent assembly of the Cerrado, a Neotropical plant diversity hotspot, by *in situ* evolution of adaptations to fire. *Proceedings of the National Academy of Science of USA* **106:** 20359–20364.

Simon MF, Grether R, de Queiroz LP, Särkinen TE, Dutra VF, Hughes CE. 2011. The evolutionary history of *Mimosa* (Leguminosae): toward a phylogeny of the sensitive plants. *American Journal of Botany* **98:** 1201–1221.

Simpson BB, Larkin LL, Weeks A. 2003. Progress towards resolving the relationships of the *Caesalpinia* group (Caesalpinieae: Caesalpinioideae: Leguminosae). In: Klitgaard BB, Bruneau A, eds. *Advances in legume systematics, Part 10. Higher level systematics*. Kew: Royal Botanic Gardens, 123–148.

Simpson GG. 1940. Mammals and land bridges. *Journal of the Washington Academy of Science* **30:** 137–163.

Stankiewicz J, Thiart C, Marsters JC, de Wit MJ. 2006. Did lemurs have sweepstake tickets? An exploration of Simpson's model for the colonization of Madagascar by mammals. *Journal of Biogeography* **33:** 221–235.

Strijk JS, Noyes RD, Strasberg D, Cruaud C, Gavory F, Chase MW, Abbott RJ, Thébaud C. 2012. In and out of Madagascar: dispersal to peripheral islands, insular speciation and diversification of Indian Ocean daisy trees (*Psiadia*, Asteraceae). *PLoS One* **7:** e42932.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17:** 1105–1109.

Thiel M, Haye PA. 2006. The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanography and Marine Biology: An Annual Review* **44:** 323–429.

Thulin M, Lavin M. 2001. Phylogeny and biogeography of the *Ormocarpum* group (Fabaceae): a new genus *Zygocarpum* from the Horn of Africa region. *Systematic Botany* **26:** 299–317.

Thulin M, Phillipson PB, Lavin M. 2013. *Peltiera* (Fabaceae), the coming and going of an 'extinct' genus in Madagascar. *Adansonia* **35:** 61–71.

Turchetto-Zolet AC, Cruz F, Vendramin GG, Simon MF, Salgueiro F, Margis-Pinheiro M, Margis R. 2012. Large-scale phylogeography of the disjunct Neotropical tree species *Schizolobium parahyba* (Fabaceae-Caesalpinioideae). *Molecular Phylogenetics and Evolution* **65:** 174–182.

Vences M, Vieitres DR, Glau F, Brinkmann H, Kosuch J, Veith M, Meyer A. 2003. Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society B: Biological Sciences* **270:** 2435–2442.

Von Der Heydt A, Dijkstra HA. 2006. Effect of ocean gateways on the global ocean circulation in the late Oligocene and early Miocene. *Paleoceanography* **21:** PA1011.

Wikström N, Avino M, Razafimandimbison SG, Bremer B. 2010. Historical biogeography of the coffee family (Rubiaceae, Gentianales) in Madagascar: case studies from the tribes Knoxieae, Naucleeae, Paederieae and Vanguerieae. *Journal of Biogeography* **37:** 1094–1113.

White F. 1983. *The vegetation of Africa. A descriptive memoir to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa*. La Chaux-de-Fonds: Courvoisier.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 315–322.

Wojciechowski MF, Lavin M, Sanderson MJ. 2004. A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *American Journal of Botany* **91:** 1846–1862.

Yahara T, Javadi F, Onoda Y, Queiroz LP de, Faith D, Prado DE, Akasaka M, Kadoya T, Ishihama F, Davies S, Slik JWF, Yi T, Ma K, Bin C, Darnaedi D, Pennington RT, Tuda M, Shimada M, Ito M, Egan AN, Buerki S, Raes N, Kajita T, Vatanparast M, Mimura M, Tachida H, Iwasa Y, Smith GF, Victor JE, Nkonki T. 2013. Global legume diversity assessment: concepts, key indicators, and strategies. *Taxon* **62:** 249–266.

Yoder AD, Nowak MD. 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecology, Evolution, and Systematics* **37:** 405–431.

