Filling in the gaps of the papilionoid legume phylogeny: The enigmatic Amazonian genus *Petaladenium* is a new branch of the early-diverging Amburaneae clade

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ABSTRACT

Recent deep-level phylogenies of the basal papilionoid legumes (Leguminosae, Papilionoideae) have resolved many clades, yet left the phylogenetic placement of several genera unassessed. The phylogenetically enigmatic Amazonian monospecific genus *Petaladenium* had been believed to be close to the genera of the Genistoid Ormosieae clade. In this paper we provide the first DNA phylogenetic study of *Petaladenium* and show it is not part of the large Genistoid clade, but is a new branch of the Amburaneae clade, one of the first-diverging lineages of the Papilionoideae phylogeny. This result is supported by the chemical observation that the quinolizidine alkaloids, a chemical synapomorphy of the Genistoids, are absent in *Petaladenium*. Parsimony and Bayesian phylogenetic analysis of nuclear ITS/5.8S and plastid *matK* and *trnL* intron agree with a new interpretation of morphology that *Petaladenium* is sister to *Dussia*, a genus comprising ∼18 species of trees largely confined to rainforests in Central America and northern South America. *Petaladenium*, *Dussia*, and *Myrospermum* have papilionate flowers in a clade otherwise with radial floral symmetry, loss of petals or incompletely differentiated petals. Our phylogenetic analyses also revealed well-supported resolution within the three main lineages of the ADA clade (Angylocalyceae, Dipterygeae, and Amburaneae). We also discuss further molecular phylogenetic evidence for the undersampled Amazonian genera *Aldina* and *Monopteryx*, and the tropical African *Amphimas*, *Cordyla*, *Leucomphalos*, and *Mildbraediodendron*.

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1. Introduction

The papilionoid legumes (Papilionoideae, Leguminosae) are among the most diverse and ecologically successful plant radiations (LPWG, 2013a), with a diversification history stemming from the Early Cenozoic (Lavin et al., 2005; Schrire et al., 2005). Their spectacular diversity, comprising ∼14,000 species and ∼484 genera (Lewis et al., 2005, 2013), is also expressed in terms of floral morphology, which includes not only the more familiar highly-differentiated papilionate flowers of the economically important soybean [*Glycine max* (L.) Merr], pea (*Pisum sativum* L.), and chickpea (*Cicer arietinum* L.), but also examples of petal loss, undifferentiated petals, numerous free stamens, closed calyx, and radial symmetry (Leite et al., 2015; Mansano et al., 2002; McMahon and Hufford, 2005; Paulino et al., 2013; Pennington et al., 2000; Tucker, 1990, 1993, 2002, 2003). Such great floral disparity is particularly common among the early-branching papilionoid genera that are traditionally placed in the broadly polyphyletic tribes Sophoreae and Swartzieae. Understanding the phylogenetic relationships among the early-diverging clades of Papilionoideae is key to revealing how the many instances of non-papilionate flow-

ers evolved or deviated from the truly papilionate flowers (Cardoso et al., 2012a, 2013a; Pennington et al., 2000, 2001). The evolutionary history of early-branching papilionoids is also critical to the understanding of biome-level biogeographical diversification in legumes (Koenen et al., 2013; Oliveira-Filho et al., 2013; Schrire et al., 2005) and the origin of their complex symbiotic association with root-nodulating bacteria (Doyle, 2011; Doyle et al., 1997), and it is essential for the production of a stable phylogenetic classification (LPWG, 2013a, 2013b).

The recent years have seen an increased phylogenetic resolution within the early-branching clades of Papilionoideae (Cardoso et al., 2012a, 2012b, 2012c, 2013a, 2013b; Lavin et al., 2001; McMahon and Hufford, 2004; Meireles et al., 2014; Pennington et al., 2001; Torke and Schaal, 2008; Wojciechowski et al., 2004). To arrive at a robust estimate of the legume phylogeny, however, requires improved taxon sampling, especially with respect to unresolved or phylogenetically enigmatic genera (LPWG, 2013a). A review of the legume systematics undertaken by the recently established Legume Phylogeny Working Group (LPWG, 2013a) revealed that 51 Papilionoideae genera still remain unplaced or without molecular phylogenetic hypothesis. Although we can use morphology to tentatively place most of these genera in some major branch of the papilionoid tree, molecular data may reveal unexpected phylogenetic positions (e.g., Cardoso et al., 2012a, 2012b, 2012c, 2013c). Resolution of the phylogenetic relationships of these 51 genera will expedite the ongoing clade-based classification of the legume family (LPWG, 2013a).

The focus of this study is to fill in the gaps of the early-branching clades of the Papilionoideae phylogeny. Recent field work in remote areas of Brazilian Amazonia was successful in targeting some poorly-known papilionoid genera (Cardoso et al., in press). Among these is the monospecific genus *Petaladenium* Ducke, which had remained uncollected since the 1980s. It is unique among the whole legume family in having fimbriate-urceolate-glandular wing petals. The papilionate flowers with subequal calyx lobes, pinkish to lilac petals, and nearly free stamens have suggested a relationship of *Petaladenium* with the genistoid genera *Clathrotropis* Harms and *Ormosia* Jacks. (Ducke, 1938; Pennington et al., 2005). If *Petaladenium* is closely related to the genera of the *Ormosia* clade, then it would be expected to accumulate quinolizidine alkaloids, an unequivocal chemical synapomorphy of the Genistoid clade (LPWG, 2013a; Kite et al., 2013; Ricker et al., 1994, 1999; Van Wyk, 2003; Wink, 2013; Wink and Mohamed, 2003).

Here we evaluate the genus *Petaladenium* for the first time in a molecular phylogenetic analysis of the Papilionoideae. New chemical and morphological evidence for the genus are also assessed in light of the phylogeny. We show that *Petaladenium* is not closely related to the Genistoids, but that it unexpectedly diverged much earlier in the Papilionoideae phylogeny. Our phylogenetic analyses of the Sophoreae and Swartzieae (Cardoso et al., 2012a, 2013a; Pennington et al., 2001) left the placement and monophyly of some genera unanswered. The *matK* phylogeny presented in this study is more comprehensive with respect to the previously undersampled papilionate-flowered *Dussia* Krug & Urb. ex Taub., radially-symmetrical-flowered *Aldina* Endl., *Amphimas* Pierre ex Dalla Torre & Harms, *Cordyla* Lour., *Leucomphalos* Benth. ex Planch., *Mildbraediodendron* Harms, and *Xanthocercis* Baill., as well as the florally divergent *Monopteryx* Spruce ex Benth., a putative branch of the Dipterygeae clade (Cardoso et al., 2012a). Furthermore, we analyze combined data from three nuclear and plastid DNA genes in an attempt to add more resolution among the poorly-supported nodes of the earliest diverging ADA clade (Angylocalyceae, Dipterygeae, and Amburaneae) as well as to evaluate the sister relationships of *Petaladenium*.

