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Flower development of *Goniorrhachis marginata* reveals new insights into the evolution of the florally diverse detarioid legumes

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● **Background and Aims** The study of floral morphology and ontogeny and the re-investigation of existing data help to uncover potential synapomorphic characters and foster our understanding of phylogenetic relationships that rely primarily on molecular analyses. *Goniorrhachis marginata* is a monotypic caesalpinioid legume (Leguminosae) that shows some interesting floral features, such as a long hypanthium and regular Rosaceae-like flowers. We studied the ontogeny and morphology of the flowers in detail and present our results in a broad phylogenetic context.

● **Methods** Flower buds were collected in the field, fixed in 70 % ethanol and investigated using scanning electron microscopy. Older buds in spirit were carefully opened to investigate the direction of style bending. Characters of the style from 131 taxa from the main legume lineages were analysed and mapped on a Bayesian molecular phylogeny.

● **Key Results** The tetramerous calyx is the result of complete loss of one sepal. The formation of the radially symmetrical corolla starts in a typical caesalpinioid pattern with the adaxial petal innermost (ascending aestivation). The young style bends in the abaxial direction, which is a character found exclusively in all studied detarioid legumes and therefore a newly described synapomorphy for the clade.

● **Conclusions** We show that investigation of unstudied taxa and reinvestigation of published data can uncover new, previously overlooked and important characters. Curvature of the style can be detected in young buds with a hand lens and therefore is an important character for field botanists. Our study reveals the importance of including poorly studied and/or phylogenetically enigmatic taxa in molecular phylogenies and in detailed morphological and ontogenetic analyses.

Key words: Caesalpinioideae, Detarieae, flower evolution, flower development, flower symmetry, Leguminosae, gynoecium, style.

INTRODUCTION

Uncovering the extraordinary diversity of floral architecture in angiosperms through developmental studies provides new insights into evolutionary history and helps us understand phylogenetically obscure or enigmatic relationships that nowadays rely almost exclusively on molecular studies (Saarela *et al.*, 2007; Cardoso *et al.*, 2012*a*, *b*, 2015; Ramos *et al.*, 2016). Plants traditionally classified in the paraphyletic subfamily Caesalpinioideae of the big and economically important bean family, Leguminosae, have long been a challenge to a phylogeny-based classification not just because of their phylogenetically unresolved deep nodes, but also because of the dramatic morphological diversity that prevents recognition of clear overall synapomorphies among clades (LPWG, 2013*a*, *b*). Chiefly because of the paraphyletic nature of the caesalpinioid lineages, ongoing progress in unravelling deeper relationships within legumes has prompted new attempts to reorganize the family classification according to its relationships as derived from molecular phylogenies (LPWG, 2017). This means that a new set of subfamilies have to be defined, which in turn are in need of good and clear morphological synapomorphies. With

this in mind, the thorough floral ontogenetic investigation of unsampled or poorly studied taxa and the reinvestigation of published data are timely and of particular interest, not only for leguminologists but also for the broad scientific community that wants to work with and understand a new legume classification system.

Improved sampling across all clades for reconstructing the legume phylogeny (e.g. Wojciechowski *et al.*, 2004; Lewis *et al.*, 2005; Bruneau *et al.*, 2008; Simon *et al.*, 2009; Cardoso *et al.*, 2012*a*, *b*, 2013) has enabled an evolutionarily comparative look at morphological characters that has revealed e.g. remarkable new insights into the evolution of extrafloral nectaries (Marazzi *et al.*, 2012), the polycarpellate gynoecium (Paulino *et al.*, 2014) and the high persistence of legume–rhizobia symbiosis (Werner *et al.*, 2015). The wealth of floral ontogenetic data available for legumes (Tucker, 1987, 1988*a*, *b*, 2003*c*; Prenner, 2004*a*, *b*; Marazzi and Endress, 2008), including recent results for phylogenetically enigmatic lineages that branch off at deep nodes (Prenner and Klitgaard, 2008; Prenner *et al.*, 2015), are likewise extremely useful for large-scale comparison and as sources for potential synapomorphic characters of still obscure relationships. The caesalpinioid Detarieae lineage is an

example of such a highly morphologically heterogeneous collection of genera marked by an exceptional lability in floral evolution (Bruneau *et al.*, 2014). The current concept of the tribe Detarieae embraces ∼750 species, of which the greatest diversity is found in Africa, but also with significant diversity in the Neotropics and tropical Asia (Mackinder, 2005). Floral morphology in Detarieae varies widely (Bruneau *et al.*, 2014), such as in the resin-producing *Copaifera*, with tiny, apetalous and radially symmetrical flowers, *Macrolobium*, with the upper petal often much larger than the others, and *Heterostemon*, with orchid-like, showy flowers with bilateral symmetry and five colourful petals usually born on cauliflorous inflorescences.

Whilst significant progress has been made in understanding the origin and evolution of the incredible floral diversity in the detarioids (Bruneau *et al.*, 2014), here we revisit their floral ontogeny by presenting the complete developmental series of the Rosaceae-like flowers of the monotypic Brazilian detarioid genus *Goniorrhachis* and also by focusing specifically on the evolution of the gynoecium and the direction of style bending. The genus is represented by just *G. marginata*, which is a 5–15(–30)-m high evergreen tree that often dominates the disjunct patches of seasonally dry tropical forests or arboreal caatinga, especially on richer soils and along temporary rivers, from Sergipe and Bahia to Minas Gerais, Espírito Santo and northern Rio de Janeiro (Fig. 2A; Queiroz, 2006, 2009; Santos *et al.*, 2012; Oliveira-Filho, 2014; Lima, 2016). The flowers of *G. marginata* are characterized by their long hypanthium with an adnate gynoecium on the rim, and by the radially symmetric corolla and diplostemonous androecium superficially resembling Rosaceae flowers (Figs 1 and 2).

The aims of this study are (1) to present a detailed ontogenetic analysis of *G. marginata*, (2) to compare ontogenetic stages with other related Detarieae and, in a broader picture, with radially symmetrical taxa from the other legume lineages, and (3) to search for potential ontogenetic synapomorphies for the morphologically complex Detarieae clade. To accomplish our goals we have implemented Bayesian and parsimony approaches to reconstruct ancestral character states of style development over a comprehensively sampled molecular phylogeny of the legume family.

MATERIALS AND METHODS

Sampling and scanning electron microscopy analysis of Goniorrhachis *flowers*

Inflorescences, flowers and flower buds of *Goniorrhachis marginata* were collected in a seasonally dry forest in Rio de Contas, Bahia, Brazil (*D. Cardoso et al. 3614*, HUEFS) and immediately fixed and stored in 70 % ethanol. For scanning electron microscopy (SEM), flowers and floral buds were dissected in 70 % ethanol, dehydrated to 100 % ethanol and critical-point dried using an Autosamdri-815B critical-point dryer. Dried samples were mounted on aluminium stubs using clear nail polish and coated with platinum in a Quorum Q150T sputter coater. SEM images were taken with a Hitachi S-4700-II cold-field emission SEM and images were processed using Adobe Photoshop CS5.

Reconstruction of ancestral states of style development

To investigate the evolution of the style curvature of *G. marginata* in the context of the major lineages of legumes, we comprehensively reviewed published data. In addition we studied some key taxa, leading to information on style curvature in 131 legume taxa (Table 1). Based on these observations, we have defined four different patterns related to style curvature in legume flowers: straight (st), bending adaxially (ad), bending abaxially (ab), and no distinct style formation (nd) (Table 1). To reconstruct the ancestral states of such style development during the evolutionary history of legumes, we assembled a molecular dataset of the phylogenetically informative plastid *matK* protein-coding gene from 107 taxa. Sequences were all obtained from publicly available data in GenBank. To avoid the possible use of misidentified or inaccurate sequences, we included only our own previously generated sequences (e.g. Cardoso *et al.*, 2012*a*, 2015) or those from comprehensive phylogenetic analyses of legumes (e.g. Wojciechowski *et al.*, 2004; Bruneau *et al.*, 2008). Also, we selected only sequences that can be taxonomically validated by voucher specimens in herbaria. We attempted to select as much as possible taxa for which there is corresponding information on flower ontogeny. However, the genera *Caesalpinia*, *Copaifera*, *Hymenaea*, *Isoberlinia*, *Tetraberlinia*, *Labichea*, *Haematoxylum* (caesalpinioids) *Calliandra* (Mimosoideae), *Aeschynomene*, *Alhagi*, *Angylocalyx*, *Astragalus*, *Dalbergia*, *Daviesia*, *Desmodium*, *Erythrina*, *Hedysarum*, *Lespedeza*, *Lonchocarpus*, *Lotus*, *Onobrychis* and *Swartzia* (Papilionoideae) are represented with a *matK* sequence from a different species, because we do not have the morphological information for the matching species. However, we believe this is not a problem since the traits investigated here are believed to be conserved at genus level (G. Prenner, unpubl. res.).

A Bayesian analysis of the *matK* dataset was performed in MrBayes v.3.2 (Ronquist *et al.*, 2012). The general time-reversible (GTR) model with γ-distributed among-site rate heterogeneity (*G*) and invariant sites was the best-fitting nucleotide substitution model selected via the Akaike information criterion (AIC), as implemented in the program MrModeltest v.2.0 (Nylander, 2004). 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 sampling parameter estimates, tree topology, and rate heterogeneity patterns every 1000 generations (Huelsenbeck *et al.*, 2001). Convergence was assessed by comparing the posterior probabilities across the MCMC runs using the online program AWTY (are we there yet?) (Nylander *et al.*, 2008; Supplementary Data Fig. S1). The Markov chains were run for 2 million generations such that 3002 non-autocorrelated Bayesian trees were sampled from likelihood stationarity for the two runs after a burn-in of 25 %. We used MrBayes to summarize trees sampled from post-burn-in generations in a 50 % majority-rule consensus tree that included clade frequencies or posterior probabilities as branch support measures (Huelsenbeck *et al.*, 2002). Visualization and partial editing of the Bayesian tree for graphical presentation were done in FigTree v.1.4.2 (Rambaut, 2014).

We employed parsimony and Bayesian approaches to reconstruct the ancestral character states of style development.
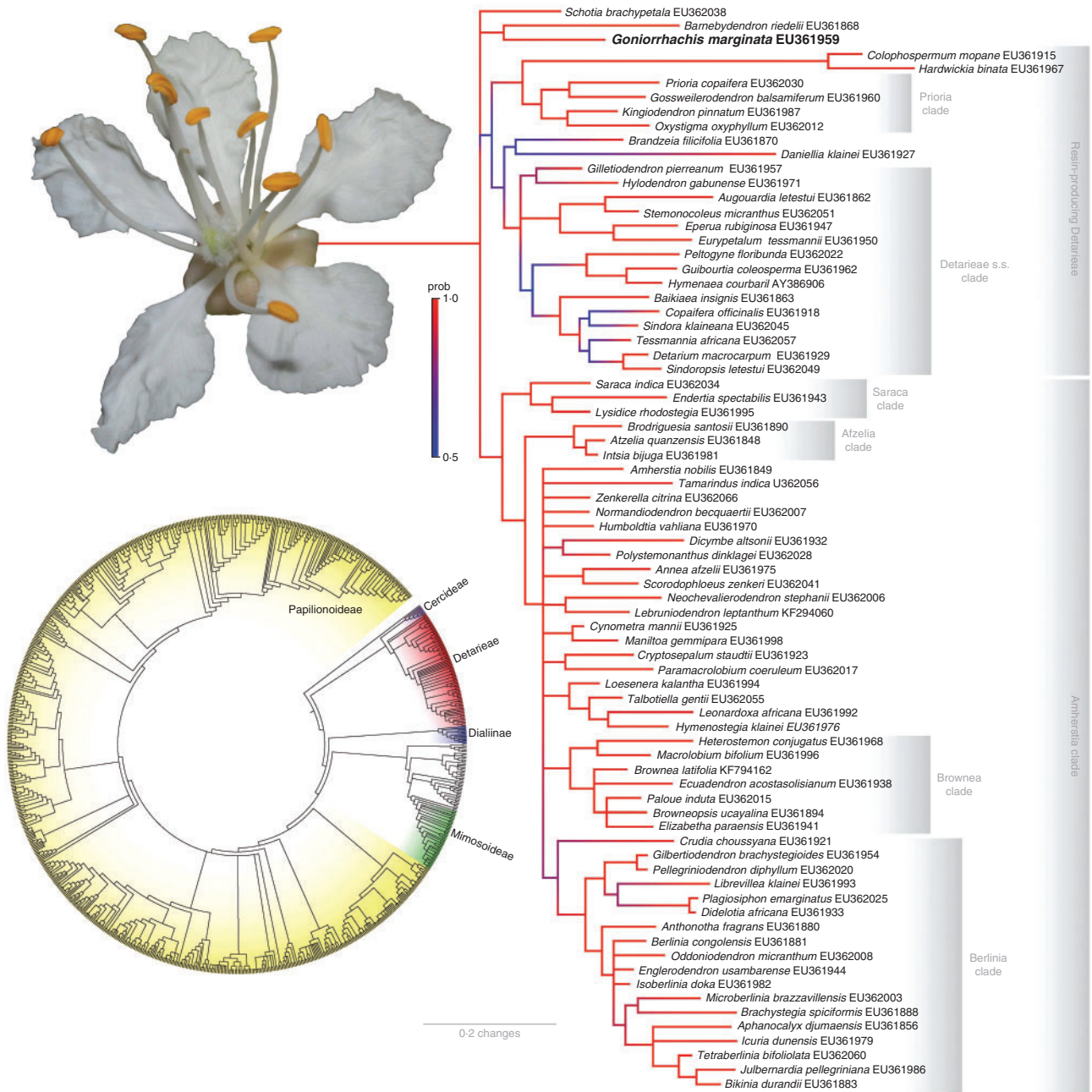
FIG. 1. Summary of the *matK* Bayesian majority-rule consensus tree broadly sampled across all major lineages of legumes as originally published by Cardoso *et al*. (2015). Here we highlight the placement of *G. marginata* in the close-up phylogeny of the caesalpinioid Detarieae. The Detarieae lineages are named after Bruneau *et al*. (2008). Photograph of the flower of *G. marginata* by Domingos Cardoso.