Yuan YM, Wohlhauser S, Möller M, Klackenberg J, Callmander M, Küpfer P. 2005. Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian ocean basin resulted from long distance dispersal and extensive radiation. *Systematic Biology* **54:** 21–34.

Zhou L, Su YCF, Thomas DC, Saunders RMK. 2012. 'Out of Africa' dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). *Journal of Biogeography* **39:** 322–335.

APPENDIX

Voucher specimen data and GenBank accessions numbers for species of *Delonix*, *Colvillea* and *Lemuropisum*, and outgroup taxa from the mimosoid clade and from the *Dimorphandra*, *Tachigali* and *Peltophorum* groups (Caesalpinioideae). Presented as follows: **Taxon**: Collection Locality, *Voucher* (herbarium acronym), GenBank accession number * for [1]*trnL-trnF*, [2]*matK*, [3]*rps16*, [4]*trnD-trnT*, [5]ITS, [6]*tRALs*, [7]*AIGP*, [8]*EIF3E*, [9]*Leafy*. Multiple GenBank accession numbers for the same sample indicate multiple clones. Previously published sequences: Bruneau *et al.* (2001)[a], Bruneau *et al.* (2008)[b], Luckow, White & Bruneau (2000)[c], Haston *et al.* (2005)[d], Luckow *et al.* (2003)[e], Miller & Bayer (2001)[f], Särkinen *et al.* (2011)[g]. Vouchers that are underlined were selected for the reduced sampling in the concatenated matrices. *Tachigali guianense* is identified as *Sclerolobium guianense* Aubl., *Tachigali densiflora* is identified as *Sclerolobium densiflorum* Benth. and *Tachigali glauca* is identified as *Tachigali myrmecophila* (Ducke) Ducke in GenBank.

PELTOPHORUM GROUP

Colvillea racemosa Bojer ex Hook.: Madagascar, *Bruneau 1397* (MT), [2]*KF379241*, [3]KY320759, [4]*KY321137*, [5]*KF379221*, [6]KY320720, [7]KF378846, [8]*KY320888*, [8]*KY320889*, [8]*KY320890*, [8]*KY320892*, [9]*KY321019*, [9]*KY321020*, [9]*KY321022*, [9]*KY321023*. Madagascar, *Bruneau 1360* (MT), [2]KX874483, [3]KY320760, [4]*KY321136*, [5]*KY321090*, [6]KY320719, [7]KY320672, [8]*KY320886*, [8]*KY320887*, [8]*KY320891*, [9]*KY321014*, [9]*KY321015*, [9]*KY321016*. Madagascar, *Bruneau 1403* (MT), [2]KX874492, [3]KY320758, [4]*KY321138*, [5]*KY321091*, [6]KY320721, [7]KY320671, [8]*KY320893*, [8]*KY320894*, [8]*KY320895*, [9]*KY321017*, [9]*KY321018*, [9]*KY321021*, [9]*KY321024*. Madagascar, *Lewis 2147* (K), [1]AY899739[c], [2]EU361916[b], [3]AY899794[c].
Delonix baccal (Chiov.) Baker f.: Somalia, *Aronson 12 (*MO), [1]KY040021, [2]KX874488, [4]*KY321155*, [5]*KY321103*, [6]KY320736, [7]KY320691, [9]*KY320980*, [9]*KY320981*, [9]*KY320982*. Kenya, *Gillett 13717* (K), [3]AY899792[c].
Delonix boiviniana (Baill.) Capuron: Madagascar, *Bruneau 1365* (MT), [1]KY040046, [2]KF379239, [3]KF379270, [4]*KY321144*, [6]KF379361, [7]KF378853, [8]*KY320874*, [8]*KY320875*, [8]*KY320876*. Madagascar, *Bruneau 1381* (MT), [1]KY040032, [2]KY010677, [3]KY320770, [4]*KY321142*, [5]*KY321098*, [6]KY320722, [7]KY320673, [8]*KY320871*, [8]*KY320872*, [8]*KY320873*, [9]*KY320946*, [9]*KY320947*, [9]*KY320953*. Madagascar, *Bruneau 1357* (MT), [1]KY040031, [2]KY010678, [3]KY320768, [4]*KY321143*, [5]*KY321102*, [7]KY320674, [8]*KY320877*, [8]*KY320878*, [8]*KY320879*, [9]*KY320944*, [9]*KY320945*, [9]*KY320948*. Madagascar, *Bruneau 1378* (MT), [1]KY040035, [2]KY010679, [3]KY320769, [4]*KY321140*, [5]*KY321101*, [7]KY320675. Madagascar, *Bruneau 1401* (MT), [1]KY040033, [2]KY010680, [3]KY320767, [4]*KY321141*, [5]*KY321097*, [6] KY320723, [7]KY320676, [8]*KY320880*, [8]*KY320881*, [8]*KY320882*, [9]*KY320949*, [9]*KY320950*, [9]*KY320951*, [9]*KY320952*. Madagascar, *Andriamihajarivo 919* (MT), [1]KY040034, [2]KY010676, [3]KY320771, [4]*KY321139*, [5]*KY321100*, [6]KY320724, [7]KY320677, [8]*KY320841*, [8]*KY320883*, [8]*KY320884*, [8]KY320885, [9]*KY320938*, [9]*KY320939*, [9]*KY320940*, [9]*KY320941*, [9]*KY320942*, [9]*KY320943*. Madagascar, *DJ Du Puy M515* (P), [5]*KY321099*. **Delonix brachy-carpa** (R.Vig.) Capuron: Madagascar, *Phillipson 3081* (P), [1]KY040041, [3]AY899790[c], [4]*KY321145*, [5]*KY321095*. Madagascar, *Randriantafika 232* (P), [1]KY040042, [5]*KY321096*. Madagascar, *Rakotovao 5671* (P), [9]*KY320959*, [9]*KY320960*, [9]*KY320961*, [9]*KY320962*, [9]*KY320963*. Madagascar, *Randriamiera 7745* (P), [9]*KY320954*, [9]*KY320955*, [9]*KY320956*, [9]*KY320957*, [9]*KY320958*. **Delonix decaryi** (R.Vig.) Capuron: Madagascar, *Rivers MR37* (MO), [4]*KY321169*, [5]*KY321094*. Madagascar, *DJ & BP Du Puy M94* (MO), [3]KY320775, [4]*KY321168*, [7]KY320684. Madagascar, *DJ Du Puy M448* (P), [1]KY040020. Madagascar, *Capuron*