2. Materials and methods

2.1. Taxon sampling and molecular data

Our study involved two sampling strategies. Because *Petaladenium* has never been evaluated in a molecular phylogeny of the Papilionoideae, the first dataset involved a dense sampling of plastid *matK* protein-coding gene sequences (Hilu and Liang, 1997). This gene has been widely used to resolve relationships across the entire Papilionoideae (e.g., Bruneau et al., 2008; Cardoso et al., 2012a, 2013a; Delgado-Salinas et al., 2011; Hu et al., 2000; Lavin et al., 2001; McMahon and Hufford, 2004; Pennington et al., 2010; Steele and Wojciechowski, 2003; Wojciechowski et al., 2004). The strategy here was to aim at sampling as many genera as possible across all major lineages of the Papilionoideae as guided by the previous phylogenies (Cardoso et al., 2012a, 2013a; Wojciechowski et al., 2004). When possible, sampling of early-branching, monospecific genera included multiple conspecific accessions in an effort to capture their morphological and geographic variation. Our *matK* data set includes 911 accessions, of which 737 are from Papilionoideae, representing 693 species and 332 of the 483 currently recognized genera within the subfamily (Lewis et al., 2005, 2013). The early-branching clades accounted for 454 accessions from 412 species and 153 genera. This sampling strategy permitted us to address with confidence the phylogenetic position of the enigmatic *Petaladenium* in the context of the Papilionoideae phylogeny. We generated new sequences not only for *Petaladenium*, but also for *Aldina*, *Dussia*, and *Monopteryx* from Central American and northern South American rain forests, as well as within *Amphimas*, *Cordyla*, *Leucomphalos*, *Mildbraediodendron*, and *Xanthocercis* from tropical Africa, all of which were under-represented or phylogenetically unresolved in previous studies. Nearly all genera (52 out of 62) of the traditionally circumscribed tribes Sophoreae and Swartzieae have been sampled, except for representatives of *Ammothamnus* Bunge, *Dalhousiea* Wall. ex Benth., *Fairchildia* Britton & Rose, *Haplormosia* Harms, *Neoharmsia* R. Vig., *Pericopsis* Thwaites, *Platycelyphium* Harms, *Sakoanala* R. Vig., *Salweenia* Baker, and *Uleanthus* Harms. Nevertheless, we can tentatively place most of these genera in some major branch of the papilionoid tree based on different DNA sequence data, chemistry or morphology (e.g., Cardoso et al., 2012a, 2013a; Edwards and Hawkins, 2007; Kajita et al., 2001; Pennington et al., 2001; Torke and Schaal, 2008). Most sequences used to build the *matK* data set are from our ongoing phylogenetic studies on the early-branching papilionoids (Cardoso et al., 2012a, 2012b, 2013a, 2013b; Queiroz et al., 2010; Winterton et al., 2014). Significant efforts of others added considerably to our broad taxon coverage (e.g., Delgado-Salinas et al., 2011; Egan and Crandall, 2008; Hu et al., 2000; Lavin et al., 2001, 2003; McMahon and Hufford, 2004; Sirichamorn et al., 2012; Steele and Wojciechowski, 2003; Wojciechowski, 2013; Wojciechowski et al., 2004). Outgroup sampling was guided by Bruneau et al. (2008) and included representatives from within different caesalpinioid and mimosoid clades.

The second set of analyses involved a densely-sampled multilocus phylogenetic approach to resolve the relationships and examine the generic limits of *Dussia*, *Petaladenium*, and *Monopteryx* within the basally divergent ADA clade (Cardoso et al., 2012a, 2013a). We performed a combined phylogenetic analysis of plastid (*matK* and *trnL* intron) and nuclear ribosomal (ITS/5.8S) DNA sequences from a sampling of 58 accessions. These represent 52 species across the broad morphological range of all genera currently recognized in the three lineages of the ADA clade. Representative sequences from the Swartzioids were used as outgroups. Most sequences used to build these three data sets are also from our recent phylogenetic studies (Cardoso et al.,

2012a; Winterton et al., 2014). The combined data set also involved some missing data, but these account for only ~11% of the matrix. Wiens (2003, 2006) showed no negative impact of including incompletely sampled taxa in a combined analysis, demonstrating that when accessions sampled for ⩽50% of the data are included they can break up long branches and improve phylogenetic accuracy (Wiens, 2005).

2.2. Molecular laboratory analyses

DNA isolations from silica-gel dried leaf material, polymerase chain reaction (PCR) amplifications, and template purifications were performed with Qiagen Kits (i.e., Qiagen, Santa Clarita, California, USA). For some samples DNA was isolated using a modified version of the $2\times$ CTAB procedure (Doyle and Doyle, 1987).

Amplification primers, sequencing primers, and reaction conditions for *matK* were described in Wojciechowski et al. (2004). The universal forward primer C (5′-CGA AAT CGG TAG ACG CTA CG-3′) was used with the reverse primer D (5′-GGG GAT AGA GGG ACT TGA AC-3′) to amplify the *trnL* intron (Taberlet et al., 1991). PCR conditions for the *trnL* intron included a 3-min denaturing step at 94 °C, followed by 40 cycles of 1 min at 94 °C (denaturation), 30 s at 50 °C (annealing), 1 min at 72 °C (extension), and further extension for 10 min at 72 °C. The forward primer 17SE (5′-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3′) was used with the reverse primer 26SE (5′-TAG AAT TCC CCG GTT CGC TCG CCG TTA C-3′) to amplify the ITS region (Sun et al., 1994). PCR involved a 5 min denaturing step at 94 °C, followed by 28–30 cycles of 1 min at 94 °C (denaturation); 1 min at 50–52 °C (annealing); 3 min at 72 °C (extension) and further extension for 7 min at 72 °C. Amplified PCR products were purified using Qiagen Kit or 20% solution of polyethylene glycol (PEG) 6000 macrogol. The same set of primers used for the PCR were also used for sequencing, except for the ITS region that was sequenced with the primers 92 (5′-AAG GTT TCC GTA GGT GAA C-3′) (Desfeux and Lejeune, 1996) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al., 1990). Sequencing reactions in both directions were performed using BigDye Terminator kit (v.3.1; Applied Biosystems/Life Technologies Corporation, Carlsbad, California, USA). The products of sequencing were analyzed on a sequencer ABI3730XL (Applied Biosystems) following the manufacture's protocol at Universidade Estadual de Feira de Santana, Bahia, Brazil, or at Edinburgh Genomics, Edinburgh, Scotland.

GenBank accession numbers and voucher details of the 89 sequences newly generated for this study are reported in Table 1. All sequences obtained from other studies through GenBank are reported with their associated accession numbers immediately beside the taxon names in the original alignments or the large *matK* phylogenetic tree that are available as online Supplementary data.

2.3. Alignment and phylogenetic analyses

Forward and reverse reads of the chromatograms were assembled with Sequencher v.4.7 (Gene Codes, Ann Arbor, Michigan, USA) or Staden Package (Staden et al., 1998). Sequences were aligned manually with the program Se–Al (Rambaut, 1996) using the similarity criterion of Kelchner (2000) and Simmons (2004) in order to avoid inconsistencies derived from automated multiple alignments.

The combined data set was subject to parsimony analysis in PAUP* version 4.0b10 (Swofford, 2002) involving the standard approaches that maximized the detection of global optima and clade stability. A first heuristic search performed 10,000 random addition sequence replicates with tree bisection-reconnection (TBR) branch-swapping and retention of up to 15 most parsimonious trees at each replicate. The trees saved from this first search

became the starting trees for the second search, which implemented TBR and saved a maximum of 10,000 trees. All character state transformations were weighted equally and unordered (Fitch, 1971). Clade support was estimated with nonparametric bootstrap resampling (Felsenstein, 1985) as implemented in PAUP*, where 5000 bootstrap replicates were each analyzed using the heuristic search parameters mentioned above but with one random addition and one tree retained per replicate.

The Bayesian analysis (Lewis, 2001) was performed in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). The best-fitting nucleotide substitution model for each partition was selected via the Akaike information criterion (AIC) (Akaike, 1974) as implemented in the program MrModeltest 2.0 (Nylander, 2004). The General Time Reversible (GTR) model with gamma-distributed among-site rate heterogeneity (G) and invariant sites was selected for the ITS and the large *matK* data sets, whereas the model GTR + G, but without invariant sites, was selected for the small *matK* and *trnL* intron data sets. Two separate runs of a Metropolis-coupled Markov Chain Monte Carlo (MCMC) permutation of parameters were each initiated with a random tree and eight simultaneous chains set at default temperatures and trees sampled every 10,000th generation (Huelsenbeck et al., 2001). The program Tracer version 1.3 (Rambaut and Drummond, 2004) was used to assess the convergence of the MCMC run and the adequacy of the burn-in length. For the large *matK* data set Markov chains were run for 7.39×10^6 generations such that 1108 nonautocorrelated Bayesian trees were sampled from likelihood stationarity for the two runs after the burn-in. For the combined data sets we subjected the Markov chains for 5×10^6 generations, which produced an after burn-in set of 752 nonautocorrelated Bayesian trees. We used MrBayes version 3.1.2 to summarize trees sampled from post burn-in generations in a 50% majority-rule consensus tree that included posterior probabilities as branch support estimates. The Bayesian majority-rule consensus tree was visualized and partially edited for graphical presentation using FigTree version 1.4.0 (Rambaut, 2012).