The parsimony reconstruction method as implemented in Mesquite v.3.04 (Maddison and Maddison, 2015) was used to trace character-state changes over the well-resolved Bayesian majority-rule consensus tree (Fig. S1). The Bayesian MCMC reconstruction of ancestral character states as implemented in the BayesMulti-State module of the program BayesTraits v.2.0 (Pagel *et al*., 2004; Pagel and Meade, 2014) was used as an alternative to parsimony optimization because it accommodates uncertainty in both phylogenetic topology and character mapping (Pagel *et al*., 2004; Ronquist, 2004). BayesTraits

optimization was conducted using 1000 randomly selected post-burn-in trees from the posterior distribution of phylogenies obtained from the MrBayes analysis. These 1000 trees are likely representative of the entire Bayesian tree pool because their resulting 50 % majority-rule consensus tree is virtually identical to that from all of the Bayesian post-burn-in trees. The addMRCA (most recent common ancestor) command was used to calculate ancestral state probabilities for deeper nodes of the backbone tree, especially that leading to the Detarieae clade. We used the reversible-jump hyperprior (rjhp) model of
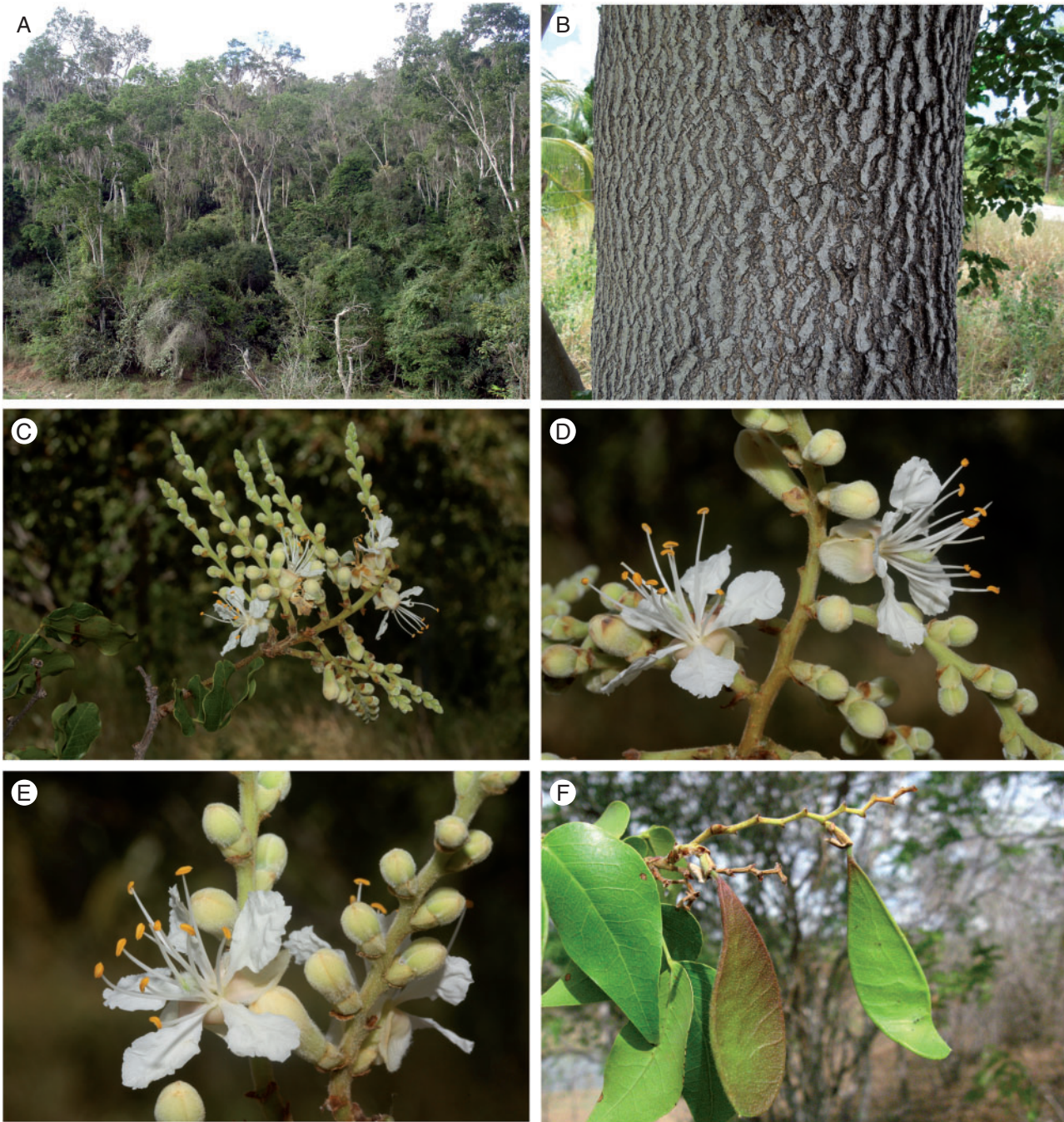
FIG. 2. (A) A *Goniorrhachis*-dominated seasonally dry tropical forest in central Bahia, Brazil, showing emergent trees of *G. marginata* with their characteristic whitish-grey bark. (B) Detail of a trunk. (C) Inflorescence with flowers just starting to open at the base of each racemose partial inflorescence. (D, E) The Rosaceae-like flowers showing recurved sepals at the base and radially symmetrical corollas with crumpled petals with more or less distinct blade and claw. Versatile anthers and stigma are exserted above the flower. (F) Flattened fruits. Photographs: Domingos Cardoso.

character evolution, with rate deviation and data deviation parameters automatically tuned (Pagel and Meade, 2014). The MCMC analyses were run for 1 010 000 generations, sampling every 1000th generation, with a burn-in of 10 %. The arithmetic mean of the resulting posterior probability distributions was used to produce the probabilities of ancestral states, which were presented as pie charts using the program TreeGraph 2 (Stöver and Müller, 2010).

RESULTS

Flower morphology and development in G. marginata

Flowers are found in five- to nine-flowered spikes, which in turn are grouped into more complex compound racemes or panicles. Flowers are formed in the axil of an abaxial bract and each flower is preceded by two lateral bracteoles. The flowers are fragrant, actinomorphic, and composed of four sepals that

TABLE 1. *Style bending during floral development in legumes*

Legume subfamilies	Species	Style bending	Reference	Voucher (unpublished data)
Caesalpinioideae	*Afzelia quanzensis*	ab	Tucker (2002b, Figs 81, 82, 85, 86–88)	
	Amherstia nobilis	ab	Tucker (2000b, Figs 68, 70, 73; 2003a, Fig. 3G)	
	Anthonotha pynaertii	ab	Prenner (unpubl. res.)	*Troupin 4269* (K)
	Aphanocalyx djumaensis	ab	Tucker (2000a, Figs 22, 23)	
	Barnebydendron riedelii	ab	Prenner (unpubl. res.)	*Lewis 1615* (K)
	Berlinia confusa	ab	Prenner (unpubl. res.)	*Polhill 5209* (K)
	Brachystegia glaucescens	ab	Tucker (2000a, Fig. 83)	
	Brodriguesia santosii	ab	Prenner and Queiroz (unpubl. res.)	*Queiroz 16163* (HUEFS)
	Brownea latifolia	ab	Tucker (2000b, Figs 31, 33)	
	Brownea longipedicellata	ab	Prenner (unpubl. res.)	*Cardoso 3831* (RB)
	Copaifera langsdorffii	ab	Prenner (unpubl. res.)	*Cardoso 3836* (HUEFS)
	Crudia choussyana	ab	Tucker (2001b, Fig. 3I)	
	Cynometra sp.	ab	Tucker (2001a, Fig. 97)	
	Gilbertiodendron brachystegioides	ab	Tucker (2002b, Fig. 122)	
	Gilbertiodendron klainei	ab	Tucker (2002b, Figs 111, 115)	
	Goniorrhachis marginata	ab	This study (Figs 4K, 6C)	
	Hymenaea stigonocarpa	ab	Prenner (unpubl. res.)	*Cardoso 3849* (ALCB)
	Annea afzelii	ab	Prenner (unpubl. res.)	*Brenan 9002* (K)
	Isoberlinia angolensis	ab	Tucker (2002a, Fig. 24)	
	Julbernardia pellegriniana	ab	Tucker (2003b, Fig. 6P)	
	Macrolobium acaciifolium	ab	Prenner (unpubl. res.)	*Lewis 1676* (K)
	Microberlinia bisulcata	ab	Prenner (unpubl. res.)	*Brenan 9319* (K)
	Microberlinia brazzavillensis	ab	Tucker (2002a, Figs 50–52)	
	Bikinia durandii	ab	Tucker (2000a, Fig. 44)	
	Neochevalierodendron stephanii	ab	Tucker (2002b, Figs 22, 23, 25)	
	Saraca declinata	ab	Tucker (2000c, Figs 4H, I, 5A–B, D–F)	
	Saraca indica	ab	Tucker (2000c, Fig. 6I, K, L)	
	Schotia afra	ab	Tucker (2001a, Figs 62, 63, 66)	
	Schotia brachypetala	ab	Tucker (2001a, Figs 32, 35, 36)	
	Schotia latifolia	ab	Tucker (2001a, Fig. 81, 82)	
	Sindora klaineana	ab	Tucker (2003a, Fig. 3E–I, K)	
	Tamarindus indica	ab	Tucker (2000b, Figs 117, 122)	
	Tetraberlinia tubmanniana	ab	Tucker (2002b, Figs 50, 53)	
	Cercis canadensis	ad	Tucker (2002c, Fig. 4F)	
	Cicer arietinum	ad	Prenner (2002, Fig. 7e, f)	
	Dialium dinklagei	ad	Zimmerman *et al.* (2013, Fig. 9b, d)	
	Dialium guianense	ad	Zimmerman *et al.* (2013, Figs 5f, 6a)	
	Dialium guineense	ad	Tucker (1998, Figs 118, 119, 121, 123)	
	Dialium orientale	ad	Zimmerman *et al.* (2013, Figs 12e, 13e)	
	Dialium pentandrum	ad	Zimmerman *et al.* (2013, Fig. 3b, c, e)	
	Duparquetia orchidacea	ad	Prenner and Klitgaard (2008, Fig. 6K)	
	Gleditsia amorphoides	ad	Prenner (unpubl. res.)	*Cardoso 3829* (RB)
	Gleditsia caspica	ad	Tucker (1991, Fig. 69)	
	Gleditsia triacanthos	ad	Tucker (1991, Fig. 73)	
	Moldenhawera blanchetiana	ad	Prenner and Queiroz (unpubl. res.)	*Queiroz 16158* (HUEFS)
	Pterogyne nitens	ad	Prenner (unpubl. res.)	*Cardoso 3852* (ALCB)
	Senna pendula	ad	Tucker (2003c, Fig. 3H)	
	Senna aciphylla	ad	Marazzi and Endress (2008, Fig. 6H–I)	
	Senna alexandrina	ad	Prenner (unpubl. res.)	*Prenner 309* (GZU)
	Senna artemisioides	ad	Prenner (unpubl. res.)	*Prenner 372* (GZU)
	Senna corymbiflora	ad	Prenner (unpubl. res.)	*Prenner 348* (GZU)
	Senna marylandica	ad	Prenner (unpubl. res.)	*Prenner 317* (GZU)
	Senna mucronifera	ad	Marazzi and Endress (2008, Fig. 7J–L)	
	Senna spectabilis	ad	Prenner (unpubl. res.)	*Prenner 457* (GZU)
	Senna tonduzii	ad	Marazzi and Endress (2008, Fig. 8G–L)	
	Senna wislizeni	ad	Marazzi and Endress (2008, Fig. 5G–K)	
	Bauhinia forficata	st	Prenner (unpubl. res.)	*Owens s.n.* (K)
	Bauhinia galpinii	st	Prenner (unpubl. res.)	*Prenner 786* (K)
	Bauhinia tomentosa	st	Prenner (unpubl. res.)	*Prenner 789* (K)
	Ceratonia siliqua	st/ad	Tucker (1992, Fig. 36)	
	Caesalpinia coccinea	st	Prenner (unpubl. res.)	HK (1989-3075)
	Caesalpinia vesicaria	st	Tucker *et al.* (1985, Figs 36, 37)	
	Chamaecrista mimosoides	st	Prenner (unpubl. res.)	*Prenner 791* (K)
	Poeppigia procera	st	Zimmerman *et al.* (2013, Fig. 16e)	
	Petalostylis labicheoides	st	Tucker (1998, Figs 28–30)	
	Labichea lanceolata	st	Tucker (1998, Figs 83, 84)	
	Haematoxylum capechianum	st	Tucker and Kantz (1997, Fig. 41)	