28520-SF (K), [1]KY040019, [5]*KY321093*. **Delonix elata** (L.) Gamble: Tanzania, *Herendeen 20-XII-97-1* (US), [1]KY040022, [4]*KY321154*, [5]*KY321105*, [6]KY320733, [7]KY320692, [8]*KY320860*, [8]*KY320862*, [8]*KY320866*, [8]*KY320867*, [9]*KY320970*, [9]*KY320971*, [9]*KY320972*. Tanzania, *Herendeen 17-XII-97-2* (US), [3]KY320774, [4]*KY321153*, [5]*KY321104*, [6]KY320734, [7]KY320696, [8]*KY320861*, [8]*KY320863*, [8]*KY320864*, [8]*KY320865*, [9]*KY320973*, [9]*KY320974*, [9]*KY320975*, [9]*KY320976*. Ethiopia, *Friis et al. 10280* (K), [1]KY040023, [2]KY010679, [4]*KY321152*, [6]KY320735, [9]*KY320977*, [9]*KY320978*, [9]*KY320979*. Yemen, *JRI Wood Y/74/449* (BM), [3]AY899787[c]. **Delonix floribunda** (Baill.) Capuron: Madagascar, *Bruneau 1393* (MT), [1]KY040047, [2]*KF379240*, [3]KY320777, [4]*KY321158*, [5]KF379224, [6]KF379359, [7]KF378850, [8]*KY320850*, [8]*KY320851*, [8]*KY320852*, [9]*KY321002*, [9]*KY321010*, [9]*KY321011*. Madagascar, *Bruneau 1398* (MT), [1]KY040036, [2]KX874489, [3]KY320779, [4]*KY321160*, [5]*KY321114*, [6]KY320729, [7]KY320686, [9]*KY321007*, [9]*KY321008*, [9]*KY321012*, [9]*KY321013*. Madagascar, *Bruneau 1405* (MT), [1]KY040037, [2]KY010684, [3]KY320780, [4]*KY321159*, [5]*KY321113*, [6]KY320730, [7]KY320688, [8]*KY320842*, [8]*KY320843*, [8]*KY320848*, [9]*KY321005*, [9]*KY321006*, [9]*KY321009*. Madagascar, *Bruneau 1409* (MT), [1]KY040038, [2]KX874490, [3]KY320778, [4]*KY321161*, [5]*KY321112*, [6] KY320718, [7]KY320687, [8]*KY320845*, [8]*KY320846*, [8]*KY320847*, [9]*KY321001*, [9]*KY321003*, [9]*KY321004*. **Delonix leucantha var gracilis** Du Puy, Phillipson & R.Rabev.: Madagascar, *DJ Du Puy M87* (MO), [5]*KY321107*. Madagascar, *McWhirter 237* (K), [1]KY040043. Madagascar, *Allorge 904* (P), [4]*KY321156*, [5]*KY321108*, [6]KY320726, [7]KY320685. **Delonix pumila** Du Puy, Phillipson & R.Rabev.: Madagascar, *Bruneau 1394* (MT), [1]KY040039, [2]KY010682, [3]KY320783, [4]*KY321164*, [5]*KY321111*, [6]KY320731, [7]KY320695, [8]*KY320844*, [8]*Y320849*, [8]*KY320853*. Madagascar, *Bruneau 1411* (MT), [1]KY040048, [2]*KF379237*, [3]KY320782, [4]*KY321163*, [5]*KF379225*, [6]KF379356, [7]KF378847, [8]*KY320854*, [8]*KY320857*, [8]*KY320858*, [9]*KY321035*, [9]*KY321036*, [9]*KY321037*. Madagascar, *Bruneau 1412* (MT), [1]KY040040, [2]KY010683, [3]KY320781, [4]*KY321162*, [5]*KY321110*, [6]KY320732, [7]KY320694, [8]*KY320855*, [8]*KY320856*, [8]*KY320859*. **Delonix regia** (Hook.) Raf.: Madagascar, *Bruneau 1320* (MT), [1]KY040024, [2]KY010675, [3]Y320773, [4]*KY321146*, [5]*KY321088*, [6]KY320740, [7]KY320682, [8]*KY320904*, [8]*KY320905*, [8]*KY320917*, [9]*KY320992*, [9]*KY320993*, [9]*KY320994*, [9]*KY320995*. Madagascar, *Bruneau 1355* (MT), [1]KY040025, [2]KX874484, [3]KY320766, [4]*KY321147*, [5]*KY321089*, [6]KY320738, [7]KY320683, [8]*KY320914*, [8]*KY320915*, [8]*KY320918*, [9]*KY320991*, [9]*KY320996*, [9]*KY320997*. Madagascar, *Bruneau s.n. A* (MT), [1]KY040026, [2]KX874485, [3]KY320762, [4]*KY321150*, [5]*KY321084*, [6] KY320737,

