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Author(s): Samuel F. Brockington, Roolse Alexandre, Jeremy Ramdial, Michael J. Moore, Sunny Crawley, Amit Dhingra, Khidir Hilu, Douglas E. Soltis, and Pamela S. Soltis

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PHYLOGENY OF THE CARYOPHYLLALES SENSU LATO: REVISITING HYPOTHESES ON POLLINATION BIOLOGY AND PERIANTH DIFFERENTIATION IN THE CORE CARYOPHYLLALES

Samuel F. Brockington,[1,*,†] Roolse Alexandre,† Jeremy Ramdial,† Michael J. Moore,‡ Sunny Crawley,§ Amit Dhingra,‖ Khidir Hilu,§ Douglas E. Soltis,* and Pamela S. Soltis†

*Department of Botany, University of Florida, Gainesville, Florida 32611, U.S.A.; †Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611, U.S.A.; ‡Biology Department, Science Center K111, Oberlin College, Oberlin, Ohio 44074, U.S.A.; §Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia 24061, U.S.A.; and ‖Horticulture and Landscape Architecture, Washington State University, Pullman, Washington 99164, U.S.A.

Molecular phylogenetics has revolutionized our understanding of the Caryophyllales, and yet many relationships have remained uncertain, particularly at deeper levels. We have performed parsimony and maximum likelihood analyses on separate and combined data sets comprising nine plastid genes (~12,000 bp), two nuclear genes (~5000 bp), and the plastid inverted repeat (~24,000 bp), giving a combined analyzed length of 42,006 bp for 36 species of Caryophyllales and four outgroups. We have recovered strong support for deep-level relationships across the order. Two major subclades are well supported, the noncore and core Caryophyllales; *Rhabdodendron* followed by *Simmondsia* are sisters to the core Caryophyllales, *Limeum* and *Stegnosperma* are successive sisters to the "globular inclusion" clade, *Gisekia* is a distinct lineage well separated from *Rivina* within the "raphide" clade, and *Rivina* and Phytolaccaceae are disparate lineages, with *Rivina* sister to Nyctaginaceae. The placement of *Sarcobatus* and relationships within the portulacaceous cohort remain problematic. Within the latter, *Halophytum* is sister to Basellaceae and Didiereaceae, and the clade comprising *Portulaca*, *Talinum*, and Cactaceae is well supported. Classical hypotheses argued that the early Caryophyllales had evolved in open, dry, marginal environments at a time when pollinators were scarce, and, as such, the ancestral caryophyllid flower was wind pollinated with an undifferentiated perianth. We reevaluated these hypotheses in light of our phylogeny and find little support for anemophily as the ancestral condition; however, the early caryophyllid flower is suggested to have possessed an undifferentiated perianth. A subsequent minimum of nine origins of differentiated perianth is inferred. We discuss the evidence for independent origins of differentiated perianth and highlight the research opportunities that this pattern offers to the field of evolutionary developmental genetics.

Keywords: character reconstruction, stochastic character mapping, petals, evo-devo, MADS-box.

Online enhancements: figures, tables.

Introduction

Research interest in Caryophyllales has a long and rich history; core members of this lineage correspond to the old Centrospermae ("central seeded"), a group long recognized by its distinctive placentation and embryology (Braun 1864; Eichler 1875–1878). Centrospermae became the focus of research and debate in the 1960s as one of the first groups whose circumscription was modified based on phytochemistry (Cronquist and Thorne 1994). All but two of the 10 families then recognized as belonging to the Centrospermae were discovered to possess betalain pigments instead of anthocyanins (Cronquist and Thorne 1994). On the basis of this chemosystematic character, Cactaceae and Didiereaceae were reassigned to the Centrospermae, and several families of dubious affiliation were excluded (Cronquist and Thorne 1994). Subsequent classifications recognized the Caryophyllales as a well-defined group on the basis of numerous morphological, ultrastructural, and chemical characters (Dahlgren 1975; Thorne 1976; Takhtajan 1980; Cronquist 1981, 1988). Just before the emergence of DNA-based molecular systematics, the Caryophyllales sensu stricto comprised 12 families (Takhtajan 1980; Cronquist 1988; Thorne 1992): Phytolaccaceae, Achatocarpaceae, Nyctaginaceae, Aizoaceae, Didiereaceae, Cactaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae, Basellaceae, Molluginaceae, and Caryophyllaceae. In addition, Polygonaceae and Plumbaginaceae have been regarded by numerous systematists as closely related to these 12 families (Cronquist and Thorne 1994).

A series of molecular phylogenetic investigations has altered the concept of Caryophyllales provided in earlier classifications. Giannasi et al. (1992) confirmed the close relationship of Polygonaceae and Plumbaginaceae with the Caryophyllales. Albert et al. (1992) and Williams et al. (1994) demonstrated the association of Droseraceae, Nepenthaceae, and Drosophyl-

[1] Author for correspondence; e-mail: sbrockington@flmnh.ufl.edu.

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laceae with Polygonaceae and Plumbaginaceae. The carnivorous clade plus Polygonaceae/Plumbaginaceae was further expanded to include Ancistrocladaceae, Dioncophyllaceae, Frankeniaceae, and Tamaricaceae (Fay et al. 1997). Numerous analyses have recognized this expanded clade, which is variously termed the noncore Caryophyllales (Cuénoud et al. 2002; APG II 2003), Caryophyllales II (Hilu et al. 2003), and Polygonales (Judd et al. 1999), as sister to Caryophyllales sensu stricto (Soltis et al. 1999, 2000).

Within Caryophyllales s.s. or the core Caryophyllales, molecular data have resulted in several refinements in phylogeny and classification. Additional families recognized as belonging to the core Caryophyllales include Physenaceae and Asteropeiaceae (Morton et al. 1997), Rhabdodendraceae, and Simmondsiaceae (Fay et al. 1997). Simmondsiaceae are supported as sister to the core Caryophyllales (Cuénoud et al. 2002), with Physenaceae and Asteropeiaceae forming a strongly supported sister group (combined *matK/rbcL* analysis; Cuénoud et al. 2002). Rhabdodendraceae have been associated with Simmondsiaceae as sister to the core Caryophyllales or as sister to both core and noncore Caryophyllales but with little support for either position (*matK* analysis; Cuénoud et al. 2002).

Molecular studies have also identified and confirmed a number of polyphyletic groups within the core Caryophyllales. Recognition that Phytolaccaceae are polyphyletic (initially by Rettig et al. [1992]) supports the delimitation of four families: Achatocarpaceae, Barbeuiaceae, Gisekiaceae, and Stegnospermataceae (Cuénoud et al. 2002; APG II 2003). Achatocarpaceae may form a clade together with Caryophyllaceae and Amaranthaceae (combined *matK/rbcL* analysis; Cuénoud et al. 2002). Stegnospermataceae are placed without support as a successive sister lineage to the remainder of the core Caryophyllales (Cuénoud et al. 2002), following the divergence of Asteropeiaceae, Physenaceae, Achatocarpaceae, Caryophyllaceae, and Amaranthaceae. *Barbeuia* represents a distinct, isolated lineage within the core Caryophyllales but of uncertain position (Cuénoud et al. 2002). *Rivina* and *Petiveria*, both formerly of Phytolaccaceae, are paraphyletic (combined *matK/rbcL* analysis; Cuénoud et al. 2002) with respect to *Phytolacca*. *Rivina* has been allied with the family Gisekiaceae, and *Petiveria* is placed sister to *Rivina* and Gisekiaceae (combined *matK/rbcL* analysis; Cuénoud et al. 2002). Similarly in the *matK* analyses, *Hilleria* is placed sister to *Rivina*, and *Ledenbergia* is sister to those two taxa. *Lophiocarpus*, originally placed within Phytolaccaceae, is now separated and placed sister to *Corbichonia* (Cuénoud et al. 2002). *Sarcobatus*, originally placed in the Chenopodiaceae, was recognized as the family Sarcobataceae (Behnke 1997; APG II 2003) on the basis of distinct sieve-element plastids with respect to Chenopodiaceae (Behnke 1997). This separation was supported by molecular analyses in which Sarcobataceae form a distinct lineage allied with the clade containing Aizoaceae, Phytolaccaceae, Nyctaginaceae, Gisekiaceae, and *Agdestis* (Downie et al. 1997; Cuénoud et al. 2002). The circumscription of Molluginaceae remains problematic; the family is likely to be polyphyletic. Previous authors have suggested that the inclusion of *Macarthuria* and *Polpoda* is unlikely on the basis of morphological observations; however, two genera have not been included in previous molecular analyses (Cuénoud et al. 2002). Genera previously included within Molluginaceae (*Corbichonia*, *Limeum*, *Gisekia*) form disparate lineages with respect to the type genus. However, the position of *Limeum* outside Molluginaceae is unsupported (*matK/rbcL*; Cuénoud et al. 2002). *Mollugo*, *Adenogramma*, *Glischrothamnus*, *Glinus*, *Pharnaceum*, and *Suessenguthiella* constitute a monophyletic group that is sister to the portulacaceous cohort (Cuénoud et al. 2002).