We examined the tree topologies from the separate plastid and nuclear parsimony analyses to assess putative incongruence. The parsimony-based partition homogeneity test (incongruence length difference test; Farris et al., 1994) was not used here, because its results have repeatedly been shown to be misleading (Barker and Lutzoni, 2002; Dolphin et al., 2000; Yoder et al., 2001). The combinability of DNA markers was assessed by comparing clade support between individual data partitions (Wiens, 1998). Because Bayesian posterior probability values are often biased high (Alfaro et al., 2003; Erixon et al., 2003; Suzuki et al., 2002), we used the more conservative parsimony bootstrap supports to identify clade conflict between the DNA partitions. Incongruent clades with bootstrap supports >80% were taken as evidence for not combining data sets. We did not find any evidence of strong conflict between the individual data partitions; hence they were combined in the concatenated phylogenetic analysis.

2.4. Chemical analysis of Petaladenium

Leaves of *Petaladenium* were screened for the presence of quinolizidine alkaloids using protocols described previously (Kite et al., 2013). Briefly, 100 mg of dry leaf of specimen *D. Cardoso* et al. *3345* (HUEFS) was ground to a powder in a pestle and mortar and extracted in 1 ml of methanol overnight. Following removal of the solid matter by centrifugation, a general screen of metabolites was first undertaken by liquid chromatography–mass spectrometry (LC–MS) using a reverse phase C18 chromatography column and a mobile phase gradient of acidified aqueous methanol. The extract was then dried and subjected to a standard method of alkaloid preparation: namely partitioning between water and dichloromethane

Table 1

DNA sequences newly generated for this study. Voucher specimen information, including collection locality, voucher collector and number, and herbarium acronym are provided.

Species	Voucher details, herbarium	Country, locality	GenBank ITS	GenBank matK	GenBank trnL intron
Aldina discolor Spruce ex Benth.	*D. Cardoso et al. 3391* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira		KP177936	
Aldina heterophylla Benth.	*D. Cardoso et al. 3288* (HUEFS)	Brazil, Amazonas, Manaus		KP177923	
Aldina kunhardtiana R.S. Cowan	*D. Cardoso et al. 3300* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira		KP177925	
Aldina latifolia Benth.	*D. Cardoso et al. 3438* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira		KP177924	
Amphimas ferrugineus Pierre ex Harms	*F.J. Breteler 15369* (WAG)	Gabon, Ogooué-Lolo, Bambidie		KP177922	
Amphimas pterocarpoides Harms	*C.C.H. Jongkind 7285* (WAG)	Liberia, Grand Gedeh, Grebo Forest		KP177921	
Baphiastrum brachycarpum Harms	*F.J. Breteler 12331* (WAG)	Gabon, Ogooué-Lolo, c. 34 km Kessipoughou-Lifouta Gare		KP177931	
Bocoa viridiflora (Ducke) R.S. Cowan	*D. Cardoso et al. 3203* (HUEFS)	Brazil, Amazonas, Rio Preto da Eva		KP177918	
Bocoa viridiflora (Ducke) R.S. Cowan	*M.A.D. Souza et al. 93* (INPA)	Brazil, Amazonas, Manaus		KP177891	
Bowringia mildbraedii Harms	*F.J. Badré 253* (WAG)	Central African Republic, Carnot		KP177930	
Cordyla africana Lour.	*D. Cardoso et al. 3275* (HUEFS)	Cultivated at Lowveld Botanical Garden, Nelspruit, South Africa		KP177913	
Cordyla densiflora Milne-Redh.	*E.B. Mhoro 1211* (WAG)	Tanzania, Iringa, Iringa Rural District, Mtera		KP177910	KP177941
Cordyla madagascariensis Viguier	*R. Randrianaivo 1180* (K)	Madagascar, Toliara, Ankilimalinika, village plus proche Ranobe	KP177952		
Cordyla somalensis J.B. Gillett	*A.A. Elmi 4019* (WAG)	Somalia, Hiiraan, c. 10 km from BulaBurti to Ceelbuur		KP177911	KP177942
Cordyla somalensis J.B. Gillett	*J.B. Gillett 24606* (WAG)	Somalia, camp site 18 km NE of El Dhere on road from Aden Yabal		KP177912	KP177943
Dalbergia sp.	*L.P. de Queiroz et al. 15784* (HUEFS)	Brazil, Amazonas, Tabatinga, Belém do Solimões		KP177932	
Dussia atropurpurea N. Zamora, R.T. Penn. & C.H. Stirt.	*R.T. Pennington 620* (E)	Costa Rica, Guanacaste, Canton de Upala	KJ813640	KP177908	KJ813675
Dussia discolor (Benth.) Amshoff	*S.A. Mori et al. 24728* (NY)	French Guiana, Inini, Saül and vicinity	KJ813619	KP177909	KJ813654
Dussia lehmannii Harms	*R.T. Pennington 550* (E)	Ecuador, Pichincha, Primary forest reserve in land of Fundacion Florestal Durini at Rio Pitzara	KJ813628	KP177903	KJ813663
Dussia macroprophyllata Harms	*R.T. Pennington 606* (E)	Costa Rica, Guanacaste, Estación San Ramon	KJ813637	KP177906	KJ813672
Dussia macroprophyllata Harms	*R.T. Pennington 601* (E)	Costa Rica, Puntarenas, Monteverde	KJ813636	KP177907	KJ813671
Dussia tessmannii Harms	*R.T. Pennington 516* (E)	Ecuador, Napo, Cantón Tena, near Estación Biológica Jatun Sacha	KJ813621	KP177905	KJ813656
Dussia sp. nov. A	*R.T. Pennington 569* (E)	Costa Rica, Puntarenas, Osa Peninsula, Cantón de Osa, Playa Chal	KJ813631	KP177897	KJ813666
Dussia sp. nov. B	*R.T. Pennington 574* (E)	Costa Rica, Puntarenas, Osa Peninsula, Cantón de Osa, Cerro Chocuaco	KJ813633	KP177898	KJ813668
Dussia sp. nov. C	*R.T. Pennington 704* (E)	Colombia, Antioquia, Road La Union to Mesopotamia	KJ813647	KP177899	KJ813682
Dussia sp. nov. D	*R.T. Pennington 544* (E)	Ecuador, Los Rios	KJ813626	KP177900	KJ813661
Dussia sp. nov. E	*R.T. Pennington 538* (E)	Ecuador, Napo, Cantón Orellana, Parque Nacional Yasuní	KJ813625	KP177901	KJ813660
Dussia sp. nov. F	*R.T. Pennington 559* (E)	Ecuador, Carchi	KJ813630	KP177902	KJ813665
Dussia sp.	*R.T. Pennington 685* (E)	Colombia, Antioquia, Autopista Medellín-Bogotá	KJ813645	KP177904	KJ813680
Leucomphalos capparideus Benth. ex Planch.	*M.S.M. Sosef 1933* (WAG)	Gabon, Woleu-Ntem, c. 70 km NE of Mitzic		KP177933	
Leucomphalos capparideus Benth. ex Planch.	*J.J. Wieringa 5213* (WAG)	Gabon, Ngounié		KP177934	
Leucomphalos libericus Breteler	*C.C.H. Jongkind 4751* (WAG)	Ivory Coast, San-Pédro, Forêt Classée de Monogaga		KP177935	
Mildbraediodendron excelsum Harms	*R. Letouzey 5413* (WAG)	Cameroon, à 2 km à l'Ouest de Masea		KP177914	KP177944
Monopteryx uaucu Spruce ex Benth.	*D. Cardoso et al. 3302* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira	KP177953	KP177915	KP177945
Monopteryx uaucu Spruce ex Benth.	*D. Cardoso et al. 3306* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira	KP177954	KP177916	KP177946
Monopteryx uaucu Spruce ex Benth.	*D. Cardoso et al. 3347* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira	KP177955	KP177917	KP177947
Muellera campestris (Mart. ex Benth.) M.J. Silva & A.M.G. Azevedo	*D. Cardoso 2320* (HUEFS)	Brazil, Bahia, Feira de Santana, Distrito de Ipuaçu, Monte Alto		KP177929	
Paramachaerium ormosioides (Ducke) Ducke	*L.P. de Queiroz et al. 15782* (HUEFS)	Brazil, Amazonas, Tabatinga, Belém do Solimões		KP177927	
Petaladenium urceoliferum Ducke	*D. Cardoso et al. 3345* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira	KP177949	KP177894	KP177938
Petaladenium urceoliferum Ducke	*D. Cardoso et al. 3352* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira	KP177951	KP177895	KP177939
Petaladenium urceoliferum Ducke	*D. Cardoso et al. 3406* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira	KP177950	KP177896	KP177940
Pterocarpus monophyllus Klitg., L.P. Queiroz & G.P. Lewis	*D. Cardoso et al. 2945* (HUEFS)	Brazil, Bahia, Barra, Distrito de Iibiraba		KP177928	
Vatairea erythrocarpa Ducke	*D. Cardoso et al. 3375* (HUEFS)	Brazil, Amazonas, São Gabriel da Cachoeira		KP177926	
Xanthocercis rabiensis Maesen	*J.J. Wieringa 2780* (WAG)	Gabon, Ogooué-Maritime, Rabi	KP177948	KP177892	KP177937
Xanthocercis zambesiaca (Baker) Dumaz-le-Grand	*D. Cardoso et al. 3276* (HUEFS)	Cultivated at Lowveld Botanical Garden, Nelspruit, South Africa		KP177893	