(continued)

TABLE 1. *Continued*

Legume subfamilies	Species	Style bending	Reference	Voucher (unpublished data)
Mimosoideae	*Calliandra angustifolia*	ad	Prenner (2004c, Fig. 6a–c)	
	Neptunia strigillosa	st	Tucker (1988b, Figs 47, 49)	
	Pithecellobium unguis-cati	ad	Prenner (unpubl. res.)	*Prenner 142* (GZU)
Papilionoideae	*Ateleia guaraya*	nd	Prenner (unpubl. res.)	*Soto 1182* (HUEFS)
	Ateleia herbert-smithii	nd	Tucker (1990, Figs 39, 40)	
	Exostyles venusta	st	Mansano *et al.* (2002, Figs 88, 89)	
	Lecointea hatschbachii	st	Mansano *et al.* (2002, Figs 119–120)	
	Abrus precatorius	ad	Prenner (2013, Fig. 2D, E, F, H)	
	Aeschynomene falcata	ad	Sampaio *et al.* (2013, Fig. 3I, K)	
	Aeschynomene sensitiva	ad	Sampaio *et al.* (2013, Fig. 6L)	
	Alhagi persarum	ad	Khodaverdi *et al.* (2014, Fig. 6H, I)	
	Amburana cearensis	ad	Leite *et al.* (2015, Fig. 6J–L)	
	Amorpha fruticosa	ad	Tucker (1987, Fig. 62)	
	Angylocalyx braunii	ad	Prenner (unpubl. res.)	HK (1992-1994)
	Apios americana	ad	Prenner (2002, Figs 88e, 89a, b)	
	Astragalus caspicus	ad	Movafeghi *et al.* (2010, Figs 36–40)	
	Astragalus compactus	ad	Naghiloo *et al.* (2012, Fig. 8f, j)	
	Astragalus lagopodoides	ad	Movafeghi *et al.* (2011, Figs 38–42)	
	Bobgunnia madagascariensis	ad	Tucker (2003b, Figs 118, 119)	
	Cadia purpurea	ad	Tucker (2002d, Fig. 29)	
	Cajanus cajan	ad	Prenner (unpubl. res.)	*Prenner 608* (GZU)
	Calopogonium sp.	ad	Prenner (unpubl. res.)	*Prenner 505* (K)
	Camoensia scandens	ad	Prenner (unpubl. res.)	*Cardoso 3812* (RB)
	Castanospermum australe	ad	Tucker (1993, Fig. 53)	
	Cochliasanthus caracalla	ad	Prenner (2002, Fig. 104f)	
	Dahlstedtia pentaphylla	ad	Teixeira *et al.* (2009, Fig. 6E, F)	
	Dahlstedtia pinnata	ad	Teixeira *et al.* (2009, Fig. 7D, E)	
	Dalbergia brasiliensis	ad	Klitgaard (1999, Fig. 19)	
	Daviesia cordata	ad	Prenner (2004d, Fig. 2J)	
	Desmodium axillare	ad	Prenner (unpubl. res.)	*Prenner 598* (K)
	Desmodium lineatum	ad	Tucker (1987, Fig. 111)	
	Dipteryx rosea	ad	Prenner (unpubl. res.)	*Cardoso 3430* (HUEFS)
	Erythrina herbacea	ad	Tucker (1987, Fig. 67)	
	Erythrina rubrinervia	ad	Prenner (2002, Fig. 94a–c)	
	Glycine max	ad	Crozier and Thomas (1993, Figs 39–41)	
	Hedysarum vartum	ad	Khodaverdi *et al.* (2014, Fig. 4L, M)	
	Hymenolobium janeirense	ad	Prenner (unpubl. res.)	*Cardoso 3828* (ALCB)
	Indigofera apicata	ad	Paulino *et al.* (2011, Fig. 39)	
	Indigofera lespedezioides	ad	Paulino *et al.* (2011, Fig. 28)	
	Indigofera suffruticosa	ad	Paulino *et al.* (2011, Fig. 50)	
	Kennedia nigricans	ad	Prenner (2002, Fig. 98d)	
	Leptolobium bijugum	ad	Prenner (unpubl. res.)	*Cardoso 3604* (HUEFS)
	Lespedeza thunbergii	ad	Prenner (2004e, Fig. 2a, b)	
	Lonchocarpus violaceus	ad	Tucker (1987, Fig. 125)	
	Lotus berthelotii × maculatus	ad	Prenner (2002, Fig. 74c)	
	Lotus corniculatus	ad	Prenner (2003, Fig. 3c, d)	
	Lotus tetragonolobus	ad	Prenner (unpubl. res.)	*Prenner 607* (GZU)
	Medicago truncatula	ad	Benlloch *et al.* (2003, Figs 3H, 4A, B)	
	Myrocarpus frondosus	ad	Prenner (unpubl. res.)	*Lima 6905* (HUEFS)
	Onobrychis melanotricha	ad	Khodaverdi *et al.* (2014, Fig. 2N, O)	
	Pearsonia cajanifolia	ad	Prenner (unpubl. res.)	*Prenner 795* (K)
	Pearsonia sessilifolia	ad	Prenner (unpubl. res.)	*Prenner 797* (K)
	Petaladenium urceoliferum	ad	Prenner *et al.* (2015, Fig. 5G, I, M)	
	Phaseolus vulgaris	ad	Prenner (unpubl. res.)	*Prenner* 614 (GZU)
	Pisum sativum	ad	Ferrandiz *et al.* (1999, Fig. 2H)	
	Poecilanthe itapuana	ad	Prenner (unpubl. res.)	*Cardoso 3512* (HUEFS)
	Sophora davidii	ad	Prenner (2002, Fig. 116a)	
	Sophora flavescens	ad	Prenner (2002, Fig. 121c, d)	
	Styphnolobium japonicum	ad	Tucker (1994, Figs 38–40), Prenner (2002, Fig. 131a)	
	Swartzia aurosericea	ad	Tucker (2003b, Figs 25–29)	
	Swartzia laurifolia	ad	Tucker (2003b, Figs 58, 69)	
	Vicia faba	ad	Prenner (unpubl. res.)	*Prenner 605* (GZU)
	Wisteria floribunda	ad	Prenner (unpubl. res.)	*Prenner 472* (GZU)
	Wisteria sinensis	ad	Naghiloo and Dadpour (2010, Figs 2G–J, 3E–K)	

ab, abaxial; ad, adaxial; st, straight; nd, no distinct style.

are recurved at anthesis, five obovate to oblanceolate white petals (9–11 × 3–6 mm), ten stamens arranged in two whorls of five, and a single carpel (Fig. 2C–E). Sepals, petals, stamens, and the carpel are adnate to the distinct hypanthium, which is about 5 × 3 mm in size. The carpel develops into a strongly compressed fruit (Fig. 2F) enclosing one to three seeds.

The initiation of individual flowers occurs in an acropetal spiral, in either an anti-clockwise (Fig. 3A) or a clockwise direction (not shown). Oval-shaped flower primordia are formed in the axil of massive bracts (Fig. 3B) and soon two lateral bracteoles arise in a short sequence (Fig. 3C, D).

The bracteoles enlarge and they soon enclose the floral primordium, on which the first sepal is formed in abaxial position (Fig. 3E). The second sepal follows only after a longer plastochron during which the first sepal enlarges distinctly (Fig. 3F). The second sepal can be found either to the right of the median plane (Fig. 3F; anticlockwise sepal formation) or to the left of the median plane (Fig. 3G; clockwise sepal formation). The third sepal arises again after a longer plastochron during which S1 and S2 enlarge (Fig. 3G, H). The last sepal arises opposite S3 after a shorter plastochron (Fig. 3I). There are no traces visible of a fifth sepal. The sepals enlarge according to their initiation pattern and soon trichomes are formed on S1 and S2. The first sepal is the outermost and the fourth sepal the innermost (Fig. 3J). S3 overlaps with S2 (Fig. 3J, K).

Petal initiation does not start until all four sepals are formed and distinctly enlarged (Fig. 3K, L). The first petal is formed in abaxial position opposite the last-formed sepal (i.e. either to the left or the right of the median plane). Individual petals are formed in relatively quick succession in either anticlockwise (Fig. 4A) or clockwise (Fig. 4B) direction, thus following the direction of sepal formation.

Soon after the last petal is formed, the first median abaxial antesepalous stamen and the carpel primordium become discernible (Fig. 4A). Soon afterwards the two lateral antesepalous stamens are formed and the carpel enlarges rapidly (Fig. 4B). The two adaxial stamens are the last antesepalous stamens to emerge (Fig. 4C). Petal growth is relatively fast, so that the petals soon touch each other and cover the antesepalous stamen primordia (Fig. 4C, D). The carpel develops also relatively fast and stays visible for a longer period, during which the adaxial cleft is formed (Fig. 4C–G).

The inner stamen whorl is the last organ whorl to be formed. The earliest observed stage indicates the simultaneous formation of all five antepetalous stamens (Fig. 4E, H).

At this developmental stage cochlear ascending petal aestivation starts to manifest, in which the first-formed petal (P1) becomes the outermost petal and the adaxial petal (P4) the innermost petal (Fig. 4F, G, J, K). Because of this and because of the distinct variation in petal size (decrease from the abaxial towards the adaxial side of the flower), the buds show a distinct dorsiventral (or bisymmetrical) symmetry (Fig. 4F, G, J, K). Besides the strict cochlear ascending petal aestivation (Figs 4K and 5A), we found some variation in petal aestivation in that one or two lateral petals are found outermost (Fig. 5B, C, E) or one lateral petal becomes covered by an adaxial petal (Fig. 5D). However, in all of these examples the adaxial petal remains the innermost petal (Figs 5A–E and 7). Young petals have a characteristic wrinkled appearance (Fig. 5F, G) and individual cells show peculiar cuticular ridges and folds (Fig. 5H).