The portulacaceous cohort of Basellaceae, Cactaceae, Didiereaceae, and Portulacaceae was initially proposed by Thorne (1976) and is supported by non-DNA characters such as presence of a floral involucre, succulent tissue, mucilage, and Crassulacean acid metabolism (Cuénoud et al. 2002; Nyffeler 2007). The monophyly of the cohort was implied by early molecular analyses (Rettig et al. 1992; Downie et al. 1997); however, relationships within the group are unclear and complicated by the gross paraphyly of Portulacaceae (suggested by Carolin [1987] and Hershkovitz [1993]). The addition of molecular data has resulted in some clarification. In an analysis of ITS sequences, Hershkovitz and Zimmer (1997) suggested that Cactaceae were embedded within Portulacaceae and sister to *Portulaca*, *Anacampseros*, and relatives and portions of *Talinum* (the ACPT clade, from Anacampseroteae, Cactaceae, *Portulaca*, and *Talinum*; Nyffeler 2007). These findings were confirmed and extended by Applequist and Wallace (2001) in an analysis of *ndhF* sequences; *Talinum* with *Talinella*, *Portulaca* with *Anacampseros*, and the Cactaceae form three distinct lineages within a well-supported clade. Although the monophyly of the ACPT clade seems clear, as summarized by Nyffeler (2007), the pattern of branching within the clade has varied among analyses. Outside of the ACPT clade, Hershkovitz and Zimmer (1997) found that Basellaceae and Didiereaceae form a distinct monophyletic group and are sister to portulacaceous genera *Portulacaria* and *Ceraria*. In addition, Applequist and Wallace (2001) described this same clade as consisting of three distinct lineages: Basellaceae; Didiereaceae with *Calyptrotheca*, *Ceraria*, and *Portulacaria*; and a strongly supported assemblage of genera including *Claytonia*, *Montia*, *Calandrinia*, *Montiopsis*, *Cistanthe*, *Calyptridium*, and *Phemeranthus*.

Despite these advances in the understanding of subordinal relationships in Caryophyllales, a number of uncertainties remain. The positions of *Limeum*, *Stegnosperma*, and *Barbeuia* are all ambiguous. The relative position of *Rhabdodendron* and *Simmondsia* as possible sisters to either the core Caryophyllales or Caryophyllales s.l. is unclear. Relationships within the portulacaceous cohort are largely unresolved, particularly within the clade containing Basellaceae, Didiereaceae, and allied genera (Applequist and Wallace 2001). The clade including Phytolaccaceae, Sarcobataceae, Nyctaginaceae, Gisekiaceae, and Agdestidaceae also lacks internal resolution. Additionally, some relationships have been suggested only on the basis of single-gene analyses (Cuénoud et al. 2002), with many nodes scattered throughout the Caryophyllales lacking strong support.

In an effort to resolve the remaining problematic deep-level relationships within Caryophyllales, we constructed a much larger data set than employed previously. Our data set comprised eight plastid genes from single-copy (SC) regions, two nuclear genes, and the entire plastid inverted repeat (IR; a combined analyzed length of 42,006 bp) for 40 taxa representing 31 families of the Caryophyllales and three families as outgroups.

The unusual morphological and biochemical variation found within the Caryophyllales has fueled much speculation as to

its evolutionary origins. Ehrendorfer (1976) outlined a plausible scenario to explain the coincident evolution of several unique characteristics found in the Caryophyllales, including floral variation and betalain pigmentation. He proposed that ancestral taxa in Caryophyllales occupied "open, warm, dry and windy habitats with mineral soils" (Ehrendorfer 1976, p. 104). Reasons for assuming this ancestral habitat derive from the observation that many of the families in Caryophyllales currently inhabit xeric, marginal environments. Ehrendorfer (1976) argued that if the ancestral habitats were xeric, there would be strong selection for anemophily because pollinating insects would have been scarce in areas of little pioneer plant growth. He states that pollinators may have been scarce because the time of the origin of the core Caryophyllales (104–111 Myr BP; Wikstrom et al. 2001) predates the major diversification of insect pollinator lineages. He then argues that much of the floral variation and novel pigmentation in the core Caryophyllales could be interpreted as the consequence of this anemophilous ancestry, with reversals to zoophilly in extant lineages. However, as this study will demonstrate, the phylogenetic concept of the Caryophyllales has changed considerably since Ehrendorfer (1976). We evaluate Ehrendorfer's hypotheses in light of a much-altered phylogeny by examining patterns of pollination biology and perianth differentiation. We discuss the evolution of perianth differentiation in the context of the literature on perianth development within Caryophyllales and use our phylogeny to identify broad trends in perianth evolution across the clade. Finally, we discuss the research opportunities that these patterns of morphological variation offer to the field of evolutionary developmental genetics.

Material and Methods

Taxon Sampling

In this analysis 31 families of Caryophyllales sensu APG II (2003; Cuénoud et al. 2002) were represented. Some families are monotypic (e.g., Drosophyllaceae, Halophytaceae, Stegnospermataceae); others comprise only one genus (e.g., Asteropeiaceae, Nepenthaceae, Ancistrocladaceae, Frankeniaceae) or two or three genera (Achatocarpaceae, Dioncophyllaceae, Droseraceae, Limeaceae, Talinaceae). For larger potentially polyphyletic or paraphyletic families (e.g., Portulacaceae), multiple genera were sampled to represent more of the phylogenetic diversity. The final data set included 36 taxa of Caryophyllales, with an additional four taxa (*Tetracera* and *Hibbertia* representing Dilleniaceae, *Berberidopsis*, and *Vitis*) sampled as outgroups. Species, voucher information, and GenBank accession numbers are given in tables A1–A9 in the online edition of the *International Journal of Plant Sciences*. In some instances sequence data were combined from multiple species to represent a family; this was judged not to significantly affect a family-level analysis, but the instances are listed here: Aizoaceae (*Delosperma napiforme*, *Delosperma echinatum*, *Delosperma cooperi*), Amaranthaceae (*Celosia argentea*, *Celosia cristata*), Cactaceae (*Opuntia microdasys*, *Opuntia dillenii*), Didiereaceae (*Alluaudia ascendens*, *Alluaudia procera*), Dilleniaceae (*Hibbertia volubilis*, *Hibbertia cuneiformis*), Gisekiaceae (*Gisekia africana*, *Gisekia pharnacioides*), Molluginaceae (*Limeum africanum*, *Limeum aetheopicum*), Plumbaginaceae (*Limonium gibertii*, *Limonium arborescens*, *Plumbago zeylanica*, *Plumbago auriculata*), Polygonaceae (*Polygonum sagittatum*, *Polygonum virginicum*), and Portulacaceae (*Claytonia virginica*, *Claytonia perfoliata*).

DNA Isolation and Amplification

We isolated DNA following standard CTAB protocols (Doyle and Doyle 1987) and using Qiagen DNA extraction kits (Qiagen, Valencia, CA). To augment depleted DNA stocks, we carried out multiple displacement amplification (MDA) using the Genomiphi kit (Amersham, Piscataway, NJ) according to the manufacturer's instructions (Brockington et al. 2008). MDA-treated DNA was diluted 1 : 10 before further PCR amplification of targeted genes.