under acidic then basic conditions. The alkaloid preparation was examined by gas chromatography–mass spectrometry (GC–MS) using a non-polar (BP-1) column. Full details of these sample preparation methods and LC–MS and GC–MS analytical conditions can be found in Kite et al. (2013).

3. Results

3.1. Phylogenetic analysis of separate data

The Bayesian analysis of the large *matK* data set did not resolve *Petaladenium* as closely related to any genus of the Genistoid clade. Rather, *Petaladenium* was placed amongst the early branches of the Papilionoideae phylogeny, where it is well supported as sister to the genus *Dussia* in the Amburaneae clade (Fig. 1; Appendix S1). The monospecific *Mildbraediodendron* is also resolved within the Amburaneae clade, but its phylogenetic placement is not clearly resolved with respect to *Cordyla*. A monophyletic *Monopteryx* is strongly supported as sister to the remaining genera traditionally circumscribed within the tribe Dipterygeae. The monophyly of *Aldina* and *Amphimas* is supported, but their relationships with respect to the main lineages of the 50-kb inversion clade still remain unclear. *Leucomphalos* is resolved within the Baphioid clade (Appendix S1).

3.2. Phylogenetic analysis of combined data

The ITS sequences of *Petaladenium* were readily aligned with representative sequences from the earliest-diverging papilionoids and much less so with sequences from the Ormosieae clade or with any other genistoid genus. The combined parsimony analysis of nuclear ITS and plastid *matK* and *trnL* intron sequences focusing on just the earliest-diverging papilionoid ADA clade yielded 10,000 equally parsimonious trees of length = 2076, CI = 0.605, and RI = 0.803. The Bayesian analysis recovered more well-resolved relationships than the parsimony analysis (Fig. 2). The backbone of the ADA clade is resolved and shows strongly supported interrelationships among the three main Angylocalyceae, Dipterygeae, and Amburaneae clades. *Petaladenium* is resolved as sister to *Dussia* with strong support in a clade that is sister to the remaining genera of the Amburaneae clade. Both *Petaladenium* and *Dussia* are separated by long stem branches, suggesting that they have long diverged from their most recent common ancestor. The monophyly and relationships of all Dipterygeae genera are strongly supported, and *Monopteryx* appears as sister to the remaining genera of the tribe. Again, *Mildbraediodendron* appears unresolved in a polytomy with the species of *Cordyla*. This clade including only African and Madagascan species is well supported as sister to the South American genus *Amburana*.

3.3. Chemistry profile of Petaladenium

No candidates for quinolizidine alkaloids could be detected in the leaf extract of *Petaladenium*, either from LC–MS analysis of the crude methanol extract, or GC–MS analysis of an alkaloid preparation from it. Rather, the LC–MS analyses revealed a major unretained chromatographic peak due to one or more compounds with the molecular formula $C_7H_{13}NO_4$ (determined from accurate mass measurement). This formula was suggestive of a non-protein amino acid – there being insufficient carbon atoms for a quinolizidine alkaloid. Further characterization of this chromatographic peak revealed that it was due to a mixture of novel isomeric non-protein amino acids that will be described elsewhere (Kite et al., unpublished results).

4. Discussion

4.1. Molecular evolution among the first-diverging papilionoids

Molecular phylogenetic analyses aimed at resolving family-wide relationships in legumes have successfully used the complete plastid *matK* protein-coding gene because of the excellent resolution it provides (Bruneau et al., 2008; Cardoso et al., 2012a, 2013a; Lavin et al., 2001; Wojciechowski et al., 2004) when compared to other commonly used markers, such as *rbcL* or the *trnL* intron (Bruneau et al., 2001; Doyle et al., 1997; Kajita et al., 2001; Pennington et al., 2001). This increased resolution reflects levels of sequence divergence up to ninefold higher for *matK* than for *rbcL*, and substitutions that are distributed more uniformly among the three codon positions in *matK* (Lavin et al., 2005). Although the *matK* gene alone has provided many new insights in legumes, we are aware that a single gene might not resolve entirely the deep-branching history of papilionoids. Most lineages within the ADA clade and the 50-kb inversion clade have always appeared unresolved or only poorly supported in phylogenetic analyses of *matK* (Fig. 1; Cardoso et al., 2012a, 2013a). Likewise, the morphologically well-defined neotropical genera of the Swartzioid and Lecointeoid clades are not resolved as monophyletic in *matK* phylogenies (Appendix S1; Cardoso et al., 2012a, 2013a). The poor support that we have seen in some specific branches of the Papilionoideae phylogeny is likely to be caused by weak phylogenetic signal because of the short branch lengths for most internodes. The relatively short branches in the early-divergence of the papilionoids may be a phylogenetic signature of an explosive early radiation (Rokas and Carroll, 2006; Rokas et al., 2005). This explanation could account for the lack of resolution or low support values within the ADA clade, Swartzioids, Lecointeoids or inside the 50-kb inversion clade (Fig. 1; Appendix S1; Cardoso et al., 2013a). In order to confidently resolve such papilionoid branches, perhaps the sequencing of the low copy nuclear gene sucrose synthase should be helpful as they have been used to resolve the early-branching caesalpinioid legume radiations (Manzanilla and Bruneau, 2012).

4.2. Petaladenium in the Papilionoideae phylogenetic tree

Petaladenium was first described by Ducke (1938) to accommodate a single tree species, *Petaladenium urceoliferum* Ducke, which is confined to terra-firme rain forests from the upper Rio Negro in the Brazilian Amazonia. *Petaladenium* is unique in Papilionoideae because of its markedly distinctive flowers with fimbriate-urceolate-glandular wing petals (Fig. 3). The generic name and specific epithet derive from this feature and refer to the concave gland-like structures along the margins of the wing petals. The genus remained poorly represented in herbaria for 75 years until collections became available in 2013 (Cardoso et al., in press). These recent collections provided the opportunity to evaluate *Petaladenium* in a molecular phylogenetic analysis for the first time.