Anther development begins relatively late, starting in the outer stamen whorl with a distal broadening of the young stamen (Fig. 4H, I). Older anthers show a distinct pointed end of the connective (Figs 4L and 6B), which develops in mature anthers into a triangular gland (Fig. 5I–K). Stamen filaments elongate gradually in bud (Fig. 6B), twisting down finally in bud while the anthers stay in their original orientation (Fig. 6D). Pollen is tricolporate with a microreticulate tectum (Fig. 5L–O).

The gynoecium remains open (with unfused adaxial cleft) for a long period (Fig. 4H, I) and later the style bends in the abaxial direction (i.e. away from the inflorescence axis). Because of this the stylar suture is facing outwards and is visible (Figs 4L and 6A–E). The carpel is adnate to the adaxial part of the hypanthium, which also carries all other floral organs on its rim (Fig. 6D, E).

Ancestral reconstruction of style morphology in legumes

Our Bayesian phylogenetic analysis of a less densely sampled *matK* dataset (Fig. S1) is highly congruent with more complete legume phylogenies (Fig. 1; Bruneau *et al.*, 2008; Cardoso *et al.*, 2015). It showed a relatively well-resolved branching history of the legumes (Fig. S1), at least in resolving most deep nodes, the main lineages (Mimosoideae, Papilionoideae and the caesalpinioid clades Cercideae, Detarieae and Dialiinae), and the placement of *G. marginata* as an early branch of the Detarieae, but inadequate resolution with respect to the resin-producing detarioids and the *Amherstia* clade. This legume phylogeny was used to reconstruct the evolution of style bending characters at each ancestral node (Table 1, Fig. 8). Based on a broad literature search and on the study of previously unsampled taxa, we collected data on the direction of style bending (i.e. abaxial, adaxial or straight, or taxa with indistinct style) for 131 taxa representing all three subfamilies and most of the major lineages within them (Table 1). Parsimony and Bayesian ancestral reconstructions concur in showing an adaxially bending style during floral development as plesiomorphic in legumes, whereas the straight style evolved independently several times. Remarkably, style bending in the abaxial direction is revealed as a new, exclusive ontogenetic synapomorphy for the Detarioids (Fig. 8).

DISCUSSION

Loss of the fifth sepal due to long plastochrons

Sepal initiation starting in the median abaxial position and following a spiral in a clockwise or anticlockwise direction is frequently found in Caesalpinioideae s.l. (Tucker, 1984). However, in *G. marginata* only four sepals are formed and the fifth sepal is completely lost (i.e. we could not verify the formation of a primordium). This loss of the fifth sepal is accompanied by long plastochrons between the formation of the first and the second sepal and between the second and the third sepal, while sepals 3 and 4 are formed with a relatively short interval. In this way the first two sepals enlarge considerably
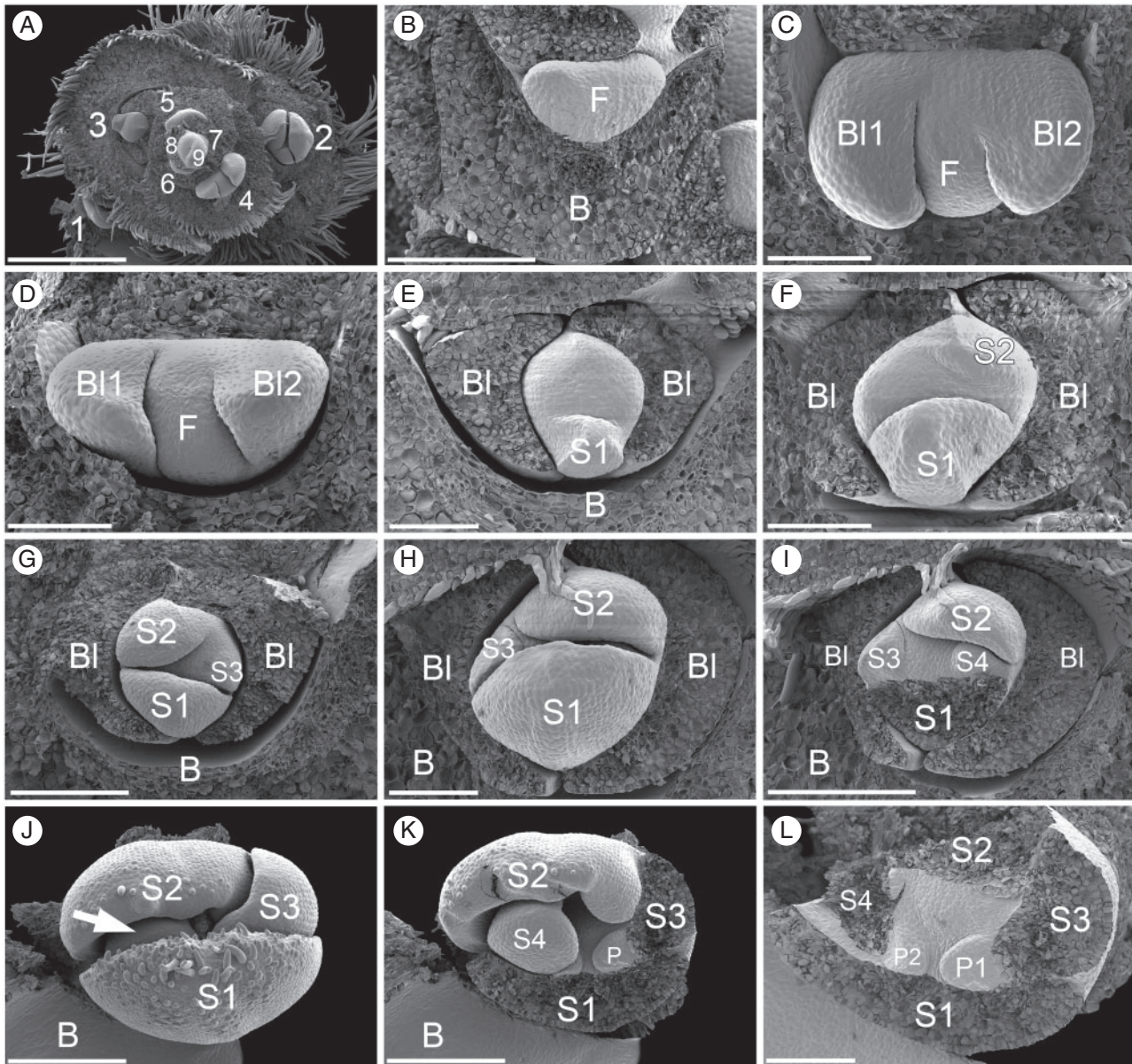
Fig. 3. Partial inflorescence and early floral development (SEM) in *G. marginata*. (A) Flowers and floral bracts (1–9) are formed in an anticlockwise spiral along the racemose partial inflorescence axis. (B) Kidney-shaped floral primordium in the axil of a bract. (C) Floral primordium flanked by two bracteoles, which arise in a short succession. (D) Frontal view of floral primordium and two lateral bracteoles. (E) The first sepal is formed in abaxial position. The sepal primordium is deformed at its flanks by the pressure of the two bracteoles (removed). (F) The second sepal arises in lateral adaxial position on the right after a longer plastochron during which S1 enlarges (pressure marks on S1 still discernible). (G) Sepal 2 is formed in lateral adaxial position on the left and the third sepal arises after a longer plastochron in transverse right position (clockwise sepal formation). (H) Anticlockwise sepal formation. S1 enlarges considerably and covers a large part of the floral meristem. S2 in lateral adaxial right position is also relatively large and S3 in transversal left position is relatively small in size. (I) Same as (H), S1 removed. A fourth sepal in transverse right position is slightly younger than S3. (J) Clockwise sepal formation. S1 has a distinct indumentum and hairs are starting to form on S2. S4 is just visible (arrow). (K) Same as (J) with S1 and S3 removed, showing the fourth sepal and the first-formed petal. (L) Same as (K) with S2 and S4 removed, showing successive formation of the two abaxial petals, of which P2 is younger than P1. B, bract; Bl, bracteole; F, floral primordium; P, petal; S, sepal. Scale bars: (A) = 500 μm; (B, G, J, K) = 200 μm; (C–F, H, L) = 100 μm.

and ultimately there is no meristem left for the formation of a fifth sepal primordium.

A strikingly similar pattern was found in the early-branching *Duparquetia orchidacea*, which also forms only four sepals and in which the first two sepals enlarge considerably and sepals 3 and 4 are formed in short succession in transverse

position (Prenner and Klitgaard, 2008). Here too, Prenner and Klitgaard (2008) concluded that there was not enough meristem left for the formation of a fifth sepal primordium. In contrast to this similarity in the sepal whorl, petal and stamen formation varies considerably among the two species (in *Duparquetia* petal formation is almost simultaneous,
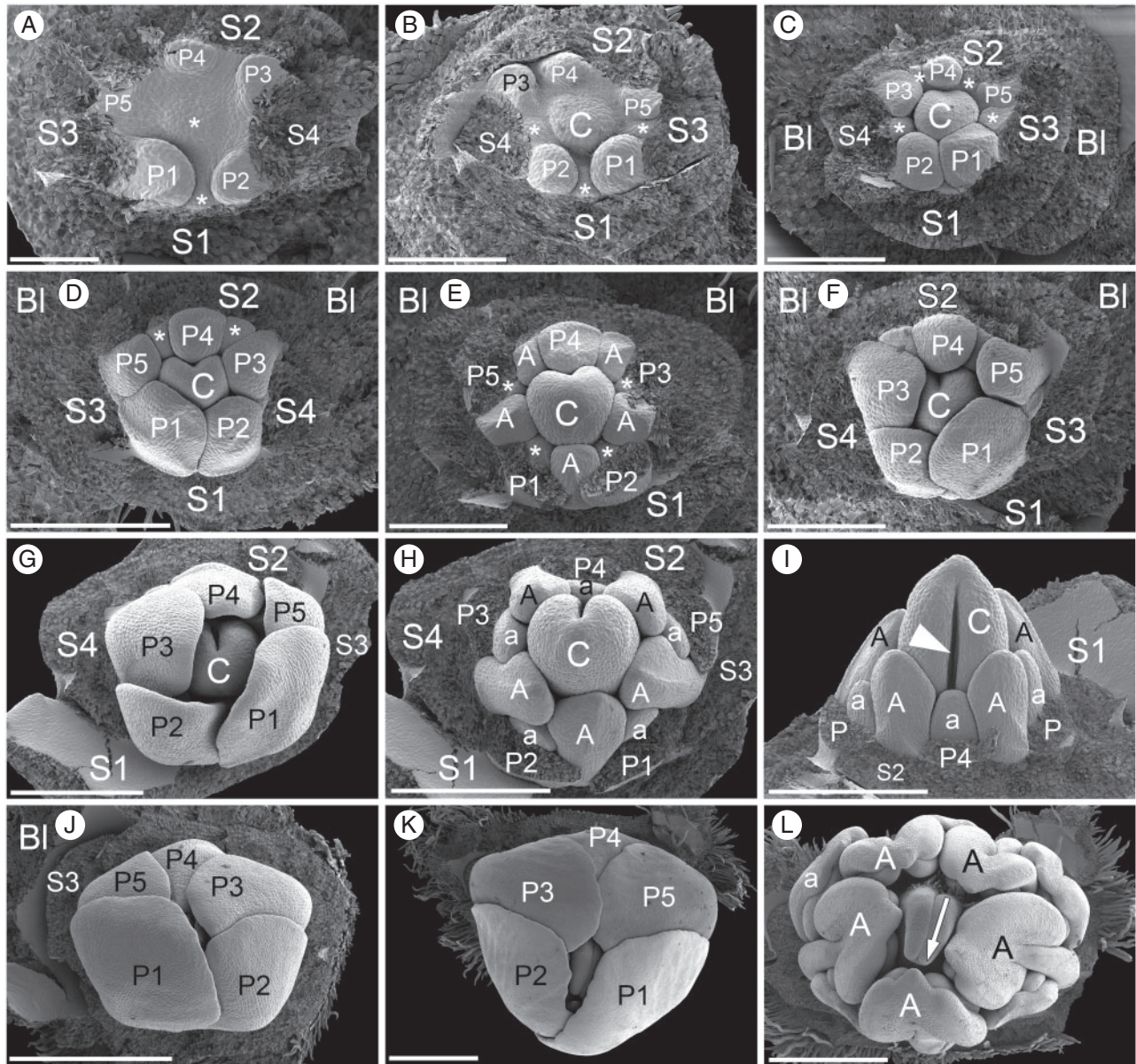
FIG. 4. Formation of petals, stamens and carpel (SEM) in *G. marginata*. (A) Young bud (sepals removed) with sequential petal formation in counter-clockwise direction. (B) Older developmental stage with petal formation in clockwise direction (sepals removed). The carpel primordium becomes visible concomitantly with the first three antesepalous stamen primordia (asterisks). (C) Somewhat older stage. Petals enlarge according to their clockwise initiation, carpel starts differentiation of adaxial cleft, and all five antesepalous stamens are formed (asterisks; abaxial stamen covered by petals). (D) Enlarging petals start to cover the floral bud. P1 and P2 are larger than the other three petals, which are similar in size. (E) Same as (D) with four petals removed to show antesepalous stamen, which are still undifferentiated, and four antepetalous stamen primordia (asterisks). The adaxial primordium is covered by the adaxial petal. (F) Petal aestivation starts to manifest. P1 overlaps P2, which overlaps P3. P4 and P5 remain small. (G) Ascending petal aestivation. The first-formed petal P1 is the outermost petal and the adaxial P4 is the innermost petal. (H) Same as (G) with petals removed. Stamens of the outer whorl broaden distally and start to differentiate anthers while inner whorl stamens remain small and undifferentiated. The carpel is the largest reproductive organ at this developmental stage. (I) Adaxial view of (H) showing stamens of the two whorls in comparison and the distinctly open carpel cleft (arrowhead) which does not extend to the top of the carpel. (J, K) Ascending petal aestivation in a flower with anticlockwise (J) and clockwise (K) petal formation. P1 is the outermost and P4 the innermost petal in both. (L) Same as (K) with petals removed. Anthers are formed in both stamen whorls and the developing style bends towards the abaxial side of the flower (arrow). A, antesepalous stamen; a, antepetalous stamen; Bl, bracteole; C, carpel; P, petal; S, sepal. Scale bars: (A) = 100 μm; (B, C, E, F, H) = 200 μm; (D, G) = 300 μm; (J–L) = 500 μm.