We targeted 11 specific genes for sequencing (nine plastid genes from the large and small SC regions and two nuclear genes); all targeted genes and primers used for PCR and sequencing are provided in figure A12 in the online edition of the *International Journal of Plant Sciences*. All PCR reactions contained *Taq* DNA polymerase (New England Biolabs, Ipswich, MA) and 10X Thermopol reaction buffer supplied by the manufacturer. The reaction volume was 25 μL, and the final concentration of the components was *Taq* buffer (pH 8.8), $MgCl_2$ (1.5 mM), 200 μM dNTP, forward and reverse primers (1 μM), 1U *Taq* polymerase, and 1 μL of DNA. PCR cycling was carried out in an Eppendorf Mastercycler (Eppendorf, Westbury, NJ) at 95°C for 3 min, followed by 30–35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension time of 7 min at 72°C. PCR products were purified using ExoSAP, and sequences were generated on an ABI 3730 XL DNA sequencer (Applied Biosystems, Fullerton, CA) following the manufacturer's protocol. Sequences were submitted to GenBank (numbers given in tables A1–A9).

The amplification, sequencing, and annotation of plastomes method (Dhingra and Folta 2005) was used to obtain the sequence of the plastid genome IR for 35 genera of Caryophyllales (the IR for *Physena* was not sequenced) and two members of Dilleniaceae. The published complete plastid sequence of *Spinacia* (Schmitz-Linneweber et al. 2001) and *Plumbago* (Moore et al. 2007) provided the IR sequence for these two taxa. The IR sequences were subsequently annotated using DOGMA (Wyman et al. 2004) and were submitted to GenBank (numbers given in tables A1–A9).

Alignment and Phylogenetic Analysis

Sequences were automatically aligned using Clustal X (Thompson et al. 1997) and then manually adjusted. Coding regions were aligned by predicted amino acid sequence. Regions at the beginnings and ends of genes for which sequences were incomplete, together with regions that were difficult to align, were excluded from the analysis. The total aligned lengths and the analyzed aligned lengths are given in table 1. Using the new sequences generated here, together with those previously published (cited in tables A1–A9), we constructed six different data partitions: (1) individual plastid genes from the SC regions, (2) combined plastid genes from the SC regions, (3) two nuclear ribosomal RNA genes (18S rDNA and 26S rDNA), (4) plastid IR, (5) combined plastid SC and nu-

Table 1

Information on Parsimony and Likelihood Analyses for Each Data Partition

Data partition	Total aligned length (bp)	Analyzed aligned length (bp)	No. MP trees	Length of MP tree	Consistency index of MP tree	Retention index of MP tree	ML tree score	ML tree length under parsimony
atpB	1497	1497	23	1296	.525	.566	8854.19	1302
matk	1650	1650	2	3360	.519	.522	18,173.93	3370
ndhF	2319	2182	20	3159	.514	.493	17,917.22	3169
psbBTN	1780	1780	15	1639	.494	.541	10,692.37	1644
rbcL	1449	1449	73	1451	.522	.601	9770.59	1459
rpoC2	3903	3652	3	4529	.576	.567	28,139.56	4533
rps4	609	609	122,155	545	.646	.623	3826.09	564
IR	29,410	23,966	1	8649	.781	.600	86,224.79	8654
18S + 26S	5221	5221	14	2713	.502	.469	21,309.04	2724
SC plastid	13,207	12,819	1	16,158	.530	.535	99,154.41	16,171
SC plastid + nuclear	18,428	18,040	1	18,939	.524	.523	121,955.45	18,962
SC plastid + nuclear + IR	47,838	42,006	1	27,604	.605	.538	210,420.96	27,613

clear genes, and (6) total evidence data set (all plastid and nuclear genes).

All data partitions were subject to the following phylogenetic analyses. We used maximum parsimony (MP) and maximum likelihood (ML) to infer phylogeny. MP analyses were implemented in PAUP*, version 4.0 (Swofford 2000). Shortest trees were obtained using a heuristic search and 1000 replicates of random taxon addition with tree-bisection-reconnection (TBR) branch swapping, saving all shortest trees per replicate. Bootstrap support (BS) for relationships (Felsenstein 1985) was estimated from 1000 bootstrap replicates using 10 random taxon additions per replicate, with TBR branch swapping and saving all trees.

For ML analyses we employed the program GARLI (Genetic Algorithm for Rapid Likelihood Inference, version 0.942; Zwickl 2000). GARLI conducts ML heuristic phylogenetic searches under the GTR model of nucleotide substitution, in addition to models that incorporate among-site rate variation, assuming a gamma distribution (Γ), a proportion of invariable sites (I), or both. Analyses were run with default options, except that the "significanttopochange" parameter was reduced to 0.01 to make searches more stringent. ML bootstrap analyses were conducted with the default parameters and 100 replicates. We performed a strict consensus of five replicate GARLI analyses, and topological differences resulting in collapsed nodes were annotated on the representative ML tree.

Bayesian analyses were performed on the combined partition to generate trees for stochastic character mapping. Models of nucleotide substitution were determined using MrModeltest (Nylander 2004). The Akaike Information Criterion was used to select GTR + I + G as an appropriate model based on the relative informational distance between the ranked models. Analyses were implemented in MrBayes, version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Two independent analyses each ran for 5 million generations, using four Markov chains, with all other parameters at default values; trees were sampled every thousandth generation, with a burn-in of 200,000 generations. Stationarity of the Markov Monte Carlo chain was determined by the average standard deviation of split frequencies between runs (after 5 million generations, the average standard deviation was 0.004%) and by examination of the posterior in Tracer, version 1.3 (Rambaut and Drummond 2003). A majority rule consensus of post-burn-in trees was generated in PAUP*, version 4.0 (Swofford 2000), using the resulting posterior distribution of the trees.

Character Reconstructions

Parsimony-based reconstructions were achieved using standard unweighted parsimony character optimization and performed within Mesquite (Maddison and Maddison 2008). Reconstructions focused on the core Caryophyllales and were carried out using the MP topology derived from the total evidence data set. Reconstructions were further modeled by means of stochastic mapping techniques as described by Huelsenbeck et al. (2003) and implemented in SIMMAP (Bollback 2006). This approach estimates the rates at which a discrete character undergoes state changes as it evolves through time. Bayesian estimation has several advantages over traditional parsimony-based reconstruction. First, it allows one to average over equally likely topologies, which is valuable because the positions of some taxa are poorly supported (e.g., *Limeum*) or poorly resolved (e.g., taxa within the portulacaeous cohort and "raphide" clade). Second, it allows more than one character change per branch and is therefore a useful methodology for character reconstruction in the Caryophyllales, a clade in which long branches are common.

Posterior mapping requires the specification of prior values. The prior on the bias parameter was fixed at $1/k$, where k is the number of states (this being the recommended approach in SIMMAP for characters of more than two states; Renner et al. 2007). We applied an empirical Bayesian approach in choosing appropriate priors for the substitution rate parameters following the method of Couvreur (2008) and Couvreur et al. (2008). The gamma distribution of the substitution rate is governed by two hyperparameters defining the mean, $E(T)$, and the standard deviation, $SD(T)$. The values of these hyperparameters for the prior gamma distribution were selected independently for each character using the "number of realizations sampled from priors" function in SIMMAP with 10,000 draws. A series of trials was performed (10,000 realizations in

each) that systematically sampled for values of $E(T)$ between 1 and 30, in combination with SD(T) values of either 1 or 5. The posterior distribution of these combinations was visualized in Tracer, version 1.3, and further plotted as graphs of frequency against rate (see fig. A11 in the online edition of the *International Journal of Plant Sciences*). The posterior distribution curves derived from these trials allowed the selection of values of $E(T)$ that gave highest sampling and allowed optimization of the $E(T)$ value (Couvreur 2008; Couvreur et al. 2008). A trial was also performed without specifying priors and allowing rates to be determined by branch lengths (as performed by Renner et al. [2007]); however, the posterior distribution curves were generally highly skewed (fig. A11), and thus this form of prior selection was not employed in subsequent analyses. Following exploration of different combinations of $E(T)$ and SD(T), the prior $E(T)$ values chosen for the characters were as follows: perianth $E(T) = 17$, and pollination $E(T) = 7$ (marked with an asterisk in fig. A11). For all of these values of $E(T)$, an SD(T) value of 5 was applied in subsequent analyses, allowing a large standard deviation to accommodate uncertainty in mean rate of substitution.

Following specification of priors, the rate and number of state transformations were estimated by 100 realizations on the 4800 post-burn-in trees (with branch lengths) from the Bayesian analyses. As recommended, branch lengths were rescaled so that the total tree length was 1 but the branch length proportions were maintained. The ancestral state at different nodes was assessed using a hierarchical Bayesian ancestral state reconstruction method implemented in the "posterior ancestral states" function of SIMMAP (Bollback 2006). The nodes for which ancestral states were estimated are labeled in figure 3. The estimations of the posterior probability of ancestral character states at each node are listed in tables A12 and A13 in the online edition of the *International Journal of Plant Sciences* and presented graphically on the nodes in figure 3.