In previous morphology-based classifications, *Petaladenium* was placed in the morphologically diverse, yet polyphyletic tribe Sophoreae (Pennington et al., 2005; Polhill, 1981a, 1994), because of its nearly free stamens. Others (Cardoso et al., 2012a, 2013a; Ducke, 1938; Pennington et al., 2005) had pointed out the affinity of *Petaladenium* with *Clathrotropis* Harms and *Ormosia* Jacks. on the basis of overall vegetative and inflorescence morphology, and the papilionate flowers with white–pinkish to light lilac petals and basally fused stamens, whereas an affinity with *Panurea* Spruce ex Benth. & Hook.f. and *Spirotropis* Tul. was suggested from the linear-oblong elastically dehiscent pods (Cardoso et al., 2012a). Recently, *Clathrotropis*, *Panurea*, and *Spirotropis* have been discovered to be part of
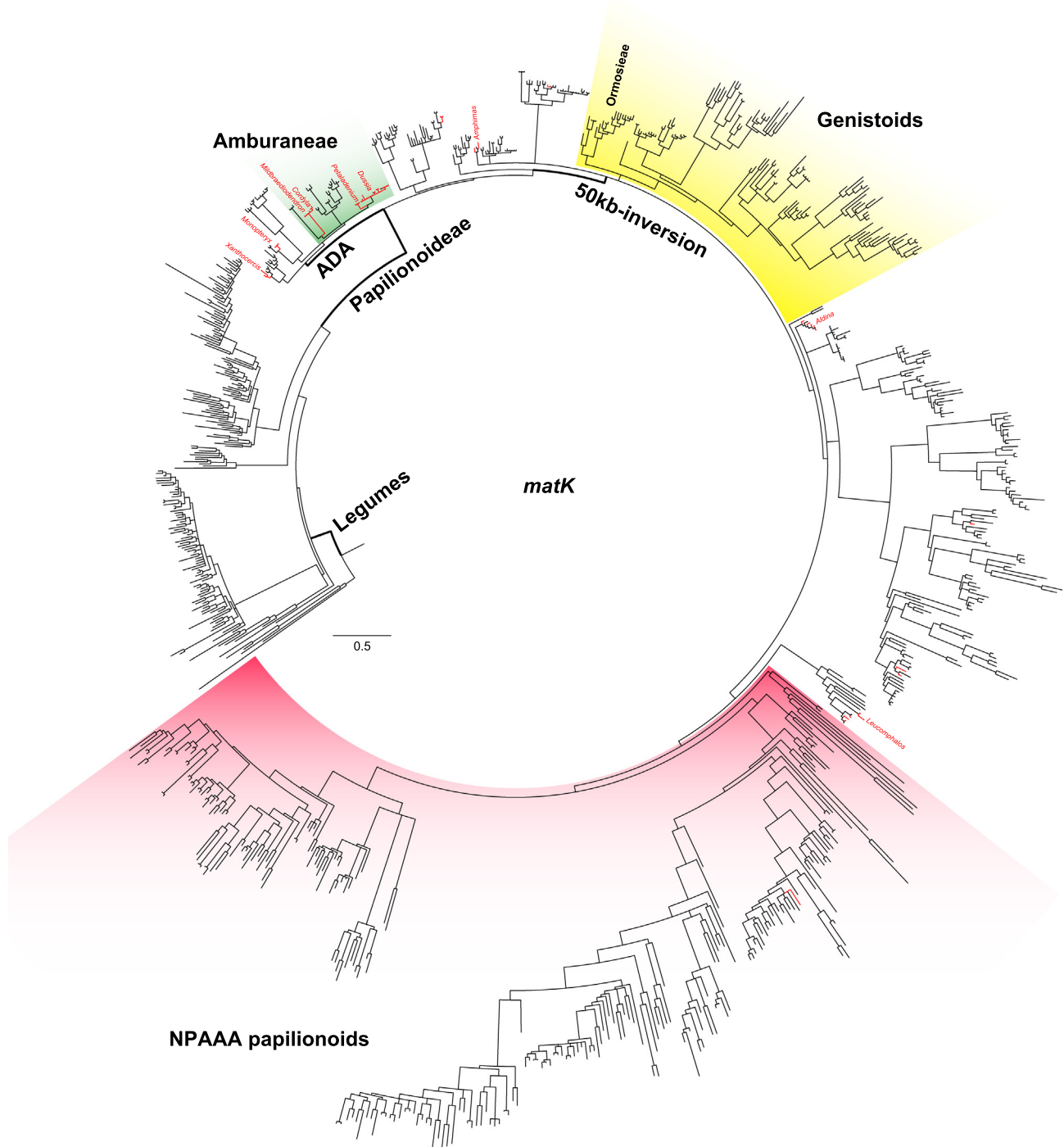
Fig. 1. A *matK* Bayesian majority-rule consensus phylogram of 911 accessions largely sampled across all major lineages of papilionoid legumes. The early-branching papilionoid clades (sensu Cardoso et al., 2012a, 2013a) are those that fall outside the species-rich NPAAA clade. Branches in red indicate new sequences generated for this study, where we also highlight the genera that were previously undersampled. See also Appendix S1 for complete version of this figure.

the Ormosieae clade, a lineage that is sister to all remaining Genistoids (Cardoso et al., 2012a, 2013a). The large Genistoid clade is one of the main lineages within the 50-kb inversion clade and contains many genera once classified in Sophoreae. Despite being a morphologically heterogeneous collection of over 2400 species and ca. 95 genera (Cardoso et al., 2013a), virtually all Genistoids are chemically defined by the presence of quinolizidine alkaloids (Pennington et al., 2001; Van Wyk, 2003; Wink, 2013; Wink and Mohamed, 2003). That *Petaladenium* is placed amongst basally divergent branches of the Papilionoideae phylogeny (Fig. 1) is consistent with its chemis-

try. We did not find evidence that the leaves of *Petaladenium* accumulate quinolizidine alkaloids, rather they accumulate non-protein amino acids, and accumulation of one or other of these two groups of nitrogen metabolites tends to be mutually exclusive, at least in legume seeds (Wink, 1997).

Finding *Petaladenium* within the Amburaneae clade near the root of the Papilionoideae phylogeny also makes more sense in the context of patterns of floral organ development. Ontogenetic data show that *Petaladenium* deviates from the common unidirectional mode of organ formation which is common in more derived
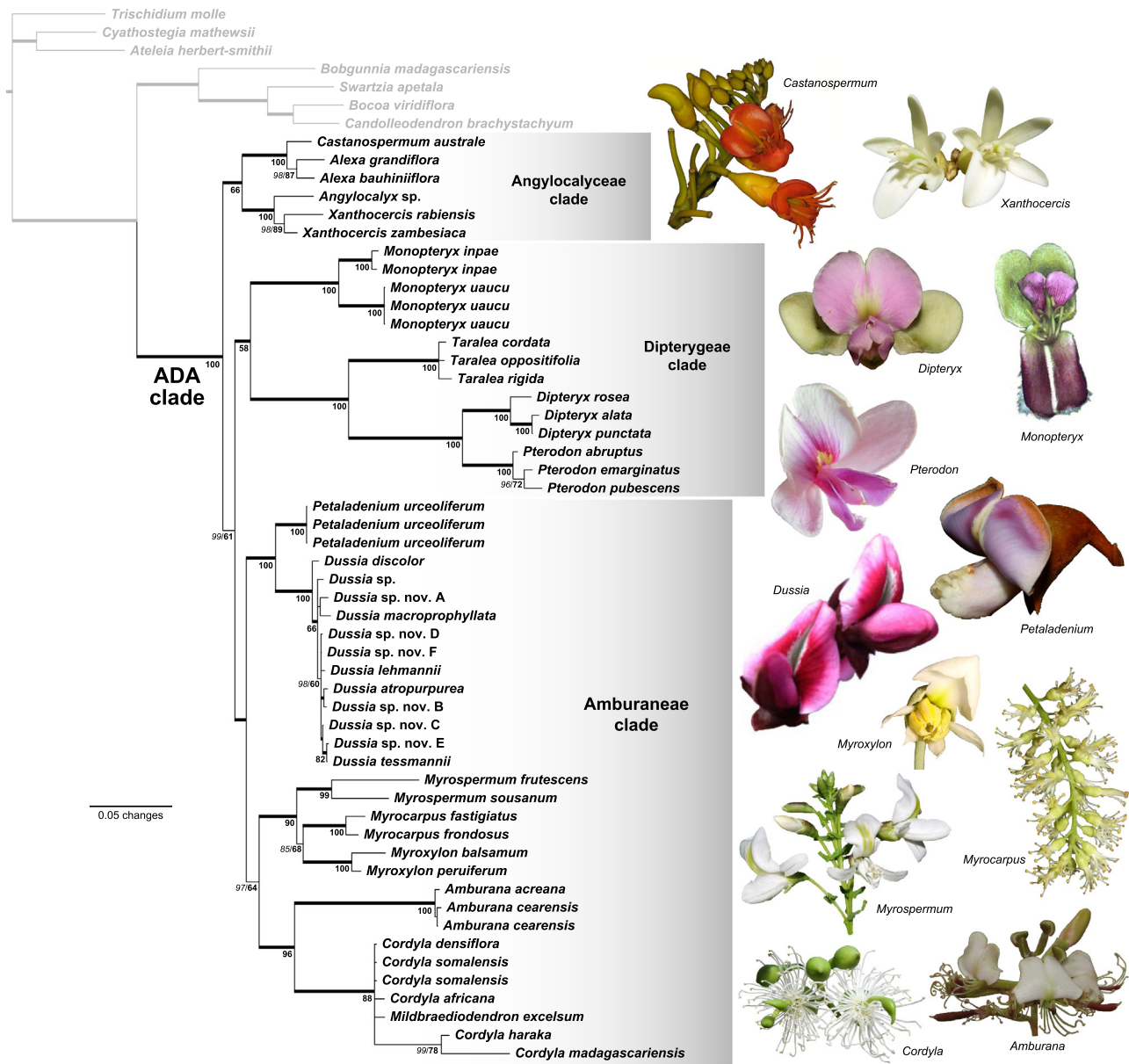
Fig. 2. Majority-rule consensus phylogram derived from the combined nuclear (ITS/5.8S) and plastid (*matK* and *trnL* intron) Bayesian analysis showing the relationships within the clades Angylocalyceae, Dipterygeae, and Amburaneae that comprise the ADA clade (Cardoso et al., 2012a, 2013a). Representative sequences from the Swartzioids were used as outgroups. Numbers on branches are Bayesian posterior probabilities (in italics) and parsimony bootstrap support values (in boldface); branches in bold are those supported by a posterior probability of 1.0. The dramatic floral diversity within ADA clade is highlighted by photographs of representative genera. Photographs: Domingos Cardoso [*Amburana cearensis* (Allemão) A.C. Sm., *Castanospermum australe* A. Cunn. & C. Fraser ex Hook., *Dipteryx rosea* Spruce & Benth., *Myrocarpus fastigiatus* Allemão, *Myroxylon peruiferum* L.f., and *Petaladenium urceoliferum* Ducke], Fidy Ratovoson and Missouri Botanical Garden (*Cordyla madagascariensis* Viguier and *Xanthocercis madagascariensis* Baill.), Carlos Velazco (*Myrospermum sousanum* A. Delgado & M.C. Johnst.), Mauricio Mercadante (*Pterodon pubescens* Benth.), Scott Mori (*Monopteryx inpae* W.A. Rodrigues), and Toby Pennington (*Dussia* sp. nov.).