petal aestivation is cochlear descending and there are only four stamens formed, as shown by Prenner and Klitgaard, 2008). This shows on the one hand that there are ontogenetic patterns such as the complete loss of an organ in taxa that are not closely related, based on similar ontogenetic patterns, and on the other hand that organ formation among different organ whorls is controlled independently in the two species (i.e. the formation of subsequent organ whorls such as petals and stamens does not seem to be influenced by the loss of a sepal).
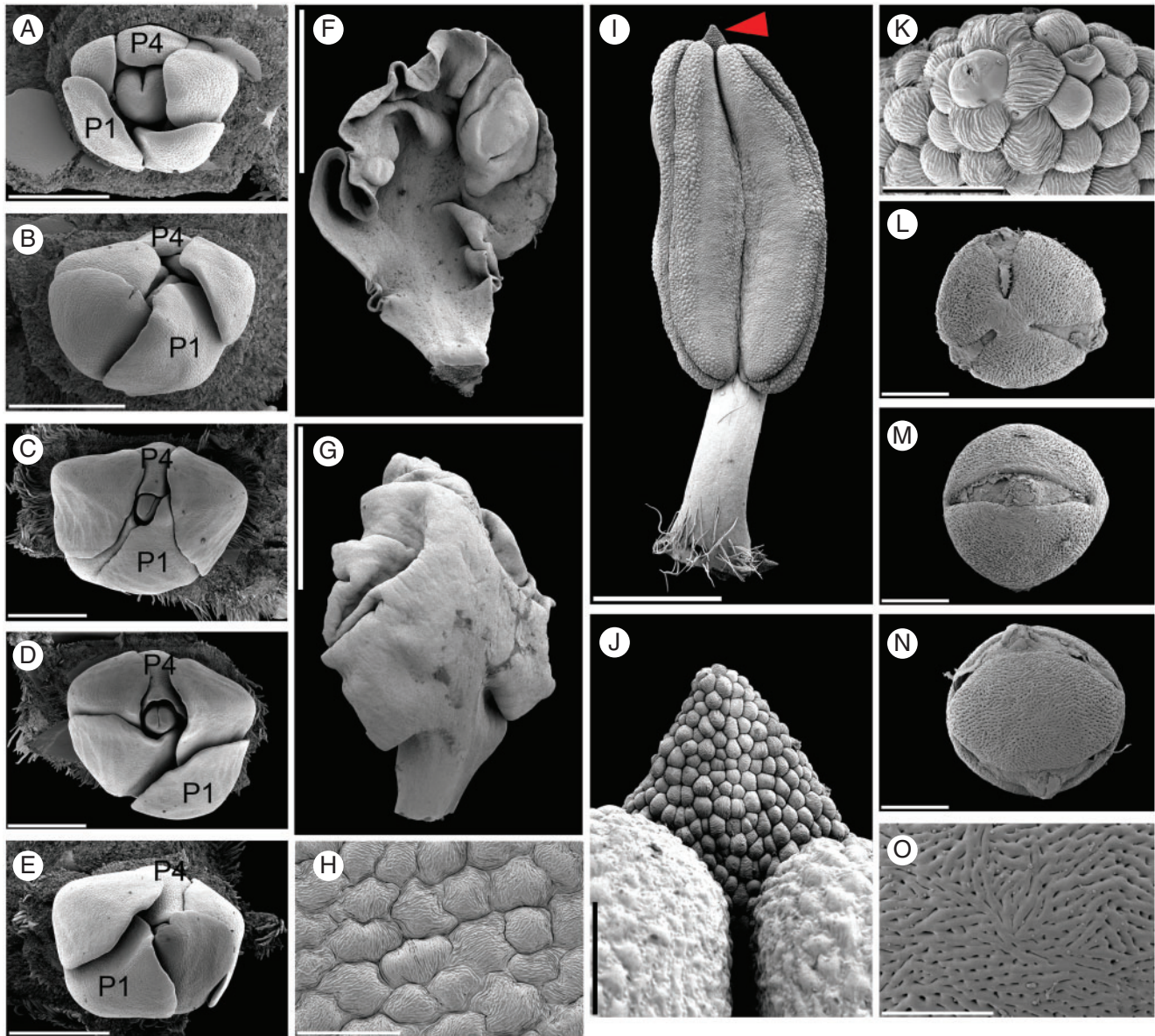
FIG. 5. Petal aestivation and anther and pollen morphology (SEM) in *G. marginata*. (A–E) Variation in petal aestivation. Note that the adaxial petal (P4) is the innermost petal in all. P1 in (A) and (E) on the left (clockwise petal initiation) and in (B–D) on the right (anticlockwise petal initiation). Two lateral petals outermost in (C). (F–H) Wrinkled petals from older flower. (F) ventral (adaxial side). (G) dorsal (abaxial side). (H) detail of (G) showing cell pattern and detail of cell surface (wrinkled cuticle). (I) Stamen from young bud showing a well-formed filament with proximal hairs, an anther composed of four thecae and a terminal glandular structure (arrowhead). (J) Detail of (I); gland on top of the anther. (K) Detail of (J) showing detail of cells and a stoma. (L–O) Tricolporate pollen grains with microreticulate tectum. (L) Polar view. (M, N) Equatorial views. (O) Detail of (N) showing microreticulate ornamentation of the exine. P, petal. Scale bars: (A) = 300 μm; (B) = 400 μm; (C–E) = 500 μm; (F, G) = 2 mm; (H, K) = 30 μm; (I) = 1 mm; (J) = 100 μm; (L–N) = 10 μm; (O) = 5 μm.

A tetramerous calyx is present in most Detarieae, except in the *Brownea* clade and most of the *Berlinia* clade (Bruneau *et al.*, 2014). However, so far it has been shown that tetramery is the result of fusion of the two adaxial sepals (Tucker, 2000*a*, *b*, *c*, 2002*a*, *b*) and that it is not based on the complete loss of a sepal, as shown for *Goniorrhachis* in this study. For *Barnebydendron*, which is sister to *Goniorrhachis* (Fig. 1), Tucker (2002*b*) mentions the helical initiation of five sepals. This indicates that the complete loss of a sepal is a rare and probably not homologous

event in Caesalpinioideae s.l. Besides *Duparquetia* mentioned above, it was only reported in *Aphanocalyx djumaensis*, in which only one sepal is formed (Tucker, 2000*a*). A tetramerous calyx is found in some Mimosoideae, such as *Mimosa* and some *Acacia* (Gemmeke, 1982; Prenner, 2011), while in the papilionoid legumes pentamery is manifested almost throughout and organ reduction occurs only in some taxa, such as *Swartzia* (Tucker, 2003*b*), *Amorpha* (McMahon and Hufford, 2005) and *Abrus* (Prenner, 2013).
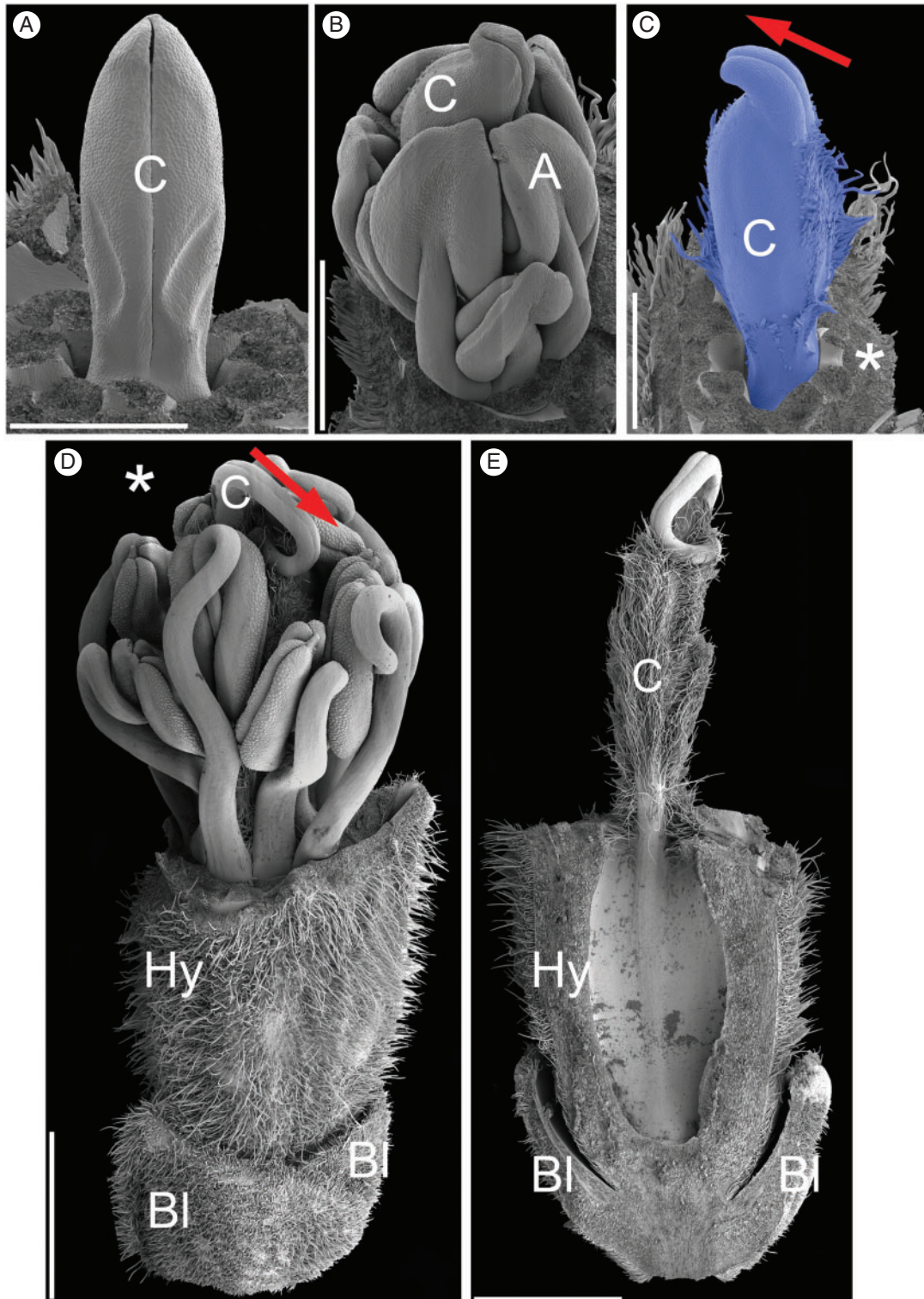
FIG. 6. Carpel and androecium development (SEM) in *G. marginata*. (A) Young carpel, adaxial view. Note that the carpel margins are still unfused at the very distal end and that the carpel shows strong imprints/pressure marks from the androecium. (B) Older bud in which a style is forming, which bends in the abaxial direction, away from the inflorescence axis, which lies on the right in this image. Anthers are differentiated in both stamen whorls, which are discernible because of their difference in length. (C) Same as (B) with stamen removed, showing the young carpel (coloured in blue) with the young style bending in abaxial direction. In this way the suture is pointing outwards and away from the flower (arrow; asterisk indicates the adaxial side of the flower). (D) Older bud with free stamens in two whorls. The filaments grow first upwards and then downwards again. The anthers, however, retain their original orientation. The carpel in the centre shows a sharply 'backward' bending of the style (i.e. in abaxial direction; arrow) (sepals and petals removed). Note two small bracteoles at the base and a long hypanthium. (E) Adaxial view of opened flower showing two lateral bracteoles and a long hypanthium, on the rim of which the carpel is adnate. A bulge running from the place of adnation to the base of the flower indicates the adnation of the carpel with the hypanthium. Style bending in abaxial direction (towards the viewer; carpellar suture facing outwards).