With parsimony reconstruction analyses, when more than one character state was present in the family, the representative taxon was coded as having more than one character state. In stochastic mapping analyses using SIMMAP, terminals cannot be coded as having more than one state, so in instances where more than one character state was present in the family, the representative taxon was coded as unknown (?). Information on pollination was derived primarily from entries in Kubitzki et al. (1993) and Kubitzki and Bayer (2003, 2007); pollination was coded as entomophilous or anemophilous. In the case where observations on pollinators have not been made, the character state determination was unknown (?). All coding information is listed in tables A10 and A11 in the online edition of the *International Journal of Plant Sciences*.

Our approach to coding perianth requires further clarification because there are many types of differentiated perianth within Caryophyllales, and their homology is not always clear. Occurrences of differentiated perianth were given different character states, where there are clear, documented differences in development of the differentiated perianth. Data on perianth and development were collated from the available literature (see fig. A11). As reviewed by Ronse De Craene (2008), criteria used to determine these differences in the literature include meristic variation, sequence of organ initiation, difference in appearance at maturity, and presence of morphological intermediates. We were, however, interested in estimating the minimum number of origins of the differentiated perianth under parsimony and therefore applied a stringent approach to character coding, minimizing the number of character states to four: undifferentiated (0), differentiated with stamen-derived petaloid organs (1), differentiated with an involucre-derived outer whorl (2), and differentiated perianth of uncertain affinity (3). We employed a conservative approach to character coding, assigning states 1 and 2 only to taxa in which developmental morphological data were most conclusive. Where we were uncertain, we assigned taxa to character state 3. We emphasize that this coding does not reflect our belief that these instances of differentiated perianth are necessarily homologous, but in coding them as identical, we ensure that estimation of the number of origins of differentiated perianth is conservative.

Results

Individual Plastid SC Data Sets

MP and ML trees from individual data sets are largely congruent with each other (figs. A1–A7 in the online edition of the *International Journal of Plant Sciences*; tree statistics shown in table 1). Consistent with the approximate nature of the GARLI approach to ML phylogeny estimation, replicate GARLI analyses on the individual gene data sets do, on occasion, recover slightly different topologies. Taking into account nodes either that are unsupported or that collapse in the strict consensus, however, there are few instances of conflicting relationships between trees derived from different individual gene data sets. These examples of conflict include the following: in the *matK* MP tree, *Delosperma* and *Gisekia* were recovered as sister groups (BS 51%); in the MP and ML *ndhF* tree, *Spinacia* and *Stellaria* were resolved as sister groups to the exclusion of *Celosia* (MP BS 100%); in the MP and ML *rbcL* tree, *Gisekia* was sister to *Rivina* (MP BS 100%), *Delosperma* was sister to *Phytolacca* (MP BS 89%), and *Stellaria* was sister to the Amaranthaceae (MP BS 53%); and in the ML *rpoC2* tree alone, *Phytolacca* and *Sarcobatus* were recovered as sister groups (ML BS 79%). Importantly, these anomalous relationships are not recovered or not supported in any other data sets, in either single-gene or combined partitions. None of the trees derived from these individual plastid gene data sets gives good resolution across the tree, and deeper-level relationships in particular are poorly supported.

Inverted Repeat Data Set

As with the individual plastid gene data sets, the IR partition generates MP and ML trees that are congruent (fig. A8 in the online edition of the *International Journal of Plant Sciences*). Parsimony analyses recovered a single tree; replicate GARLI analyses recovered trees that differed only in the topology of the "succulent" clade. The IR tree differs from the previous analyses in the placement of *Sarcobatus*, which is resolved as sister to Nyctaginaceae and Phytolaccaceae with strong support (ML BS 100%). Furthermore, analyses of individual plastid genes resolve *Talinum* as sister to *Portulaca* and Cactaceae whereas the IR data set recovers *Portulaca* and *Talinum* as sister to each other.

Combined Plastid Genes from the SC Region

The combined plastid gene data set generated a single most parsimonious tree. Replicate GARLI analyses recovered the same ML topology (fig. A9 in the online edition of the *International Journal of Plant Sciences*). Levels of BS are higher in general in the ML tree than in the MP tree. Again, the MP and ML trees are largely congruent, although in the MP tree *Sarcobatus* and *Rivina* are sister to each other whereas in the ML tree *Sarcobatus* is placed without support with Phytolaccaceae and Nyctaginaceae. In the MP tree *Limeum* is placed without support as sister to *Mollugo* and the "succulent" clade, and *Stegnosperma* is placed as sister to the "globuloid inclusion" clade; in the ML tree, *Limeum* and *Stegnosperma* are placed as successive sisters to the "globular inclusion" clade. As with the individual plastid gene trees, *Talinum* is resolved as sister to *Portulaca* and Cactaceae, but relationships among Didiereaceae, Basellaceae, *Halophytum*, and *Claytonia* are either poorly supported or unresolved.

Combined Plastid SC and Nuclear Genes

The addition of the nuclear gene data set to the combined plastid genes has little effect on topology (fig. A10 in the online edition of the *International Journal of Plant Sciences*). In contrast to analyses of the combined plastid gene data set, both the MP and the ML analyses with the nuclear data recover *Stegnosperma* and *Limeum* as successive sisters to the globular inclusion clade. Again the MP and ML trees differ in their placement of *Sarcobatus* in the same way as in the combined plastid tree topologies, i.e., as sister to *Rivina* in the MP tree but sister to *Rivina* and Nyctaginaceae in the ML tree; both placements have low BS (~60%). As in the IR ML tree topology, the MP recovers *Portulaca* and *Talinum* as sister to Cactaceae but without support; in the ML tree, however, *Talinum* is sister to *Portulaca* plus Cactaceae.

Total Evidence Data Set

The total evidence data partition generated a single MP (fig. 2) tree that agrees in topology with the ML tree (fig. 1), except for the placement of *Sarcobatus*. The MP and ML trees derived from the total evidence data set show more congruence with each other than the congruence found between MP and ML trees derived for any other data partition. As in previous combined analyses, in the MP tree, *Sarcobatus* and *Rivina* are placed sister to each other (BS 63%) while in the ML tree, *Sarcobatus* is placed without support as sister to Nyctaginaceae plus *Rivina*. The position of *Sarcobatus* therefore remains uncertain in these analyses. The ML topology was chosen as the basis of subsequent character reconstruction analyses because it is less prone to the problem of long-branch attraction (Felsenstein 1978) and because the bootstrap values are higher than in the MP tree. The full topology of the tree is therefore described in detail here.

The noncore Caryophyllales form a strongly supported (BS 100%) monophyletic group with two subclades. One clade comprises Plumbaginaceae with Polygonaceae resolved as sister to Frankeniaceae plus Tamaricaceae (all with BS 100%). The second clade, containing the carnivorous taxa and relatives, comprises Drosophyllaceae with Ancistrocladaceae and Dioncophyllaceae (BS 100%) and Nepenthaceae with Droseraceae (BS 59%).

The core Caryophyllales form a strongly supported group (BS 100%), with Rhabdodendraceae as sister to the rest (BS 100%). Following the divergence of Rhabdodendraceae, the backbone of the tree is strongly supported and characterized by a grade of successively branching taxa, in the following order: Simmondsiaceae; Asteropeiaceae with Physenaceae; a clade comprising Caryophyllaceae, Achatocarpaceae, and Amaranthaceae (BS 100%); and Stegnospermataceae (BS 100%). Subsequently, *Limeum* is placed as sister to the remaining members of Caryophyllales, which form two clades. In the first of these two clades, the topology is as follows: the earliest-diverging group is Aizoaceae, followed by *Gisekia*, *Phytolacca*, *Sarcobatus*, *Rivina*, and Nyctaginaceae. In the second clade, Molluginaceae are sister to a group comprising Cactaceae, Portulacaceae, Didiereaceae, Basellaceae, *Halophytum*, and *Claytonia*. Within this group, *Portulaca* and *Talinum* are strongly supported as sister to Cactaceae; however, relationships among Didiereaceae, Basellaceae, *Halophytum*, and *Claytonia* are poorly supported.