papilionoid lineages (Prenner et al., unpublished results). Floral ontogeny in *Petaladenium* points towards an experimental phase of organ formation (cf. Prenner and Klitgaard, 2008) and therefore it fits very well into the early branching lineages of papilionoid legumes in which similar patterns are evident (see Leite et al., 2015). With the inclusion of *Petaladenium*, the Amburaneae clade now comprises ca. 38 species in eight florally disparate genera that are mostly of neotropical distribution (Fig. 2). *Petaladenium* along with *Dussia* and *Myrospermum* Jacq. are the only genera in the clade having strongly differentiated papilionate flowers, in which the five petals can be readily distinguished as a standard, two keel petals, and two wing petals. However, among other genera in the Amburaneae clade, *Myroxylon* L.f. has a well-differentiated stan-

dard with the remaining petals being undifferentiated and much reduced (Polhill, 1981a), *Myrocarpus* Allemão has radially symmetrical, mimosoid-like flowers, and *Amburana* Schwacke & Taub. bears a distinctive floral morphology in which a long tubular hypanthium is formed and only the standard petal is fully developed whereas the other four petals are aborted during early ontogeny (Leite et al., 2015). The African genera *Cordyla* and *Mildbraediodendron* exhibit swartzioid-like flowers that have an entire calyx, no petals, and numerous exserted free stamens. The Amburaneae clade lacks a clear non-molecular synapomorphy, although some genera share characters such as the production of balsams (*Myrocarpus*, *Myrospermum*, *Myroxylon*), red exudate from cut bark and twigs (*Dussia* and *Petaladenium*), and glandular punc-

Fig. 3. The unique petal morphology in the papilionate flowers of *Petaladenium urceoliferum*. (A) Inflorescence. (B) Close-up of the inflorescence showing a floral bud and flower. (C) Close-up of a flower showing the intriguing urceolate-glandular margins of the wing petals. (D) Close-up of the lower part of the flower to reveal a distinct view of the urceolate-glandular wings. Photographs: Domingos Cardoso.

tate leaflets (*Cordyla*, *Mildbraediodendron*, *Myrocarpus*, *Myrospermum*, *Myroxylon*). Some of these features, however, are shared with genera of its sister the Dipterygeae clade (Cardoso et al., 2012a; Pennington et al., 2001).

The sister relationship of *Petaladenium* with *Dussia*, a genus of about 18 tree species in the Chocó biogeographical province, Central American, and Amazonian rain forests (Winterton et al., 2014), is supported not only by their bilaterally-symmetrical papilionate flowers, but also other morphological traits (Table 2). Within the Amburaneae clade, they are the only genera in which the calyx has five distinct subequal lobes. Other general similarities include the deeply vertically fissured trunk, non-glandular punctate leaflets, and inflorescences in ramiflorous or axillary poorly-branched racemes that are densely covered by reddish-brown tomentose indumentum. Flowers with white–pinkish to pale lilac petals are not observed in any member of the Amburaneae clade other than in *Petaladenium* and *Dussia*. The intriguing stalked glands on the

margins of bracts and bracteoles of *Dussia macroprophyllata* Harms are reminiscent of the glands found on the margins of the wing petals of *Petaladenium*. The parallel tertiary veins at right angles to the secondary veins that are very characteristic of all *Dussia* species are more or less evident in *Petaladenium*. The fruits of *Petaladenium* and *Dussia* are dehiscent, whereas other genera in the Amburaneae clade bear indehiscent fruits with the exception of *Amburana*. The fruits of *Amburana* are unique among Papilionoideae, in that the seeds are dispersed within a thin paleaceous, endocarp envelope that resembles a basal-winged samara (except for a still to be published new species which has free red seeds that lacks the paleaceous endocarp envelope), so that the *Amburana* fruit has been commonly referred to as a cryptosamara (Barroso et al., 1999; Queiroz, 2009).

Despite the morphological similarities shared by *Petaladenium* and *Dussia*, we are confident in maintaining a monospecific circumscription of *Petaladenium* and not combining it in a wide concept of

Table 2
Foremost morphological similarities and differences (traits highlighted in bold) between *Petaladenium* and its phylogenetically related *Dussia* within the early-branching Amburaneae clade of Papilionoideae.

Trait	*Petaladenium*	*Dussia*
Trunk	Deeply vertically fissured and producing small quantities of reddish exudate	Deeply vertically fissured and producing reddish exudate
Leaflet secondary venation	Eucamptodromous	Craspedodromous
Leaflet tertiary venation	Tertiary veins more or less parallel and at right angles to the secondary veins	Parallel tertiary veins more conspicuously at right angles to the secondary veins
Inflorescence	Ramiflorous or axillary in poorly-branched racemes that are densely covered by densely reddish-brown hairs	Ramiflorous or axillary in poorly-branched racemes that are densely covered by densely reddish-brown or golden hairs
Bract and bracteoles	Linear-lanceolate, entire, never glandular	Linear-lanceolate to broadly ovate or triangular on a distinct stalk, entire to serrate, occasionally erose with some conspicuous stalked white glands
Flower	Zygomorphic, papilionate	Zygomorphic, papilionate
Calyx	Distinctly composed of five subequal lobes	Distinctly composed of five subequal lobes
Petal color	Lilac to pinkish	Purple, or lilac to pinkish
Wing petals	Margin urceolate-glandular	Margin entire
Stamens	Ten, basally fused	Ten, basally fused
Fruit	An elongate, flattened elastically dehiscent legume	An ellipsoidal, dehiscent legume
Seed coat	Uncolored and non-fleshy	Colored and fleshy

Dussia for several reasons. The eucamptodromous secondary venation, elongate elastically dehiscent pods, and non-fleshy seeds of *Petaladenium* greatly contrast with the craspedodromous leaflets, ellipsoidal pods, and colored, fleshy seeds that traditionally define the generic concept of *Dussia* (Table 2). Perhaps more interesting in terms of floral morphology is that, if we preserve the generic status of *Petaladenium*, it will be chiefly distinguished among all Papilionoideae genera on the basis of its striking urceolate-glandular wing petals. The recognition of monospecific or poorly-diverse, yet morphologically-diagnosable genera has been a common practice in Papilionoideae systematics (e.g., Torke and Schaal, 2008; Queiroz et al., 2010; Cardoso et al., 2012c, 2013c; Meireles et al., 2014).