C, carpel; A, outer whorl stamen; Bl, bracteole; Hy, hypanthium. Scale bars: (A) = 400 µm; (B, C) = 500 µm; (D, E) = 2 mm.
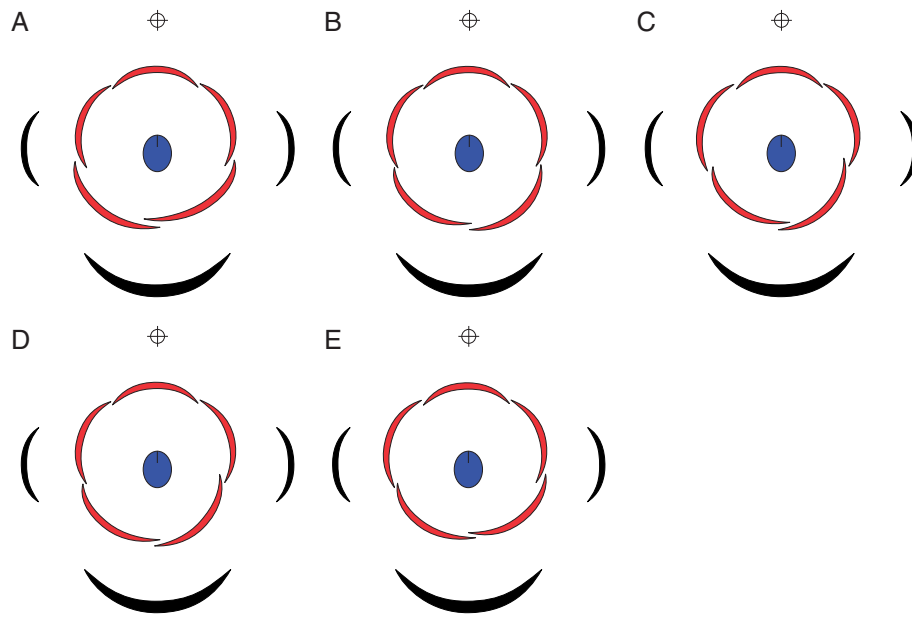
FIG. 7. Variable petal aestivation depicted in floral diagrams, showing the subtending bract in abaxial/lowermost position, two lateral bracteoles, five petals in red and the carpel in blue. Note that the adaxial petal is the innermost in all. (A, B) Ascending petal aestivation with either the abaxial left (A) or the abaxial right (B) petal outermost. (C) The two lateral petals are outermost. (D, E) Either the lateral left (D) or the lateral right (E) petal is in outermost position.

The Goniorrhachis *corolla: a typically dorsiventral caesalpinioid morphology transformed into a Rosaceae-like radially symmetrical corolla at anthesis*

In *Goniorrhachis* the first petal follows the direction of the sepals and therefore is formed in abaxial position, either to the left or to the right of the median plane. The second petal is also formed in abaxial position, indicating unidirectional organ formation from the abaxial towards the adaxial side of the flower, which is frequently found in caesalpinioid and particularly in papilionoid legumes (Tucker, 1984). But strict unidirectionality, in which the two lateral petals would appear next, is disturbed in *Goniorrhachis*, because the remaining three petals are formed sequentially, in either a clockwise or an anticlockwise direction. In this way the last-formed petal is either the left or the right petal in transverse position.

Nonetheless, the adaxial petal, which is always the second to last-formed petal, remains the smallest during early and mid-developmental stages. It is covered by the two lateral petals and thus it becomes the innermost petal in a dorsiventral corolla with cochlear ascending petal aestivation (Figs 4J, K, 5A–E and 7). This is the typical caesalpinioid pattern of petal aestivation and it contrasts with the descending petal aestivation in papilionoid legumes, valvate petal aestivation in Mimosoideae and contort petal aestivation in Rosaceae (Eichler, 1878; Ronse De Craene, 2010). Based on our ontogenetic data, we cannot verify the oblique position of the flowers, as shown in a diagram by Taubert (1892, Table III-4). We can rather confirm that the flowers are oriented in the conventional caesalpinioid way with a petal in median adaxial/uppermost position. The dorsiventral symmetry, which is evident during early petal formation, is lost during later flower development, so that the anthetic flowers display almost perfect radial symmetry and the mature flowers strongly resemble flowers of Rosaceae, such as

cherries (*Prunus* sp.) or apples (*Malus* sp.) (Ronse De Craene, 2010). Bruneau *et al.* (2014) showed that within Detarieae there were repeated switches from zygomorphy to actinomorphy and vice versa, but, interestingly, in Caesalpinioideae s.l. zygomorphy is not clearly associated with a higher diversification rate (contrary to Papilionoideae; e.g. Cardoso *et al.*, 2013).

While in all studied buds the adaxial median petal is in the innermost position, there are some variations in the strictly cochlear ascending aestivation (Fig. 7). Nonetheless, our results show that certain caesalpinioid characters, such as the position of the innermost adaxial petal, are deeply grounded in the plant genome. Deviations from ascending cochlear aestivation are rare in Caesalpinioideae s.l. In *Duparquetia orchidacea* petal aestivation is cochlear descending (Prenner and Klitgaard, 2008), which is the common pattern in Papilionoideae. However, in Papilionoideae there is some variation in petal aestivation among early-branching taxa (e.g. Mansano *et al.*, 2002; Tucker, 2002*d*).

The androecium in Goniorrhachis

The androecium in *G. marginata* consists of ten stamens in two whorls of five, which matches the floral ground plan for the legume family. Stamen initiation is slightly unidirectional in the outer whorl and more or less simultaneous in the inner whorl. These are relatively common developmental pathways among caesalpinioid legumes (Tucker, 1987). However, the mature androecium is noteworthy because of the triangular anther glands, which are a distal outgrowth from the anther connective and which are here reported for the first time in the genus. Reports of anther glands are rare in Caesalpinioideae s.l. and in Papilionoideae. Anther glands in the caesalpinioid *Burkea* are described as relatively simple in comparison with
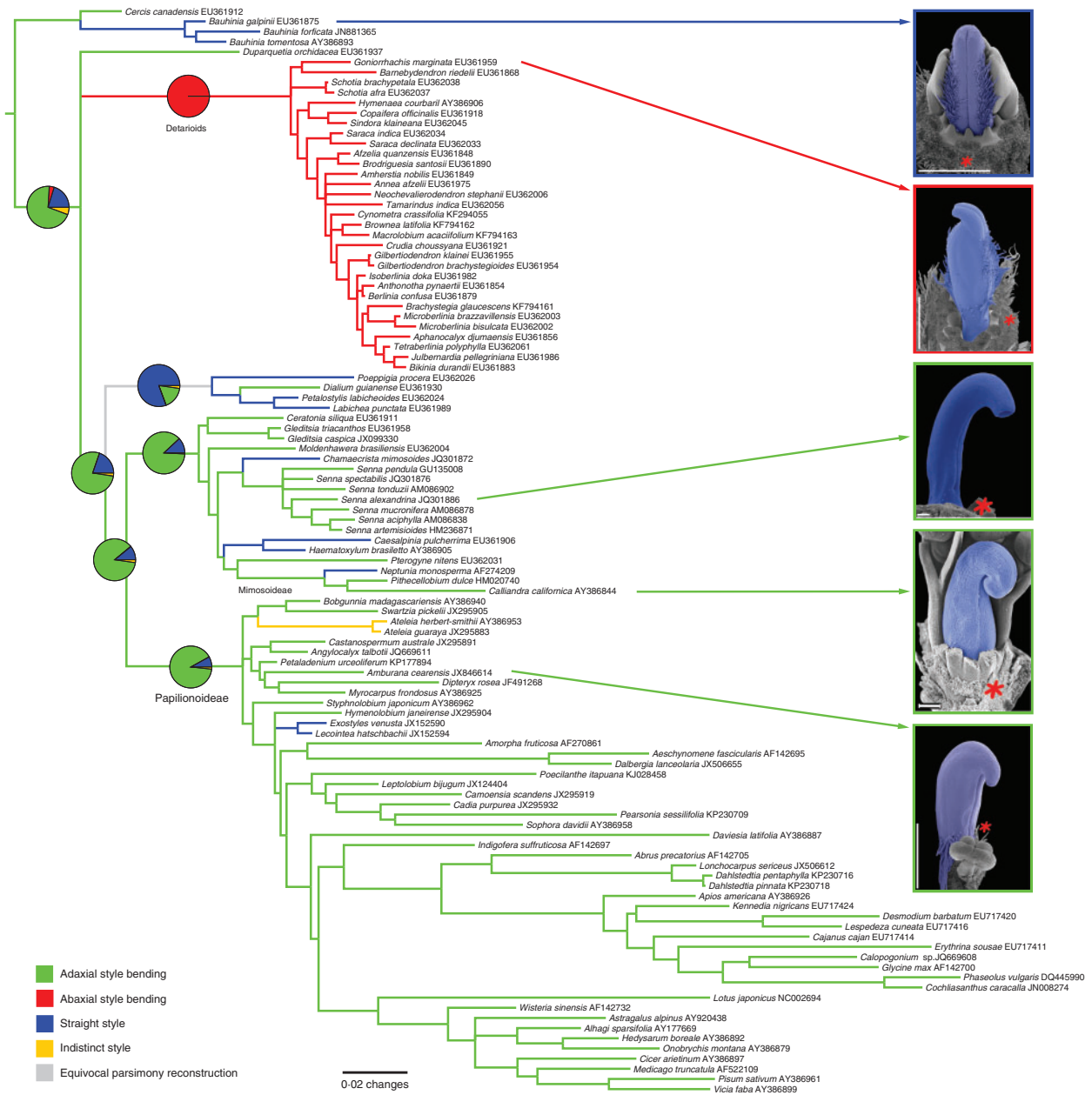
FIG. 8. A *matK* Bayesian majority-rule consensus tree of 1000 randomly selected trees used to reconstruct the evolution of ancestral states in style development in legumes. The coloured branches represent parsimony optimization as implemented in Mesquite. The pie charts on specific nodes show the proportions of the mean posterior probability from BayesTraits optimization. The detarioid legumes or Detarieae as circumscribed by Bruneau *et al.* (2008) are uniquely marked by the evolution of style bending abaxially during flower ontogeny (red optimization and red-framed SEM image). The red asterisk (*) on the SEM images marks the position of the adaxial petal. See Fig. S1 for a complete version of this figure that includes branch supports as posterior probabilities.