For each of the character reconstructions, multiple state transitions are inferred within the core Caryophyllales. The patterns of character evolution derived from parsimony reconstruction and the inferred ancestral states derived from stochastic mapping analyses are illustrated in figure 3.

Discussion

Several broad molecular phylogenetic analyses have examined intraordinal relationships across the entire Caryophyllales sensu lato. Rettig et al. (1992) conducted an *rbcL* analysis of 12 families, Downie and Palmer (1994) inferred phylogeny from chloroplast genome structural changes and IR restriction site variation in 11 families of Caryophyllales, and Downie et al. (1997) compared sequences of ORF2280 (*ycf2*) across 11 families. However, the most comprehensive study is that of Cuénoud et al. (2002), who generated a partial *matK* sequence phylogeny (30 families, 121 genera). In Cuénoud et al. (2002) a subset of the *matK* data was combined with previously published genes to generate a combined *matK/rbcL* phylogeny (19 families, 53 genera) and a four-gene analysis that also incorporated *atpB* and 18S rDNA sequences (19 families, 25 genera). Although the taxonomic sampling of the *matK* phylogeny was extensive and dramatically improved our understanding of the Caryophyllales phylogeny, the study suffered from restricted taxon sampling in the combined analyses, with just over half of the families in the core Caryophyllales represented in the *matK/rbcL* and *matK/rbcL/atpB*/18S data sets. Parsimony was the only optimality criterion used in these analyses, and there were several soft incongruences among the *matK*, *matK/rbcL*, and *matK/rbcL/atpB*/18S trees. Our analyses resolve many of these remaining uncertainties.

Phylogenetic Analyses

The earliest-diverging lineages in the core Caryophyllales are clarified and well supported. Notably, Rhabdodendraceae followed by Simmondsiaceae are supported as sisters to the rest of the core Caryophyllales (both with BS 100%; fig. 1). The position of *Rhabdodendron* had previously been ambiguous, recovered either as sister to both core and noncore Car-

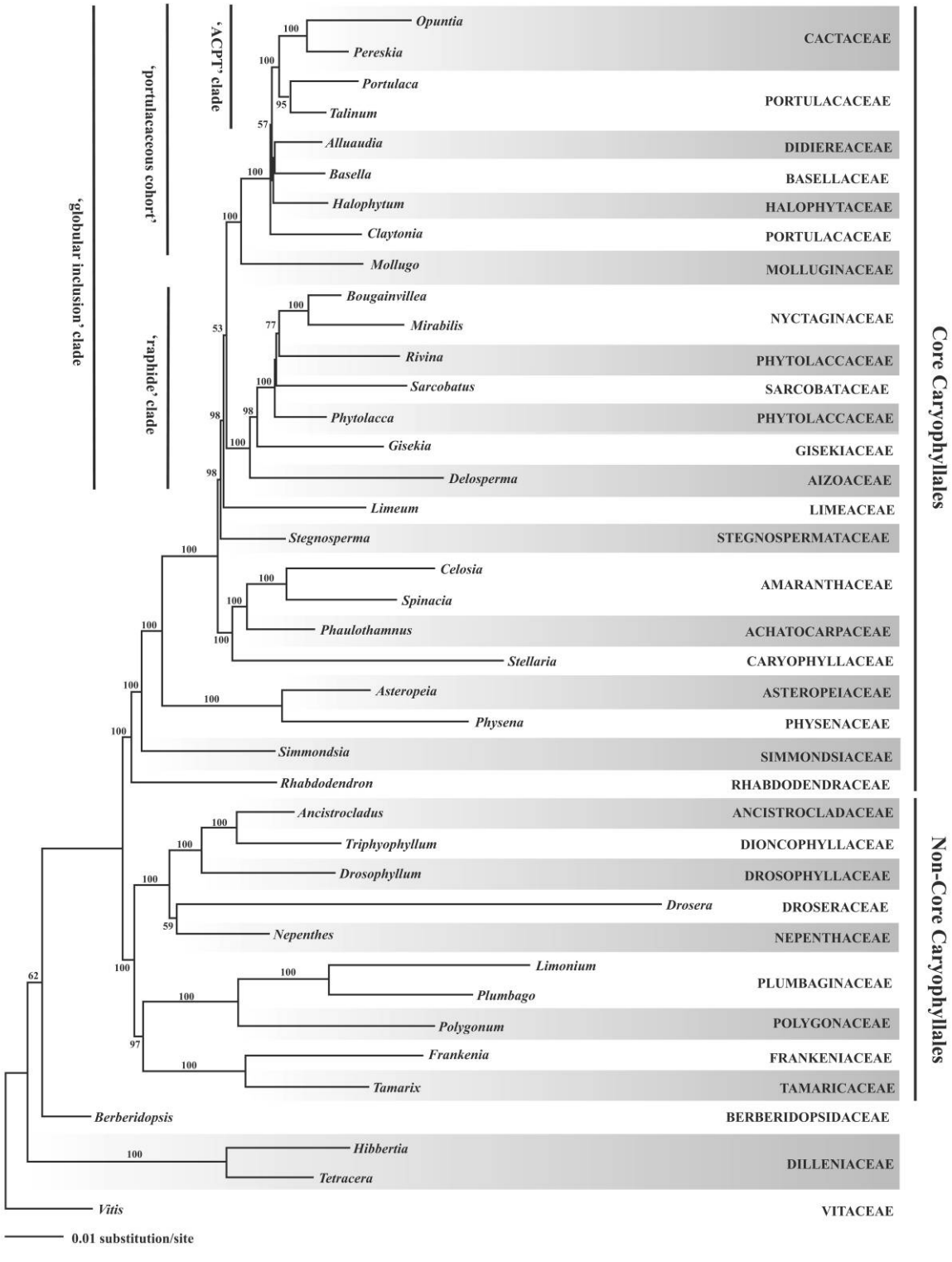

Fig. 1 Maximum likelihood (ML) tree resulting from GARLI analysis of total evidence data set (two nuclear genes, nine plastid genes from the single-copy region, and the inverted repeat) for 36 members of the Caryophyllales and four outgroups. Numbers above branches are bootstrap values (−lnL score 210,420.96).