[7] KY320678, [8]*KY320909*, [8]*KY320910*, [8]*KY320911*. Madagascar, *Bruneau s.n. B* (MT), [1]KY040027, [2]KX874493, [3]KY320761, [4]*KY321151*, [5]*KY321085*, [6]KY320739, [7]KY320679, [8]*KY320906*, [8]*KY320907*, [8]*KY320908*. Madagascar, *Bruneau s.n. C* (MT), [1]KY040028, [2]KX874486, [3]KY320763, [4]*KY321149*, [5]*KY321086*, [7]KY320680. Mauritius, *Archambault 69* (MT), [1]KY040044, [2]*KF379238*, [3]KY320765, [4]*KY321148*, [5]*KF379222*, [6]KF379350, [7]KF378851, [8]*KY320903*, [8]*KY320912*, [8]*KY320913*, [8]*KY320916*, [9]*KY320998*, [9]*KY320999*, [9]*KY321000*. Madagascar, *T. Bradley 31902* (MT), [1]KY040029, [2]KX874487, [3]KY320764, [5]*KY321087*, [7]KY320681. **Delonix tomentosa** (R.Vig.) Capuron: Madagascar, *Perrier de la Bâthie 1325* (K), [1]KY040018, [3]KY320776, [4]*KY321157*, [5]*KY321106*, [6]KY320727, [7]KY320690, [9]*KY320967*, [9]*KY320968*, [9]*KY320969*. **Delonix velutina** Capuron: Madagascar, *Bruneau 1349* (MT), [1]KY040030, [2]KX874491, [3]KY320772, [4]*KY321165*, [5]*KY321109*, [6]KY320725, [7]KY320693, [8]*KY320896*, [8]*KY320897*, [8]*KY320898*, [9]*KY320984*, [9]*KY320985*, [9]*KY320986*. Madagascar, *Bruneau 1354* (MT), [1]KY040045, [2]*KF379236*, [3]KF379271, [4]*KY321166*, [5]*KF379220*, [6]KF379358, [7]KF378852, [8]*KY320869*, [8]*KY320870*, [8]*KY320899*, [9]*KY320983*, [9]*KY320987*, [9]*KY320988*, [9]*KY320989*, [9]*KY320990*.