4.3. Confirming the phylogenetic placement of other previously undersampled genera

Early classifications of the Papilionoideae placed *Monopteryx*, a small genus of three Amazonian species, within the Sophoreae (Pennington et al., 2005; Polhill, 1981a, 1994). We have demonstrated in phylogenetic analyses of plastid *matK* and *trnL* intron sequences (Cardoso et al., 2012a) that the genus could be closely related to *Dipteryx* Schreb., *Pterodon* Vogel, and *Taralea* Aubl., all these traditionally placed in the tribe Dipterygeae. Our previous analyses, however, only sampled *M. inpae* W.A. Rodrigues, the most morphologically divergent species in the genus (Barneby and Grimes, 1984; Rodrigues, 1975). Our recent field work in remote areas of the Brazilian Amazon resulted in the collection of a second species, *M. uaucu* Spruce ex Benth., which is a common tree in the upper Rio Negro, but which was poorly represented in herbaria (Cardoso et al., in press). Unlike *M. inpae*, the newly sampled *M. uaucu* matches with the original description of the genus because it bears an extrafloral nectary on the leaf rachis, brochidodromous leaflets with a conspicuous intramarginal vein, and large (>20 cm long), falcate pods with crimped wings on the margin. Therefore, the inclusion of *M. uaucu* in the current phylogenetic analysis was critical to confirm the monophyly and placement of the genus as sister to the remaining Dipterygeae (Fig. 2). Except for *Monopteryx*, all other Dipterygeae have a two-lipped calyx, monadelphous androecium, and the typical papilionate corolla differentiated into standard, keel, and wing petals (Leite et al., 2014). *Monopteryx* was never formally associated before with the Dipterygeae genera because of its nonpapilionate corolla in which the wing petals are reduced and the keel petals are connate and open out exposing the free stamens. We are also not aware of extrafloral nectaries on the leaf rachis of any other Dipterygeae species other than in *M. uaucu* and *M. angustifolia* Spruce ex Benth.

It is now clear that a new tribal phylogenetic classification of the Dipterygeae should encompass the genus *Monopteryx* (Cardoso et al., 2012a, 2013a), a long-standing opinion indeed shared by others (Barham, 2005; Bentham, 1862; Pennington et al., 2005; Polhill, 1981b), based upon the shared floral character of a two-lipped calyx. The two upper calyx lobes observed in *Dipteryx*, *Pterodon*, and *Taralea* are completely separate to their base so that they appear wing-like, whereas in *Monopteryx* the upper enlarged lobes assume a standard-like position because they are fused into a single structure right behind the smaller standard petal. A distinctly lipped calyx formed by enlarged petaloid lobes can be frequently found in Polygalaceae (although see the ontogenetic homologies in Prenner, 2004), but is rather uncommon in Papilionoideae. A notable exception is the African millettioid genus *Platysepalum* Welw. ex Baker, where the upper sepal lobe is almost as large as the standard petal.

Given the strongly supported phylogenetic relationships within the Dipterygeae clade (Fig. 2), we can now infer that indehiscent fruits are a synapomorphy of the subclade comprised of *Dipteryx*

and *Pterodon*, whereas the elastically dehiscent flattened pods with woody valves of *Monopteryx* and *Taralea* are plesiomorphic. *Pterodon* is defined by the flattened samaras with a wing around the raised central seed-chamber, and *Dipteryx* by drupaceous fruits; ovate to suborbicular dehiscent pods are synapomorphic for *Taralea*. The elongate to long-obovate and falcate dehiscent pods of *Monopteryx* help to further differentiate this genus from the other Dipterygeae, but whether this trait is synapomorphic for *Monopteryx* would need a closer look outside the Dipterygeae clade, since such elongate elastically dehiscent pods are also found variously within the Angylocalyceae and Amburaneae clades. Recent data on floral ontogeny, seed and embryo morphology for some representatives of Dipterygeae showed interesting features that differentiate *Dipteryx*, *Pterodon*, and *Taralea* (Leite et al., 2014; Pinto et al., 2014). For example, *Dipteryx* presents asynchronous initiation of bracteoles, modified unidirectional initiation of sepals, a truncate stigma, appendices in the anther and bracteole, seeds with a lateral or subapical hilum and a rugose embryo bearing conspicuous plumule; *Pterodon* has simultaneous initiation of bracteoles, helical initiation of sepals, a capitate stigma, appendices in the anther and bracteole, seeds with a lateral hilum and covered by an aril, and the smooth embryo has a conspicuous plumule; *Taralea* species have simultaneous initiation of bracteoles, sequential or modified sequential initiation of sepals, a punctiform stigma, no appendices in the anther and bracteole, seeds with a basal hilum, and the embryo has an inconspicuous plumule (Leite et al., 2014; Pinto et al., 2014). Nevertheless, at the moment we are unable to infer any putative synapomorphies with either floral development or seed features because of the missing data for *Monopteryx*. Regrettably this genus was never considered in morphological studies, despite early indications of its close affinity to the other Dipterygeae genera (Barham, 2005; Bentham, 1862; Pennington et al., 2005; Polhill, 1981b).

The combined phylogenetic analysis confirmed the relationship of the tropical African monospecific *Mildbraediodendron* with the African and Madagascan species of *Cordyla* (Fig. 2; Cardoso et al., 2012a; Herendeen, 1995; Pennington et al., 2001). These are the only genera of the Amburaneae clade that have indehiscent globose or elongate fruits with a fleshy endocarp and flowers with an entire calyx and no petals, both floral features being more common in the Swartzioid clade. Unlike their most closely related genus *Amburana*, *Mildbraediodendron* and *Cordyla* also share glandular punctate leaflets, although this feature might be plesiomorphic in the Amburaneae clade since it also occurs in *Myrocarpus*, *Myrospermum*, *Myroxylon*, and most Dipterygeae genera. *Mildbraediodendron* and some species of *Cordyla* share a similar flavonoid chemistry in producing flavonol *O*-glycosides (cordylasins and mildbraedin) characterized by having a unique *O*-linked tetrasaccharide (Veitch et al., 2008). The main morphological differences between *Mildbraediodendron* and *Cordyla* include only a few floral characters (Polhill, 1981a). *Mildbraediodendron* does not have a hypanthium and has less than 20 stamens basally fused into a single whorl surrounding a large fleshy disc. *Cordyla* has flowers with a hypanthium and numerous stamens (usually well over 50) that are basally united into three or four whorls; the staminal disc is absent. Despite the unresolved placement of *Mildbraediodendron* either within or as sister to *Cordyla*, and their shared morphological and chemical features, we are not confident in suggesting their amalgamation because the phylogenetic signal might be obscured by the high percentage of missing data (~40% of a total of 21 sequences that should have been included for both genera in the three data sets). We suggest that more comprehensive sampling, also including fast-evolving genes, is needed before any formal taxonomic decision is made about synonymisation of *Mildbraediodendron* with *Cordyla*. Such a densely-sampled phylogeny would also permit evaluation of whether the Madagascan species of *Cordyla*

(*C. haraka* Capuron and *C. madagascariensis* R. Vig.), earlier transferred to the new genus *Dupuya* J.H. Kirkbr. on the basis of seed characters and presence of staminodes (Kirkbride, 2005), should be maintained in *Cordyla* as indicated by chemistry analyses (Veitch et al., 2008). The Madagascan *Cordyla* species formed a strongly supported clade in our combined analysis (Fig. 2), but they are not resolved with respect to *Mildbraediodendron* and the remaining *Cordyla* species, likely because they were not sampled for *matK* and were incompletely sampled for ITS.