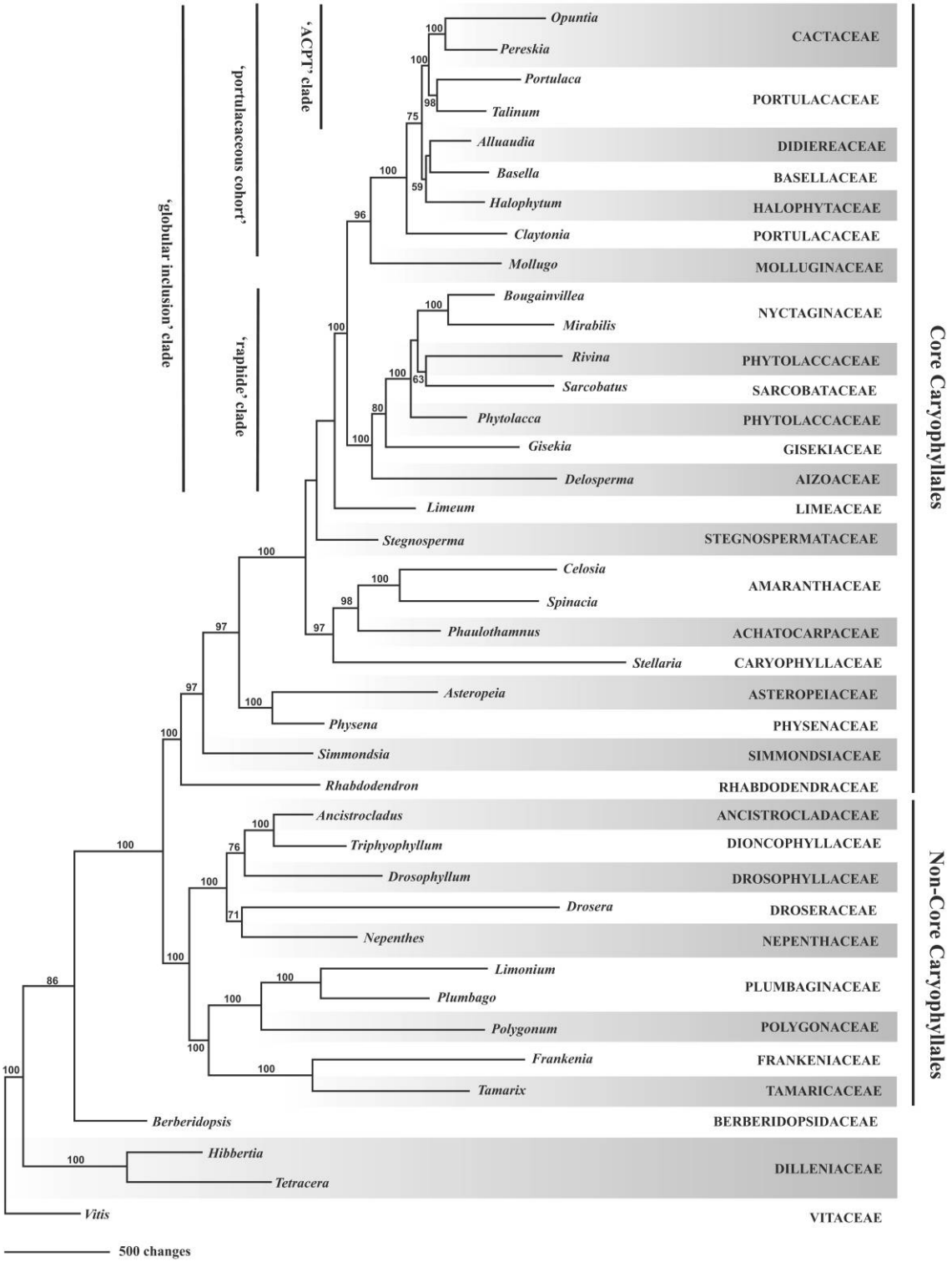

Fig. 2 Phylogram of single most parsimonious tree based on the total evidence data set (two nuclear genes, nine plastid genes from the single-copy region, and the inverted repeat) for 36 members of the Caryophyllales and four outgroups. Numbers above branches are bootstrap values (length 27,604, consistency index 0.605, retention index 0.538).

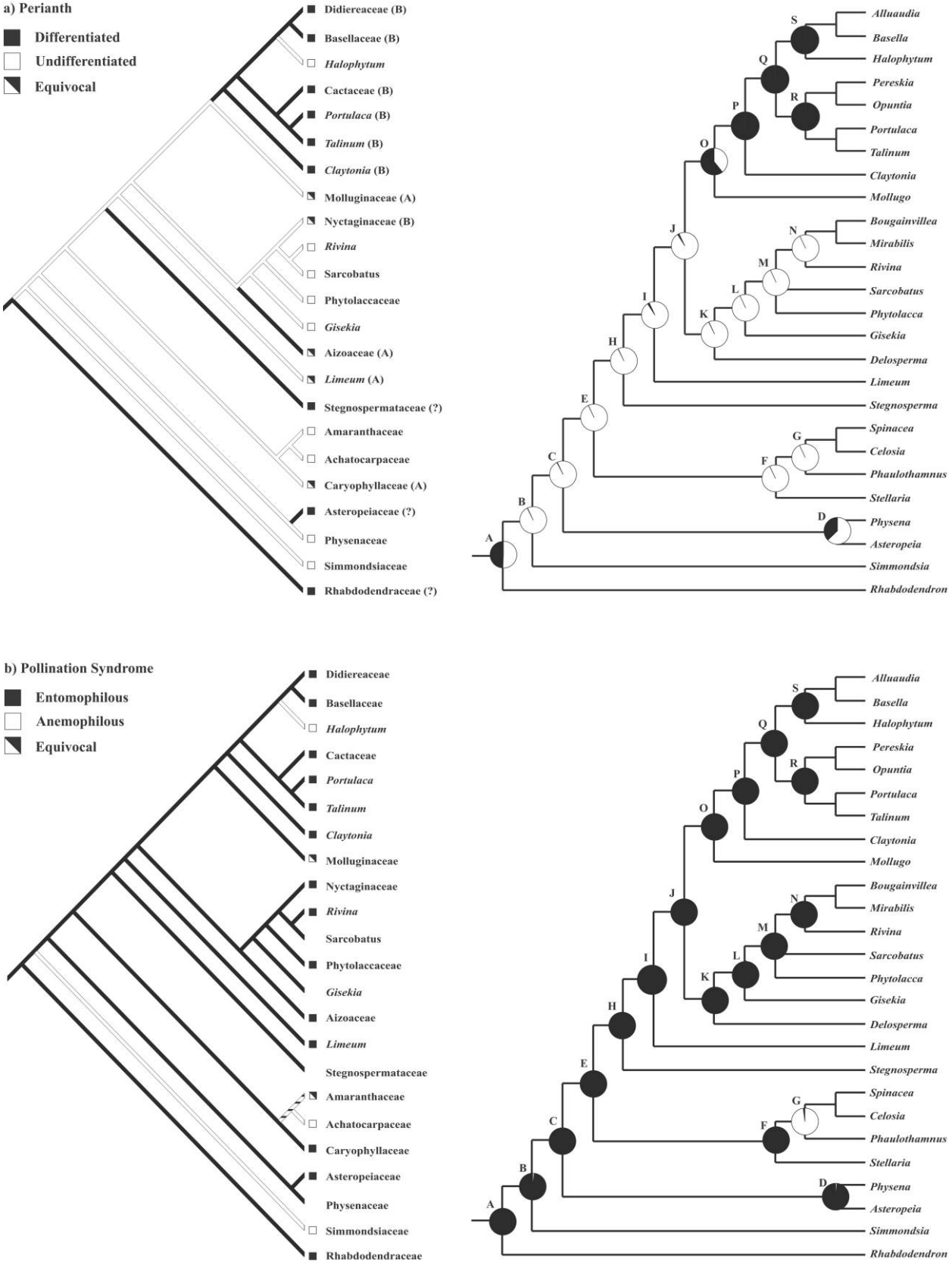

Fig. 3 Parsimony reconstruction (illustrated on a maximum parsimony tree) and stochastic character mapping (illustrated on a Bayesian consensus tree). *a*, Reconstruction of perianth evolution. *b*, Pollination syndromes.

yophyllales (in combined *matK* and *rbcL* analyses; Cuénoud et al. 2002) or as weakly supported as sister to *Simmondsia*, at the base of the core Caryophyllales (in *matK* analysis; Cuénoud et al. 2002). Following the divergence of Rhabdodendraceae and Simmondsiaceae, our analyses strongly support a clade of Physenaceae and Asteropeiaceae (BS 100%) as sister to the remaining core Caryophyllales. Relatively little is known about these early-diverging lineages of core Caryophyllales from the perspective of morphology, and lack of data for these critical early lineages prevents a clear understanding of ancestral states within the core Caryophyllales.

In the *matK* analysis of Cuénoud et al. (2002), Caryophyllaceae and Achatocarpaceae plus Amaranthaceae s.l. branch successively as sister to the rest of the core Caryophyllales and do not form a clade with Achatocarpaceae and Amaranthaceae s.l., as suggested in the *matK/rbcL* analyses. In our analyses, the clade comprising Caryophyllaceae, Achatocarpaceae, and Amaranthaceae s.l. receives strong support (BS 100%), agreeing with the combined analyses of Cuénoud et al. (2002). Morphological synapomorphies for this clade remain elusive, probably in part because Achatocarpaceae are poorly studied.

Molecular studies have consistently recovered a distinct clade within the core Caryophyllales (Giannasi et al. 1992; Rettig et al. 1992; Downie et al. 1997; Cuénoud et al. 2002), termed the globular inclusion clade (Aizoaceae, Phytolaccaceae, Nyctaginaceae, Gisekiaceae, Molluginaceae, Portulacaceae, Didiereaceae, Basellaceae, Cactaceae; Cuénoud et al. 2002), on account of distinctive P plastid characteristics (as found by Behnke [1994]). Consistent with previous analyses, *Stegnosperma* and *Limeum* are recovered as successive sisters to this globular inclusion clade but with greater support (BS 98%) than in earlier studies. Within the globular inclusion clade, two subclades are recovered that correspond to the raphide clade (Judd et al. 1999) and the succulent clade (Rettig et al. 1992). Molluginaceae are maximally supported as sister to this succulent clade (BS 100%). In Cuénoud et al. (2002), Molluginaceae were placed as sister to the succulent clade but with no support in the *matK* and *matK/rbcL* analyses and moderate support (BS 70%) in the four-gene analysis.