Lemuropisum edule H.Perrier: Madagascar, *Du Puy M1033* (K), [1]*EU361818*[b], [4]*KY321167*, [5]*KY321092*, [6]KY320728, [7]KY320689, [8]*KY320868*, [8]*KY320900*, [8]*KY320901*, [8]*KY320902*, [9]*KY320964*, [9]*KY320965*, [9]*KY320966*. Madagascar, *Phillipson 3460* (K), [1]AF365070[a], [2]EU361991[b]. Madagascar, *T. Willings s.n.* (K), [3]AY899795[c].

PELTOPHORUM OUTGROUP

Bussea perrieri R.Vig: Madagascar, *Randrianasolo 527* (P), [1]*EU361757*[b], [2]*EU361896*[b], [4]*KY321132*, [6]KY320710, [7]KY320669, [8]*KY320795*, [8]*KY320796*, [8]*KY320797*. **Bussea sakalava** Du Puy & R.Rabev: Madagascar, *Du Puy M500* (NY), [5]*KY321081*. Madagascar, *R. Capuron 23.331_SF* (K), [1]AY899708[c], [3]AY899766[c]. **Conzattia multiflora** Robinson: Mexico, *Hughes 1815* (NY), [2]KX874494, [4]*KY321135*, [5]*KF379219*, [6]KY320717, [7]KF378848, [8]*KY320828*, [8]*KY320829*, [8]*KY320830*, [8]*KY320831*, [9]*KY321025*, [9]*KY321026*, [9]*KY321027*, [9]*KY321028*, [9]*KY321029*, [9]*KY321030*, [9]*KY321031*. Mexico, *Sahagun s.n.* (RNG), [1]AY899729[c], [2]AY386918[b], [3]AY899786[c]. **Parkinsonia aculeata** L.: New Mexico, *Spellenberg 12704* (MT), [1]AF365072[a], [2]EU362019[b], [4]*KY321134*, [5]*KF379226*, [6]KY320716, [7]KF378849, [8]*KY320825*, [8]*KY320826*, [8]*EKY320827*, [9]*KY321032*, [9]*KY321033*, [9]*KY321034*. Mexico, *Hawkins 94/5* (FHO), [1]AY899715[c], [3]AY899772[c]. **Peltophorum pterocarpum** (DC.)

Bayer ex K.Heyne: Tanzania, *Goyder 3719* (K), [2]EU362023[b], [6] KY320712. Tanzania, *Herendeen 12-XII-97-2* (US), [7]KY320662. *Grierson & Long 2884* (E), [1]AY899713[c], [3]AY899770[c]. **Peltophorum dubium var. dubium** (Spreng.) Taub.: Bolivia, *Hughes 2436* (K), [4]*KY321131*, [8]KY320785, [8]KY320786, [8]KY320787, [9]*KY321038*, [9]*KY321039*, [9]*KY321040* (3). Brazil, *Savassi et al. 1303*, [4]*KY321130*, [5]*KY321082*, [9]*KY321041*, [9]*KY321042*, [9]*KY321043*. Mexico, *Hughes 1685* (FHO), [1]AY904432[c], [2]AY386846[c], [3]AY899769[c].