Mimosoideae (Luckow and Grimes, 1997), and Tucker (1996) shows tufts of hairs on the connective tip in the papilionoid *Indigofera incarnata* and narrow and attenuate connective tips in *Indigofera hirsuta*. But there is no evidence that these structures are glandular.

In contrast to this, anther glands are frequently found in Mimosoideae, where a broad range of structures has been described (Luckow and Grimes, 1997; Barros and Teixeira, 2016). The gland-like outgrowths found in *Goniorrhachis* resemble the *Prosopis africana* type *sensu* Barros and Teixeira

(2016), which occurs in *P. africana* only (and which does not occur in the other members of this genus, in which the anther gland is stalked).

Another interesting character of the androecium in the mature bud is the coiling of the filaments, which make a full turn in the bud while the anthers stay in their original position. In the anthetic flower, filaments straighten quickly and the versatile anthers are presented towards flower visitors.

The hypanthium is a common character among Detarieae, where it is only missing in *Gilbertiodendron* (Tucker, 2002*b*)

and some species of *Cynometra* (Tucker, 2001*a*). Hypanthia can be also found in the caesalpinioids Cercideae, *Ceratonia* and *Cassia* and frequently in the Caesalpinieae. The attachment of the ovary to the side of the hypanthium is also a common character found in most Detarieae, except in *Didelotia*, *Librevillea*, *Barnebydendron*, *Sindora* and *Tessmannia* (cf. Tucker, 2002*b*, 2003*a*). From these taxa, a report on the neotropical monotypic genus *Barnebydendron* is of special interest because it is the sister taxon to *Goniorrhachis* (Fig. 1). The putatively bird-pollinated flowers of *Barnebydendron* do not share many characters with the flowers of *Goniorrhachis*. Mature flowers also show four sepals, but according to Tucker (2002*b*) five sepals are developmentally formed in helical direction, whereas only four sepals are formed in *Goniorrhachis*. The corolla of *Barnebydendron* is dorsiventral and formed of three petals, with occasionally two vestigial petals. The androecium consists of ten stamens, but they are fused in a diadelphous pattern (9+1), and both stamens and anthers are dimorphic (Warwick *et al*., 2008). According to Arroyo (1981) the pink to crimson red flowers of *Barnebydendron* are likely to be pollinated by hummingbirds. This is in contrast to *Goniorrhachis*, which is most probably insect-pollinated.

The abaxially bending style as a new synapomorphy for Detarieae

The carpel in *Goniorrhachis* terminates the flower and uses up all of the remaining meristematic tissue in the centre of the flower. Carpel formation starts early, simultaneously with the onset of antepetalous stamen formation. This precocious formation of the carpel is a common character in legumes and has not been fully explored so far. In mid-development it is noteworthy that the adaxial cleft remains open over a longer period so that the carpel can be classified as 'open'. Open carpels were recently reviewed by Endress (2015), who has reservations against the description of this character in legumes, in which the long ovaries could be the reason for an aberrant opening of the carpel during material preparation.

Another remarkable character of *Goniorrhachis* floral development is the bending of the young style away from the inflorescence axis and towards the abaxial side of the flower. In this way the adaxial suture becomes visible in top view and the character is therefore easily recognizable in younger buds. Only during anthesis does the style straighten and the character becomes less obvious.

A review of the literature and an investigation of spirit-preserved material permitted us to reconstruct the evolution of style bending across all main legume clades. We show that the bending of the style in abaxial direction is restricted to Detarieae (Fig. 8). In the majority of the studied taxa the style bends in the adaxial direction (i.e. towards the inflorescence axis) and the adaxial suture is not visible in top view (Table 1). This feature is inferred to be plesiomorphic within the legume family (Fig. 8). Taxa with styles that remain more or less straight in bud have evolved at least six times independently (Fig. 8) and the papilionoid genus *Ateleia* is a rare example with an indistinct style (Tucker, 1990; Fig. 8). We therefore propose the abaxially bending style as a valuable and newly described synapomorphy for Detarieae. Studies that will focus on the physics and a spatial analysis of floral buds in Leguminosae are currently under way and will help us understand this peculiar behaviour of the style in Detarieae. The morphological background of this character, which has not received special attention so far, is unknown and further work is necessary to elucidate potential reasons for the difference and to understand why it evolved exclusively in the Detarioid clade.

Outlook

In the present study we show that in-depth analysis of the floral ontogeny and morphology of previously unstudied taxa can reveal a wealth of characters that can be used for classification on various taxonomic levels. We propose 'style bending towards the abaxial side' as a new exclusive synapomorphy for Detarieae that can be readily used as a diagnostic character for all included genera in the clade. The new character can be easily detected with a hand lens in opened flower buds and therefore is of interest for a broad range of scientists, from field botanists to herbarium-based taxonomists. It remains to be studied why style bending in the abaxial direction was manifested exclusively in a lineage of caesalpinioid legumes and why this character does not show up more frequently within the family. We speculate that the morphology of the carpel, spatial conditions within the bud and/or spatiotemporal processes during bud and carpel development are potentially responsible for the formation of this character.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: a *matK* Bayesian majority-rule consensus tree of 107 accessions across major lineages of legumes that have either published information on style bending or that were investigated in the course of this study. The placement of the dry forest-inhabiting *G. marginata* is highlighted. The graph was generated using the online tools in AWTY (Nylander *et al*., 2008) and shows the convergence of posterior probabilities for all taxon bipartitions, as derived from MCMC runs in MrBayes. Photograph of the flower of *G. marginata* by Domingos Cardoso.

ACKNOWLEDGEMENTS

LITERATURE CITED

Arroyo MTK. 1981. Breeding systems and pollination biology in Leguminosae. In: Polhill RH, Raven PH, eds. *Advances in legume systematics, part 1.* Kew: Royal Botanic Gardens, 723–769.

Barros TC, Teixeira SP. 2016. Revisited anatomy of anther glands in mimosoids (Leguminosae). *International Journal of Plant Sciences* **177**: 18–33.

Benlloch R, Navarro C, Beltran JP, Canas LA. 2003. Floral development of the model legume *Medicago truncatula*: ontogeny as a tool to better characterize homeotic mutations. *Sexual Plant Reproduction* **15**: 231–241.

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

Bruneau A, Klitgaard BB, Prenner G, Fougere-Danezan M, Tucker SC. 2014. Floral evolution in the Detarieae (Leguminosae): phylogenetic evidence for labile floral development in an early-diverging legume lineage. *International Journal of Plant Sciences* **175**: 392–417.

Cardoso D, Queiroz LP, Pennington RT, et al. 2012a. Revisiting the phylogeny of papilionoid legumes: new insights from comprehensively sampled early-branching lineages. *American Journal of Botany* **99**: 1991–2013.

Cardoso D, Lima HC, Rodrigues RS, Queiroz LP, Pennington RT, Lavin M. 2012b. The realignment of *Acosmium* sensu stricto with the dalbergioid clade (Leguminosae, Papilionoideae) reveals a proneness for independent evolution of radial floral symmetry among early branching papilionoid legumes. *Taxon* **61**: 1057–1073.

Cardoso D, Pennington RT, Queiroz LP, et al. 2013. Reconstructing the deep-branching relationships of the papilionoid legumes. *South African Journal of Botany* **89**: 58–75.

Cardoso D, São-Mateus WMB, Cruz DT, et al. 2015. Filling in the gaps of the papilionoid legume phylogeny: the enigmatic Amazonian genus *Petaladenium* is a new branch of the early-diverging Amburaneae clade. *Molecular Phylogenetics and Evolution* **84**: 112–124.

Crozier TS, Thomas JF. 1993. Normal floral ontogeny and cool temperate-induced aberrant floral development in *Glycine max* (Fabaceae). *American Journal of Botany* **80**: 429–448.

Eichler AW. 1878. *Blüthendiagramme*. Leipzig: W. Engelmann.

Endress PK. 2015. Patterns of angiospermy development before carpel sealing across living angiosperms: diversity, and morphological and systematic aspects. *Botanical Journal of the Linnean Society* **178**: 556–591.

Ferrandiz C, Navarro C, Gomez MD, Canas LA, Beltran JP. 1999. Flower development in *Pisum sativum*: from the war of the whorls to the battle of the common primordia. *Developmental Genetics* **25**: 280–290.

Gemmeke V. 1982. Entwicklungsgeschichtliche Untersuchungen an Mimosaceen-Blüten. *Botanische Jahrbücher für Systematik* **103**: 185–210.

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**: 2310–2314.

Huelsenbeck JP, Larget B, Miller RE, Ronquist F. 2002. Potential applications and pitfalls of Bayesian inference in phylogeny. *Systematic Biology* **51**: 673–688.

Khodaverdi M, Movafeghi A, Dadpour MR, Naghiloo S, Ranjbar M, Prenner G. 2014. Comparative study of floral development in *Onobrychis melanotricha*, *Hedysarum varium* and *Alhagi persarum* (Leguminosae: Papilionoideae: Hedysareae). *Flora* **209**: 23–33.

Klitgaard BB. 1999. Floral ontogeny in tribe Dalbergieae (Leguminosae: Papilionoideae): *Dalbergia brasiliensis*, *Machaerium villosum* s.l., *Platymiscium floribundum*, and *Pterocarpus rotundifolius*. *Plant Systematics and Evolution* **219**: 1–25.

Leite VG, Teixeira SP, Mansano VF, Prenner G. 2015. Floral development of the early branching papilionoid legume *Amburana cearensis* (Leguminosae) reveals rare and novel characters. *International Journal of Plant Sciences* **176**: 94–106.

Lewis G, Schrire B, Mackinder B, Lock M. eds. 2005. *Legumes of the world.* Kew: Royal Botanic Gardens.

Lima HC. 2016. *Goniorrhachis*. In: Forzza RC, Leitman PM, Costa A, *et al.*, organizers. *Lista de espécies da flora do Brasil*. Rio de Janeiro: Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB79069 (last accessed 3 November 2016).

LPWG (Legume Phylogeny Working Group). 2013a. Towards a new classification system for legumes: progress report from the 6th International Legume Conference. *South African Journal of Botany* **89**: 3–9.

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

LPWG (The Legume Phylogeny Working Group). 2017. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon, in press.

Luckow M, Grimes J. 1997. A survey of anther glands in the mimosoid legume tribes Parkieae and Mimoseae. *American Journal of Botany* **84**: 285–297.

Mackinder B. 2005. Detarieae. In: Lewis G, Schrire B, Mackinder B, Lock M, eds. *Legumes of the world.* Kew: Royal Botanic Gardens, 69–109.

Maddison WP, Maddison DR. 2015. *Mesquite: a modular system for evolutionary analysis.* Version 3.04. http://mesquiteproject.org (last accessed 3 November 2016).

Mansano VF, Tucker SC, Tozzi AMGA. 2002. Floral ontogeny of *Lecointea*, *Zollernia*, *Exostyles*, and *Harleyodendron* (Leguminosae: Papilionoideae: Swartzieae s.l.). *American Journal of Botany* **89**: 1553–1569.

Marazzi B, Endress PK. 2008. Patterns and development of floral asymmetry in *Senna* (Leguminosae, Cassiinae). *American Journal of Botany* **95**: 22–40.

Marazzi B, Ané D, Simon M, Delgado-Salinas A, Luckow M, Sanderson MJ. 2012. Locating evolutionary precursors on a phylogenetic tree. *Evolution* **66**: 3918–3930.

McMahon M, Hufford L. 2005. Evolution and development in the amorphoid clade (Amorpheae: Papilionoideae: Leguminosae): petal loss and dedifferentiation. *International Journal of Plant Sciences* **166**: 383–396.

Movafeghi A, Dadpour MR, Naghiloo S, Farabi S, Omidi Y. 2010. Floral development in *Astragalus caspicus* Bieb. (Leguminosae: Papilionoideae: Galegeae). *Flora* **205**: 251–258.

Movafeghi A, Naghiloo S, Dadpour MR. 2011. Inflorescence and floral development in *Astragalus lagopoides* Lam. (Leguminosae: Papilionoideae: Galegeae). *Flora* **206**: 219–226.