Within the raphide clade, *Gisekia* is strongly supported as sister to Phytolaccaceae, *Rivina*, *Sarcobatus*, and Nyctaginaceae (BS 100%). This contradicts the findings of Cuénoud et al. (2002), whose single-gene analyses variously place *Gisekia* as sister to Aizoaceae (*matK*) or *Rivina* (*rbcL* and *matK/rbcL*). Out of the eight plastid genes that we analyzed, only *rbcL* supports a sister relationship between *Gisekia* and *Rivina*. In addition, we provide further evidence and support for the separation of *Rivina* (Rivinioideae) from Phytolaccaceae and its placement as sister to Nyctaginaceae (BS 77%). The placement of *Sarcobatus* in our analyses is problematic, and its position varies in relation to Nyctaginaceae, *Rivina*, and Phytolaccaceae. The raphide clade is undersampled in this study, and while we suggest alternative placements for *Gisekia* and *Sarcobatus*, we recognize that increased taxon sampling (e.g., *Agdestis*, which has been associated with *Sarcobatus* by Cuénoud et al. [2002]) could affect these findings.

Taxa within the portulacaceous cohort have traditionally been treated at the rank of family; however, the degree of paraphyly in Portulacaceae suggests that phylogenetic resolution should be conceptually envisioned as an intrafamilial problem (Hershkovitz and Zimmer 1997). For example, in analyses of ITS, the genetic divergence of Cactaceae from Portulacaceae is equal to or less than that between many pairs of genera in Portulacaceae (Hershkovitz and Zimmer 1997). Two methodological constraints limited our ability to address the question of phylogenetic relationships among Cactaceae and its portulacaceous relatives. First, the large amount of sequencing for each taxon limited the total number of taxa that could be sampled; this undersampling is particularly acute in the succulent clade, given the degree of paraphyly inherent in Portulacaceae. Second, the broad scope of the taxon sampling, i.e., the whole of Caryophyllales s.l., meant that only slower-evolving coding genes rather than more rapidly evolving regions, such as intergenic spacers, could be sampled to permit alignment across the order. Consistent with the low levels of genetic divergence in this clade, very little informative variation was obtained for members of the portulacaceous cohort from these coding regions (despite sequencing more than 40,000 bp). There are fewer than 20 substitutions on the branches leading to the clade containing *Halophytum*, *Alluaudia*, and *Basella* and fewer than 50 substitutions on the branch leading to *Portulaca* and *Talinum*.

Limitations aside, within the portulacaceous cohort, the monophyly of the ACPT clade comprising Cactaceae (*Pereskia* plus *Opuntia*), *Portulaca*, and *Talinum* is strongly supported (BS 100%) in analyses of all partitions, with the exception of the IR partition. Historically, however, different analyses have recovered different patterns within the ACPT clade, depending on taxon sampling and phylogenetic markers used (reviewed in Nyffeler 2007). In our analyses, different partitions and analytical methods also gave different branching patterns within the ACPT clade. Parsimony analyses of the IR partition recovered a branching pattern (with support less than 50%) similar to the morphological cladistic analysis of Carolin (1987). Parsimony and GARLI analyses of the plastid gene partition found *Talinum* sister to *Portulaca* plus Cactaceae, as proposed by the morphological cladistic analyses of Hershkovitz (1993) and the Bayesian molecular analysis of Nyffeler (2007). Parsimony and GARLI analyses of the total evidence and GARLI analyses of IR data sets recovered a well-supported branching pattern not found in previous analyses: Cactaceae sister to *Portulaca* plus *Talinum*.

Total evidence, plastid gene, and plastid plus nuclear and IR data sets all place *Halophytum* as sister to Basellaceae and Didiereaceae. This affiliation is consistent with Savolainen et al.'s (2000) analysis of *rbcL* sequences (albeit with low taxon sampling in the Caryophyllales) and was suggested by Bittrich (1993) on the basis of pollen morphology and by Hunziker et al. (2000) because of shared similarities in basic chromosome number (x=12). The position of *Claytonia*, however, is unstable in our analyses and is generally not in agreement with studies with better taxon sampling (Hershkovitz and Zimmer 1997; Applequist and Wallace 2001; Nyffeler 2007). *Claytonia* together with associated portulacaceous genera were placed in a clade with Basellaceae and Didiereaceae with reasonable support (BS 80%; Applequist and Wallace 2001). However, in our study only MP analyses of the *matK* and IR data sets were able to recover this relationship. Individual plastid genes placed *Claytonia* in a variety of positions while the combined data sets invariably placed *Claytonia* as

sister to the rest of the portulacaceous cohort. Notably, our phylogeny derived from *ndhF* alone (the same gene employed by Applequist and Wallace [2001]) also recovered *Claytonia* as sister to the rest of the portulacaceous cohort. This suggests that the apparent instability in the placement of *Claytonia* may be the result of limited taxon sampling in our study; pruning the data set from Applequist and Wallace (2001) to match our taxon sampling generated a similarly anomalous placement of *Claytonia* (data not shown).

Reconstruction of Pollination Mechanism

Our phylogeny differs considerably from the concept of the Caryophyllales that stimulated the speculations of Ehrendorfer (1976). The Caryophyllales sensu Ehrendorfer essentially correspond to the core Caryophyllales presented in this study; however, the composition and phylogeny of this clade have changed considerably. None of the four currently recognized early-diverging lineages was recognized as belonging to the Caryophyllales in the 1970s. Ehrendorfer was strongly influenced by the idea that the Chenopodiaceae (Amaranthaceae s.l.), with their reduced anemophilous flowers, were representative of the ancestral Caryophyllid type. Consequently, he argued that anemophily was the ancestral condition because the early Caryophyllales had evolved in open, dry, marginal environments at a time when pollinators were scarce. These hypotheses are difficult to prove or disprove (Clement and Mabry 1996); however, our phylogeny confirms that the Amaranthaceae constitute a relatively derived lineage. If pollinators were scarce at the time of origin of the Caryophyllales, this might also apply as a general limitation to other lineages of eudicots diverging at that time, but in any case, the relative timing and location of diversification in eudicot lineages and their respective pollinator lineages are unclear at present. Friedman and Barrett (2008) demonstrate a strong correlation between the occurrence of open habitat and anemophily and provide support for the prevalence of anemophily in open habitats; however, this correlation may not necessarily be due to pollinator scarcity but rather to the selective advantage of wind pollination in an open environment. Moreover, parsimony reconstruction of the ancestral habitat would be ambiguous, given that the extant members of early-diverging lineages of the core Caryophyllales occupy tropical understory (Asteropeiaceae and Rhabdodendraceae) or have a global holoarctic distribution (Caryophyllaceae).

Using the current phylogeny, parsimony-based character reconstruction and stochastic character mapping do not provide support for the hypothesis that the Caryophyllales were ancestrally wind pollinated. *Rhabdodendron*, which is sister to all other core Caryophyllales, is described as visited by pollen-collecting bees (Prance 2003) while extensive field observations suggest that Asteropeiaceae are also entomophilous (Birkinshaw et al. 2004). It is notable, however, that together with the wind-pollinated *Simmondsia*, two other early-diverging lineages do at least exhibit morphological characteristics that are reminiscent of wind-pollinated flowers. Despite reports of bee visitation, *Rhabdodendron* exhibits very long anthers and sepaloid petals, lacks a nectary, possesses a gynoecium with only one or two ovules and a single seed in fruit, and has a relatively long stigma (P. K. Endress, personal communication); perianth parts also fall off as the flower opens (Nelson and Prance 1984). *Physena* exhibits very long anthers and no petaloid organs, lacks a nectary, and has a large stigmatic surface. Coding *Physena* as wind pollinated, however, does not alter the conclusion of the character mapping analyses, and thus there is little support for an anemophillous ancestry in the core Caryophyllales. Indeed, as noted by Clement and Mabry (1996), even if one accepts the highly reduced inconspicuous flowers of Amaranthaceae s.l. as archetypal, it is not necessary to invoke wind pollination because it has already been noted that many of the diminutive flowers in Amaranthaceae are probably entomophilous (Blackwell and Powell 1981; Kuhn 1993).