Schizolobium parahyba (Vell.) Blake: South Africa, *Klitgaard 694* (K), [2]EU362036[b], [4]*KY321133*, [5]*KY321083*, [6]KY320715, [7]KY320670, [8]*KY320788*, [8]*KY320789*, [8]*KY320790*, [8]*KY320791*, [8]*KY320792*, [8]*KY320793*, [8]*KY320794*, [9]*KY321044*, [9]*KY321045*, [9]*KY321046*. Mexico, *Hughes 1880* (FHO), [1]AY899710[c], [3]AY899767[c].

TACHIGALI GROUP

Jacqueshuberia brevipes Barneby: Guyana, *Redden 1240* (US), [1]*EU361815*[b], [2]*EU361984*[b], [4]*KY321129*, [5]*KY321078*, [6]KY320700, [7]KY320666, [8]*KY320808*, [8]*KY320809*, [8]*KY320810*, [9]*KY321062*, [9]*KY321063*, [9]*KY321064*. **Jacqueshuberia loretensis** R.S.Cowan: Peru, *M Rimachi Y 9050* (NY), [3]AY899761[c]. **Tachigali guianense** (Benth.) Zarucchi & Herend.: South Africa, *Klitgaard 687* (K), [1]AF365111[a]. **Tachigali densiflora** (Benth.) L.F.Gomes da Silva & H.C.Lima: Brazil, *de Carvalho 4095* (K), [3]AY899763[c]. **Tachigali vasquezii** Pipoly: Ecuador, *Neill 13998* (K), [1]AF365113[a], [2]EU362054[b], [6]KY320709, [7]KY320667, [8]*KY320803*, [8]*KY320804*, [8]*KY320811*, [8]*KY320812*, [8]*KY320813*, [9]*KY321065*, [9]*KY321066*, [9]*KY321067*. **Tachigali amplifolia** (Ducke) Barneby: French Guyana, *S. Mori et al. 23868* (NY), [5]*KY321077*, [6]KY320708, [8]*KY320805*, [8]*KY320806*, [8]*KY320807*. **Tachigali paniculata** Aubl.: Guyana, *T.W Henkel 657* (NY), [6]KY320707, [7]KY320668, [9]*KY321057*, [9]*KY321058*, [9]*KY321059*, [9]*KY321060*, [9]*KY321061*. **Tachigali glauca** Tul.: Brazil, *R.S. Cowan 38220* (K), [1]AY899706[c], [3]AY899764[c].

DIMORPHANDRA GROUP

Campsiandra comosa Benth.: *T. Pennington 341* (K), [3]KY320755, [7]KY320652. Guyana, *Maas et al. 5431* (MO), [5]*KY321079*. Guyana, *Redden 1100* (US), [1]EU361780[b], [2]EU361908[b], [3]KY320754, [4]*KY321126*, [6]KY320714, [8]*KY320798*, [8]*KY320799*, [8]*KY3207800*, [8]*KY320801*.

Dinizia excelssa Ducke: Guyana, *Jansen-Jacob 1900* (NY), [3]KY320753, [5]*KY321076*, [6]KY320702, [9]*KY321053*, [9]*KY321054*, [9]*KY321055*, [9]*KY321056*. *Sergio de Faria*