An interesting phylogenetic pattern of reciprocal monophyly of South American seasonally dry forest genus *Amburana* and the African *Cordyla* + *Mildbraediodendron* might be explained by population coalescence processes acting at the clade level (Barraclough, 2010). The mean age estimate of ~50.8 Ma for the crown ADA clade (see node 16 in Lavin et al., 2005), postdating the split of west Gondwana by ~50 Ma, and the mostly neotropical distribution of the genera within the Amburaneae clade and its sister clade Dipterygeae, suggest that the African distribution of *Cordyla* and *Mildbraediodendron* resulted from long distance dispersal from a New World ancestor. The relatively long stem branches of the reciprocally monophyletic clades indicate that the neotropical clade might have experienced much species turnover without subsequent long distance dispersal to Africa (Barraclough, 2010). This pattern of reciprocal monophyly is also observed in Amphi-Atlantic distributed legume clades, such as *Hymenaea-Guibourtia* clade in caesalpinioid Detarieae, *Acacia* subgen. *Aculeiferum* (currently *Senegalia*) in the mimosoids, and *Chapmannia-Stylosanthes* in papilionoid Dalbergieae (Lavin et al., 2004; Schrire et al., 2005), all of which postdate the split of west Gondwana.

Our recent *matK* phylogenetic analysis of the early-branching papilionoids shed some light on the previously obscure relationship of the African *Amphimas* and neotropical *Aldina* (Cardoso et al., 2012a, 2013a). The additional sequences generated here within these genera support their monophyly (Appendix S1), but we acknowledge that our sampling of the morphological diversity of *Aldina* is still far from complete. The phylogenetic placement of *Amphimas* and *Aldina* within the 50-kb inversion clade still remains enigmatic. The unique combination of floral features that make up the radially-symmetrical flowers of *Amphimas* (Pennington et al., 2000; Polhill, 1981a) precludes assigning this genus to any main papilionoid lineage based upon floral morphology. The globose indehiscent drupaceous fruits of *Aldina* are reminiscent of those in *Andira* Lam., which has been always resolved as sister to *Hymenolobium* Benth., but this small clade is also unresolved within the 50-kb inversion clade (Cardoso et al., 2012a, 2013a).

Our study evaluated for the first time the monospecific genus *Leucomphalos* s.s. (Appendix S1) in a phylogenetic framework and corroborates its affinities with the Baphioids as suggested by vegetative, floral, and fruit morphologies (Cardoso et al., 2013a; Pennington et al., 2005; Polhill, 1981a). The Baphieae clade (sensu Cardoso et al., 2013a) is comprised of seven genera of predominantly African, Madagascan, and Asian trees and lianas characterized by the combination of unifoliolate leaves, free stamens with basifixed anthers, and the calyx usually splitting in one or two main lobes. The Baphioids appear as sister to the so-called nonprotein-amino-acid-accumulating (NPAAA) clade, the largest radiation of legumes that includes more than 60% of the species and generic diversity of the Papilionoideae.

Clarification of the generic delimitation in the Baphioids depends upon a more complete phylogenetic analysis of the clade, especially within the species rich, yet clearly polyphyletic genus *Baphia* (Appendix S1; Cardoso et al., 2013a). At least with respect to *Leucomphalos* s.s. and related genera we have seen now a clearer pattern of relationships. Breteler's (1994) broad concept of *Leucomphalos* that also encompasses *Bowringia* and the monospecific *Baphiastrum* is supported. *Leucomphalos* s.s. indeed shares with *Bowringia* and *Baphiastrum* the transverse ovule orientation, a character unique within papilionoids. This unique character was the basis for the merger by Breteler (1994), and its occurrence is found as synapomorphic for the clade. Nevertheless, the current preliminary Baphieae phylogeny also suggests that *Leucomphalos* s.s. could deserve generic level status since *L. capparideus* Benth. ex Planch. (the type species of *Leucomphalos*) is placed on a relatively long branch that is sister to the *Bowringia* + *Baphiastrum* clade, which also has a relatively long stem branch; both branch lengths are comparable to the length of other branches of papilionoids that bear genera (Appendix S1). Still, whether to recognize a single *Leucomphalos* s.l. based on the unique transverse ovule character, or two distinct genera based on other characters remains a matter of taste.

This pattern of relationships where species-poor or monospecific non-papilionate-flowered genera are often sister to a more species-diverse papilionate-flowered clade occurs recurrently in early-branching papilionoid clades (Cardoso et al., 2012a, 2012b, 2012c, 2013a, 2013c; McMahon and Hufford, 2004). The pattern that early-branching clades contain relatively many monospecific genera is a trend that can also be observed in one of the earliest branches of legumes, the Detarieae. Of the 84 currently recognized genera, 27 (32%) are monospecific (Mackinder, 2005; Mackinder and Wieringa, 2013; Wieringa et al., 2013). Older clades likely have had more time to accumulate changes in monospecific lineages, resulting in such large differences that it becomes impossible to lump such basally branching species into the more speciose sister clade. Therefore preserving the identity of a monospecific *Leucomphalos* will maintain an evolutionarily interesting pattern frequently observed at the genus level in the legume phylogeny. In addition to the radial corolla of subequal, unclawed petals, the genus *Leucomphalos* is distinguished from all Baphioids by possessing 11 or 12 stamens, whereas almost all Baphieae exhibit the more common fixed number of ten stamens (Breteler, 1994; Brummitt, 1968; Soladoye, 1985). Also, the calyx in *Leucomphalos* splits into two segments without teeth or lobes, and the stamens have distinctly apiculate anthers that are disproportionally much larger than the filaments (Breteler, 1994). That at least *Bowringia* and *Baphiastrum* should be congeneric is reasonable, since *Baphiastrum* is nested within the paraphyletic *Bowringia* (here represented by the species *B. mildbraedii* Harms and *Leucomphalos* [=*Bowringia*] *libericus* Breteler) and they share several morphological features, chiefly those that make up their papilionate corolla (Breteler, 1994). At the molecular level it is obvious that the branch leading to this clade is longer than the internal branches of this clade.

5. Conclusions and future prospects

The phylogenetic data presented here suggest phylogenetic placements for the previously poorly-sampled or unsampled genera *Aldina*, *Amphimas*, *Dussia*, *Leucomphalos*, *Mildbraediodendron*, *Monopteryx*, and *Petaladenium*. Additionally, the monophyly of *Monopteryx* as sister to the remaining Dipterygeae, as well as the sister relationship between *Dussia* and *Petaladenium* within the Amburaneae clade, were revealed for the first time. The unexpected finding that *Petaladenium* is placed amongst the basally divergent branches of the Papilionoideae phylogeny was supported by its chemistry (of non-protein amino acids rather than any type of quinolizidine alkaloids). Comprehensively-sampled molecular phylogenies of papilionoid legumes have repeatedly revealed the non-monophyletic circumscription of tribes or genera originally defined largely on floral features (Cardoso et al., 2012a, 2013b; Pennington et al., 2001), a pattern seen here where *Petaladenium* and *Ormosia*, which share similar papilionate-flowers, are unrelated.

The lesson from this study and a wealth of recent molecular phylogenies in papilionoid legumes is clear: better resolved and more strongly supported relationships can be revealed with more

complete taxon sampling (Cardoso et al., 2012a, 2012b, 2013b; Gagnon et al., 2013; Meireles et al., 2014; Silva et al., 2012; Sirichamorn et al., 2012, 2014). Hence, devoting efforts to building densely-sampled molecular data-sets across as much as possible of the morphological variation in heterogeneous groups such as the early-branching papilionoids should precede formalization of any new taxonomic classification. The LPWG (2013a) flagged a list of 66 legume genera that lack DNA sequence data. Here we have gathered new information for some morphologically unique genera to fill in the gaps of the early evolutionary history of the Papilionoideae. Our future work will focus on the Amazonian *Uleanthus* and the Old World genera *Pericopsis* from Africa and Asia, *Haplormosia* from Africa, and *Neoharmsia* and *Sakoanala* from Madagascar, now the only genera formerly placed in the tribe Sophoreae whose relationship remain yet undetermined. A complete genus-level molecular phylogeny of the Papilionoideae will help to resolve a number of outstanding taxonomic questions, as well as provide a better understanding of the evolution of the astonishing floral diversity in the early-branching papilionoid clades.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.12.015.

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