Naghiloo S, Dadpour MR. 2010. Floral ontogeny in *Wisteria sinensis* (Fabaceae: Faboideae: Millettieae) and its systematic implications. *Australian Systematic Botany* **23**: 393–400.

Naghiloo S, Dadpour MR, Movafeghi A. 2012. Floral ontogeny in *Astragalus compactus* (Leguminosae: Papilionoideae: Galegeae): variable occurrence of bracteoles and variable patterns of sepal initiation. *Planta* **235**: 793–805.

Nylander JAA. 2004. *MrModeltest v2. Evolutionary Biology Centre*, Uppsala University. Program distributed by the author.

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**: 581–583.

Oliveira-Filho AT. 2014. *NeoTropTree, Flora arbórea da Região Neotropical: um banco de dados envolvendo biogeografia, diversidade e conservação.* Universidade Federal de Minas Gerais. http://www.icb.ufmg.br/treeatlan/ (last accessed 3 November 2016).

Pagel, MD, Meade A. 2014. *BayesTraits v2.0.* http://www.evolution.reading.ac.uk/BayesTraits.html (last accessed 3 November 2016).

Pagel M, Meade A, Barker D. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* **53**: 673–684.

Paulino JV, Groppo M, Teixeira SP. 2011. Floral developmental morphology of three *Indigofera* species (Leguminosae) and its systematic significance within Papilionoideae. *Plant Systematics and Evolution* **292**: 165–176.

Paulino JV, Prenner G, Mansano VF, Teixeira SP. 2014. Comparative development of rare cases of a polycarpellate gynoecium in an otherwise monocarpellate family, Leguminosae. *American Journal of Botany* **101**: 572–586.

Prenner G.2002. *Floral development in Fabaceae – new aspects in selected genera*. PhD Thesis, Karl Franzens University of Graz, Austria.

Prenner G. 2003. A developmental analysis of the inflorescence and the flower of *Lotus corniculatus* (Fabaceae-Loteae). *Mitteilungen des Naturwissenschaftlichen Vereines Steiermark* **133**: 99–107.

Prenner G. 2004a. New aspects in floral development of Papilionoideae: initiated but suppressed bracteoles and variable initiation of sepals. *Annals of Botany* **93**: 537–545.

Prenner G. 2004b. The asymmetric androecium in Papilionoideae (Leguminosae): definition, occurrence, and possible systematic value. *International Journal of Plant Sciences* **165**: 499–510.

Prenner G. 2004c. Floral ontogeny in *Calliandra angustifolia* (Leguminosae: Mimosoideae: Ingeae) and its systematic implications. *International Journal of Plant Sciences* **165**: 417–426.

Prenner G. 2004d. Floral development in *Daviesia cordata* (Leguminosae: Papilionoideae: Mirbelieae) and its systematic implications. *Australian Journal of Botany* **52**: 285–291.

Prenner G. 2004e. Floral ontogeny in *Lespedeza thunbergii* (Leguminosae: Papilionoideae: Desmodieae): variations from the unidirectional mode of organ formation. *Journal of Plant Research* **117**: 297–302.

Prenner G. 2011. Floral ontogeny of *Acacia celastrifolia*: an enigmatic mimosoid legume with pronounced polyandry and multiple carpels. In: Wanntorp L, Ronse De Craene LP, eds. *Flowers on the tree of life*. Cambridge: Cambridge University Press, 256–278.

Prenner G. 2013. Floral development in *Abrus precatorius* (Leguminosae: Papilionoideae: Abreae) and a review of androecial characters in Papilionoideae. *South African Journal of Botany* **89**: 210–218.

Prenner G, Klitgaard BB. 2008. Towards unlocking the deep nodes of Leguminosae: floral development and morphology of the enigmatic *Duparquetia orchidacea* (Leguminosae, Caesalpinioideae). *American Journal of Botany* **95**: 1349–1365.

Prenner G, Cardoso D, Zartman CE, Queiroz LP. 2015. Flowers of the early-branching papilionoid legume *Petaladenium urceoliferum* display unique morphological and ontogenetic features. *American Journal of Botany* **102**: 1780–1793.

Queiroz LP. 2006. The Brazilian caatinga: phytogeographical patterns inferred from distribution data of the Leguminosae. In: Pennington RT, Lewis GP, Ratter JA, eds. *Neotropical savannas and dry forests: plant diversity, biogeography, and conservation*. Oxford: Taylor & Francis CRC Press, 113–149.

Queiroz LP. 2009. *Leguminosas da Caatinga*. Feira de Santana: Universidade Estadual.

Rambaut A. 2014. *FigTree, a graphical viewer of phylogenetic trees*. http://tree.bio.ed.ac.uk/software/figtree/.

Ramos G, Lima HC, Prenner G, Queiroz LP, Zartman CE, Cardoso D. 2016. Molecular systematics of the Amazonian genus *Aldina*, a phylogenetically enigmatic ectomycorrhizal lineage of papilionoid legumes. *Molecular Phylogenetics and Evolution* **97**: 11–18.

Ronquist F. 2004. Bayesian inference of character evolution. *Trends in Ecology and Evolution* **19**: 475–481.

Ronquist F, Teslenko M, van der Mark P, *et al.* 2012. MrBayes 3·2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Ronse de Crane LP. 2010. *Floral diagrams*. Cambridge: Cambridge University Press.

Saarela JM, Rai HS, Doyle JA, *et al.* 2007. Hydatellaceae identified as a new branch near the base of the angiosperm phylogenetic tree. *Nature* **446**: 312–315.

Sampaio DS, Moco MCC, Mariath JEA. 2013. Floral ontogeny of *Aeschynomene falcata* and *A. sensitiva* (Leguminosae: Papilionoideae) supports molecular phylogenetic data. *Plant Systematics and Evolution* **299**: 499–513.

Santos RM, Oliveira-Filho AT, Eisenlohr PV, Queiroz LP, Cardoso DBOS, Rodal MJN. 2012. Identity and relationships of the arboreal Caatinga among other floristic units of seasonally dry tropical forests (SDTFs) of north-eastern and Central Brazil. *Ecology and Evolution* **2**: 409–428.

Simon MF, Grether R, 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 Sciences of the USA* **106**: 20359–20364.

Stöver BC, Müller KF. 2010. TreeGraph 2. Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**: 7 http://treegraph.bioinfweb.info/ (last accessed 3 November 2016).

Taubert P. 1892. Leguminosae novae v. minus cognitae austro-americanae. *Flora* **75**: 68–86.

Teixeira SP, Ranga NT, Tucker SC. 2009. Inflorescence and floral development of *Dahlstedtia* species (Leguminosae: Papilionoideae: Millettieae). *Flora* **204**: 769–781.

Tucker SC. 1984. Unidirectional organ initiation in leguminous flowers. *American Journal of Botany* **71**: 1139–1148.

Tucker SC. 1987. Floral initiation and development in legumes. In: Stirton CH ed. *Advances in legume systematics, Part 3*. Kew: Royal Botanic Gardens, 183–239.

Tucker SC. 1988a. Loss versus suppression of floral organs. In: Leins P, Tucker SC, Endress PK. eds. *Aspects of floral development*. Berlin: J. Cramer, 69–82.

Tucker SC. 1988b. Heteromorphic flower development in *Neptunia pubescens*, a mimosoid legume. *American Journal of Botany* **75**: 205–224.

Tucker SC. 1990. Loss of floral organs in *Ateleia* (Leguminosae: Papilionoideae: Sophoreae). *American Journal of Botany* **77**: 750–761.

Tucker SC. 1991. Helical floral organogenesis in *Gleditsia*, a primitive caesalpinioid legume. *American Journal of Botany* **78**: 1130–1149.

Tucker SC. 1992. The role of floral development in studies of legume evolution. *Canadian Journal of Botany* **70**: 692–700.

Tucker SC. 1993. Floral ontogeny in Sophoreae (Leguminosae: Papilionoideae): I. *Myroxylon* (Myroxylon group) and *Castanospermum* (Angylocalyx group). *American Journal of Botany* **80**: 65–75.

Tucker SC. 1994. Floral ontogeny in Sophoreae (Leguminosae, Papilionoideae). 2. *Sophora* sensu-lato (Sophora group). *American Journal of Botany* **81**: 368–380.

Tucker SC. 1996. Trends in evolution of floral ontogeny in *Cassia* sensu stricto, *Senna*, and *Chamaecrista* (Leguminosae: Caesalpinioideae: Cassieae: Cassiinae): a study in convergence. *American Journal of Botany* **83**: 687–711.

Tucker SC. 1998. Floral ontogeny in legume genera *Petalostylis*, *Labichea*, and *Dialium* (Caesalpinioideae: Cassieae), a series in floral reduction. *American Journal of Botany* **85**: 184–208.

Tucker SC. 2000a. Evolutionary loss of sepals and/or petals in detarioid legume taxa *Aphanocalyx*, *Brachystegia*, and *Monopetalanthus* (Leguminosae: Caesalpinioideae). *American Journal of Botany* **87**: 608–624.

Tucker SC. 2000b. Floral development in tribe Detarieae (Leguminosae: Caesalpinioideae): *Amherstia*, *Brownea*, and *Tamarindus*. *American Journal of Botany* **87**: 1385–1407.

Tucker SC. 2000c. Floral development and homeosis in *Saraca* (Leguminosae: Caesalpinioideae: Detarieae). *International Journal of Plant Sciences* **161**: 537–549.

Tucker SC. 2001a. Floral development in *Schotia* and *Cynometra* (Leguminosae: Caesalpinioideae: Detarieae). *American Journal of Botany* **88**: 1164–1180.

Tucker SC. 2001b. The ontogenetic basis for missing petals in *Crudia* (Leguminosae: Caesalpinioideae: Detarieae). *International Journal of Plant Sciences* **162**: 83–89.

Tucker SC. 2002a. Comparative floral ontogeny in Detarieae (Leguminosae: Caesalpinioideae). 1. Radially symmetrical taxa lacking organ suppression. *American Journal of Botany* **89**: 875–887.

Tucker SC. 2002b. Comparative floral ontogeny in Detarieae (Leguminosae: Caesalpinioideae). 2. Zygomorphic taxa with petal and stamen suppression. *American Journal of Botany* **89**: 888–907.

Tucker SC. 2002c. Floral ontogeny of *Cercis* (Leguminosae: Caesalpinioideae: Cercideae): does it show convergence with papilionoids? *International Journal of Plant Sciences* **163**: 75–87.

Tucker SC. 2002d. Floral ontogeny in Sophoreae (Leguminosae: Papilionoideae). III. Radial symmetry and random petal aestivation in *Cadia purpurea*. *American Journal of Botany* **89**: 748–757.

Tucker SC. 2003a. Comparative floral ontogeny in Detarieae (Leguminosae: Caesalpinioideae). 3. Adaxially initiated whorls in *Julbernardia* and *Sindora*. *International Journal of Plant Sciences* **164**: 275–286.

Tucker SC. 2003b. Floral ontogeny in *Swartzia* (Leguminosae: Papilionoideae: Swartzieae): distribution and role of the ring meristem. *American Journal of Botany* **90**: 1271–1292.

Tucker SC. 2003c. Floral development in legumes. *Plant Physiology* **131**: 911–926.

Tucker SC, Kantz KE. 1997. Comparative floral development and evolution in tribe Caesalpinieae (Leguminosae: Caesalpinioideae). *Haematoxylum*. *American Journal of Botany* **84**: 1047–1063.

Tucker SC, Stein OK, Derstine KS. 1985. Floral development in *Caesalpinia* (Leguminosae). *American Journal of Botany* **72**: 1424–1434.

Warwick MC, Lewis GP, Lima HC. 2008. A reappraisal of *Barnebydendron* (Leguminosae: Caesalpinioideae: Detarieae). *Kew Bulletin* **63**: 143–149.

Werner GDA, Cornwell WK, Cornelissen JHC, Kiers ET. 2015. Evolutionary signals of symbiotic persistence in the legume–rhizobia mutualism. *Proceedings of the National Academy of Sciences of the USA* **112**: 10262–10269.

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.

Zimmerman E, Prenner G, Bruneau A. 2013. Floral ontogeny in Dialiinae (Caesalpinioideae: Cassieae), a study in organ loss and instability. *South African Journal of Botany* **89**: 188–209.