sn (BH), [1]AF278479[d], [2]AF521827[e]. **Dinizia sp.** (taxon nov.): Brazil, *Folli 4889* (K), [1]EU361809[b], [2]EU361952[b], [3]KY320751, [4]KY321128, [7]KY320660, [8]*KY320838*, [8]*KY320839*, [8]*KY320840*, [9]*KY321050*, [9]*KY321051*, [9]*KY321052*. Brazil, <u>*Folli 4884*</u> (K), [1]EU361808[b], [2]EU361951[b], [3]KY320752, [4]*KY321127*, [5]*KY321075*, [6]KY320701, [7]KY320661, [9]*KY321047*, [9]*KY321048*, [9]*KY321049*. **Dimorphandra conjugata** Sandwith: Guyana, *Breteler 13800* (WAG), [1]AF365099[a], [2]EU361934[b], [3]KY320756, [4]*KY321123*, [6]KY320711, [7]*KY320663*, [8]*KY320802*, [8]*KY320832*, [8]*EKY320833*, [8]*KY320834*, [8]*KY320835*, [8]*KY320836*, [8]*KY320837*. **Diptychandra aurantiaca** Tul.: Brazil, *Klitgaard 70* (NY), [1]EU361799[b], [2]EU361935[b], [3]KY320741, [4]*KY321115*, [5]*KY321068*, [6]KY320697, [7]KY320650, [8]KY320784. **Erythrophleum suaveolens** (Guill. & Perr.) Brenan: Tanzania, <u>*Herendeen 11-XII-97-3*</u> (US), [1]AF365103[a], [2]EU361949[b], [4]*KY321117*, [6]KY320698, [8]*KY320821*, [8]*KY320822*, [8]*KY320823*, [8]*KY320824*, [8]*KY320820*. Tanzania, *Herendeen 11-XII-97-2* (US), [3]KY320743, [8]*KY320816*, [8]*KY320817*, [8]*KY320818*, [8]*KY320819*. **Erythrophleum ivorense** A. Chev.: Gabon, *Breteler 15446* (WAG), [6]KY320699, [7]KY320651. **Moldenhawera floribunda** Schroder.: Brazil, *Klitgaard 30* (K), [3]KY320742, [4]*KY321116*, [7]KY320664. **Moldenhawera brasiliensis** Yakovlev: Brazil, *Queiroz 5530* (K), [1]EU361824[b], [2]EU362004[b]. **Mora gonggrijpii** (Kleinh.) Sandwith: Guyana, *Breteler 13792* (WAG), [1]AF365104[a], [2]EU62005[b], [3]KY320757, [4]*KY321124*, [5]*KY321080*, [6]KY320713, [7]KY320665.

MIMOSOID CLADE

Vachellia caven (Molina) Seigler & Ebinger: Canada, *Montreal Botanical Garden no.386–89* (MT), [1]AF365041[a], [3]KY320745, [4]*KY321118*, [5]*KY321070*, [6]KY320705, [7]KY320654, [8]*KY320919*. Argentina, *CANB 615552* (DLEP), [2]AF274131[f]. **Calliandra inaequilatera** Rusby: Canada, <u>*Archambault sn*</u> (MT), [3]KY320747, [4]*KY321121*, [5]*KY321072*, [7]KY320659, [8]*KY320926*, [8]*KY320927*, [8]*KY320928*, [8]*KY320929*. **Calliandra trinervia** Benth. **var. carbonaria** (Benth.) Barneby: Ecuador, *Klitgaard 622* (K), [1]AF278516[a], [2]AF521815[e], [3]KY320746, [7]KY320658, [8]*KY320923*, [8]*KY320924*, [8]*KY320925*. **Inga sp**: Ecuador, *Klitgaard 677* (K), [1]AF365046[a], [2]EU361980[b], [3]KY320750, [4]*KY321122*, [5]*KY321073*, [6]KY320703, [7]KY320656, [8]*KY320935*, [8]*KY320936*, [8]*KY320937*. **Mimosa quitensis** Benth.: Ecuador, *Klitgaard 647* (K), [1]AF278514[a], [2]AF521844[e], [3]KY320748, [5]*KY321071*, [7]KY320655, [8]*KY320920*, [8]*KY320921*, [8]*KY320922*. *Lewis 2856* (K), [4]HM353116[g]. **Parkia multijuga** Benth.: South Africa, *Klitgaard 697* (K), [1]AF365050[a], [2]EU362018[b], [3]KY320749, [4]*KY321119*, [5]*KY321069*, [6]KY320704, [7]KY320657, [8]*KY320930*, [8]*KY320931*, [8]*KY320932*, [8]*KY320933*, [8]*KY320934*. **Pentaclethra macrophylla** Benth.: Gabon, *Wilde & Maessen 11496* (WAG), [1]AF278485[a], [3]KY320744, [4]*KY321120*, [5]*KY321074*, [6]KY320706, [7]KY320653. Congo, *BNBG no. 87–1143* (BR), [2]AF521853[e].