Reconstruction of Perianth Differentiation

Despite recovering entomophily as ancestral, our character reconstruction analyses suggest that an undifferentiated perianth arose early within the core Caryophyllales (in agreement with Ehrendorfer 1976; Ronse De Craene 2008). This perianth type has been strongly correlated with anemophily (Friedman and Barrett 2008). Parsimony reconstruction infers that the evolution of this undifferentiated condition evolved after the divergence of *Rhabdodendron*, while the stochastic mapping analyses recover the basalmost node in core Caryophyllales as either undifferentiated or differentiated, with equal probability. Subsequent nodes along the backbone of the tree until the divergence of Molluginaceae are recovered as undifferentiated (with greater than 0.99 posterior probability).

A discussion of perianth evolution within the core Caryophyllales is complicated by the great diversity of floral structure within the order and the uncertainty in defining the correspondence of these structures both within Caryophyllales and with respect to floral organs in other eudicots. Observations by Ronse De Craene (2007, 2008) suggest that although petal organs in eudicots may appear homologous with respect to position and superficial appearance, the variable expression of features reminiscent of either stamens or bracts means that petals in different lineages of core eudicots are of uncertain homology and may have been differently derived. This viewpoint argues against the widespread notion that the petals within eudicots are invariably derived from stamens and makes it difficult to homologize between perianth parts even within the core eudicots (Ronse De Craene 2007, 2008). In the Caryophyllales, these difficulties are compounded by different floral structures and the limitations of established terminology. The specific terms "petal," "sepal," "corolla," and "calyx" are not usefully applied to Caryophyllid taxa because they imply not only the characteristics and function of an organ but also the position of the organ (Endress 1994; Jaramillo and Kramer 2007). In core eudicots, for example, the term "petal" implies both the showiness of the perianth part and the position of the organ in the second whorl of the flower (Jaramillo and Kramer 2007). However, in comparing the differentiated perianth found in many Caryophyllales with the bipartite perianth of most core eudicots, the positional feature alone is not necessarily a sufficient criterion of homology, and terms such as "petal" that imply positional correspondence are misleading. Similarly, the term "bipartite perianth" should also be avoided because this implies the presence of distinct perianth whorls. While distinct perianth whorls may be found in some families in the Caryophyllales, e.g., Limeaceae (Hofmann

1973) and Caryophyllaceae (Rohweder 1967), differentiation in a spiral phyllotaxis occurs in Cactaceae. For the purposes of this discussion, therefore, we use the term "differentiated" to describe a perianth that comprises at least two distinct types of organ that perform the functions commonly ascribed to the calyx and corolla. We refer to members of a differentiated perianth as either petaloid or sepaloid (i.e., resembling the petals or sepals of other core eudicots and putatively performing similar functions without necessarily being homologous by positional criterion alone). Finally, because the terms "petaloid" and "sepaloid" refer only to a superficial resemblance and putatively similar function, within Caryophyllales we apply these terms to structures that are clearly nonhomologous in other respects. The terms "sepaloid tepal" and "petaloid tepal" are applied to the quincuncial perianth parts that are present in core Caryophyllales, while "petaloid staminodes" refer to perianth parts that are clearly androecium derived.

Multiple Origins of Perianth Differentiation

Our analyses suggest that there have been a minimum of nine independent origins of a differentiated perianth within the Caryophyllales. This is more than the minimum suggested by Ronse De Craene (2008), who, in a broad survey of eudicots, cites five origins of petals in the core Caryophyllales, occurring in Stegnospermataceae, Aizoaceae, Portulacaceae clade, Caryophyllaceae, and Molluginaceae. Considering the reconstructions provided by Ronse De Craene (2008), the difference in our respective estimations of perianth differentiation can be attributed to several factors. Most significantly, coding and definition of the perianth differ in our studies; e.g., Nyctaginaceae and the portulacaceous cohort are coded as petals absent (Ronse De Craene 2008; fig. 3), but by our definition, both *Mirabilis jalapa* (Nyctaginaceae) and the portulaceous cohort have a differentiated perianth and are listed as polymorphic and differentiated, respectively. Similarly, *Glinus* is a member of the Molluginacaeae that possesses putatively staminodial petals (Hofmann 1994, pp. 137, 141) but is coded as petals absent by Ronse De Craene (2008). In our analysis, Molluginaceae are coded as polymorphic, exhibiting both taxa with a uniseriate, undifferentiated perianth and taxa with differentiated perianth. A different tree topology may also be a factor contributing to the different results, e.g., *Rhabdodendron* is sister to the core Caryophyllales (this study), and the placement of *Corbichonia* and *Glinus* as successive sisters to the "globuloid" clade by Ronse De Craene (2008) is erroneous based on current understanding; *Corbichonia* is most likely sister to the raphide clade (Cuénoud et al. 2002), and *Glinus* (Molluginaceae) is sister to the portulacaceous cohort (BS 70% [according to Cuénoud et al. 2002] and BS 100% [this study]). Finally, because of the broader scope of the study by Ronse De Craene (2008; i.e., all eudicots), lineages of core Caryophyllales with differentiated perianth are also undersampled, with both *Asteropeia* and *Limeum* excluded from his study. Consequently, differences in emphasis, sampling, coding, and tree topology may all have contributed to the differences between Ronse De Craene's (2008) results and those we report here.

Despite these differences, the five independent origins of petals described by Ronse De Craene (2008) are included in the nine independent derivations of differentiated perianth inferred in our study. These nine origins occur in Asteropeiaceae, Caryophyllaceae (although several genera do not have differentiated perianth), Stegnospermataceae, some species of *Limeum*, *Corbichonia* (not sequenced in this study), the subfamilies Mesembryanthemoideae and Ruschioideae within Aizoaceae, *Mirabilis* in Nyctaginaceae, *Glinus* in Molluginaceae, and the portulacaceous cohort (Portulacaceae, Didiereaceae, Basellaceae), including Cactaceae. The number of origins of differentiated perianth could well increase, depending on the final placement of the enigmatic *Macarthuria* and increased resolution of phylogenetic relationships within Caryophyllaceae. Developmental evidence (where available) is consistent with these independent origins of differentiated perianth indicated by character reconstruction analyses. We discuss the developmental evidence for perianth differentiation by different mechanisms in these nine lineages: through differentiation of putatively homologous organs and through the recruitment of floral structures derived either from the androecium or from the preceding bracts.

Recruitment of Preceding Bracts

The secondary recruitment of preceding bracts to from perianth parts has occurred twice within the globular inclusion clade, once in Nyctaginaceae and again in the portulacaceous cohort (fig. 3). Developmental studies suggest that the mechanism underlying the recruitment of preceding bracts is different in these distinct lineages. Within Nyctaginaceae, an involucre may have evolved more than once, occurring also in *Abronia*, *Allionia*, *Boerhavia*, *Bougainvillea*, *Mirabilis*, *Nyctaginia*, and *Tripterocalyx* (Douglas and Manos 2007). In *Boerhavia*, Sharma (1963) describes an involucre surrounding five lateral flowers and one central flower. In *Mirabilis*, however, there appears to be a tendency toward reduction in floral number. In *Mirabilis nyctagineus*, only the first three leaves of the involucre subtend axillary flowers (Hofmann 1994). In *Mirabilis jalapa*, each flower has a differentiated perianth with a calyx of five fused parts that has been secondarily derived from an involucre of bracts (fig. 4*L*). This variation within *Mirabilis* suggests that the apparent bipartite perianth in *M. jalapa* may have been derived through reduction in floral number. Subsequent loss of five lateral flowers is inferred, leaving a single central flower with the involucre appearing as a pseudocalyx (Vanvinckenroye et al. 1993). In Nyctaginaceae, floral loss within an involucre of bracts would appear to result in an apparently differentiated perianth, although the association of the involucre (calyx) with the rest of the flower is weak (Rohweder and Huber 1974).

Portulacaceae s.l., Didiereaceae, and Basellaceae share a distinct floral morphology that emerges following the divergence of Molluginaceae (fig. 3). These lineages possess flowers with an involucre that, in contrast to *Mirabilis* (Nyctaginaceae), comprises two leafy phyllomes that are inserted below the petaloid members of the perianth. Importantly, their developmental origin is probably different from the involucre found in Nyctaginaceae because Hofmann (1994) comments that axillary products are never formed in the axes of these phyllomes. They are termed "involucral phyllomes" by Hofmann (1994), reflecting the belief that these organs are additional phyllomes inserted be-

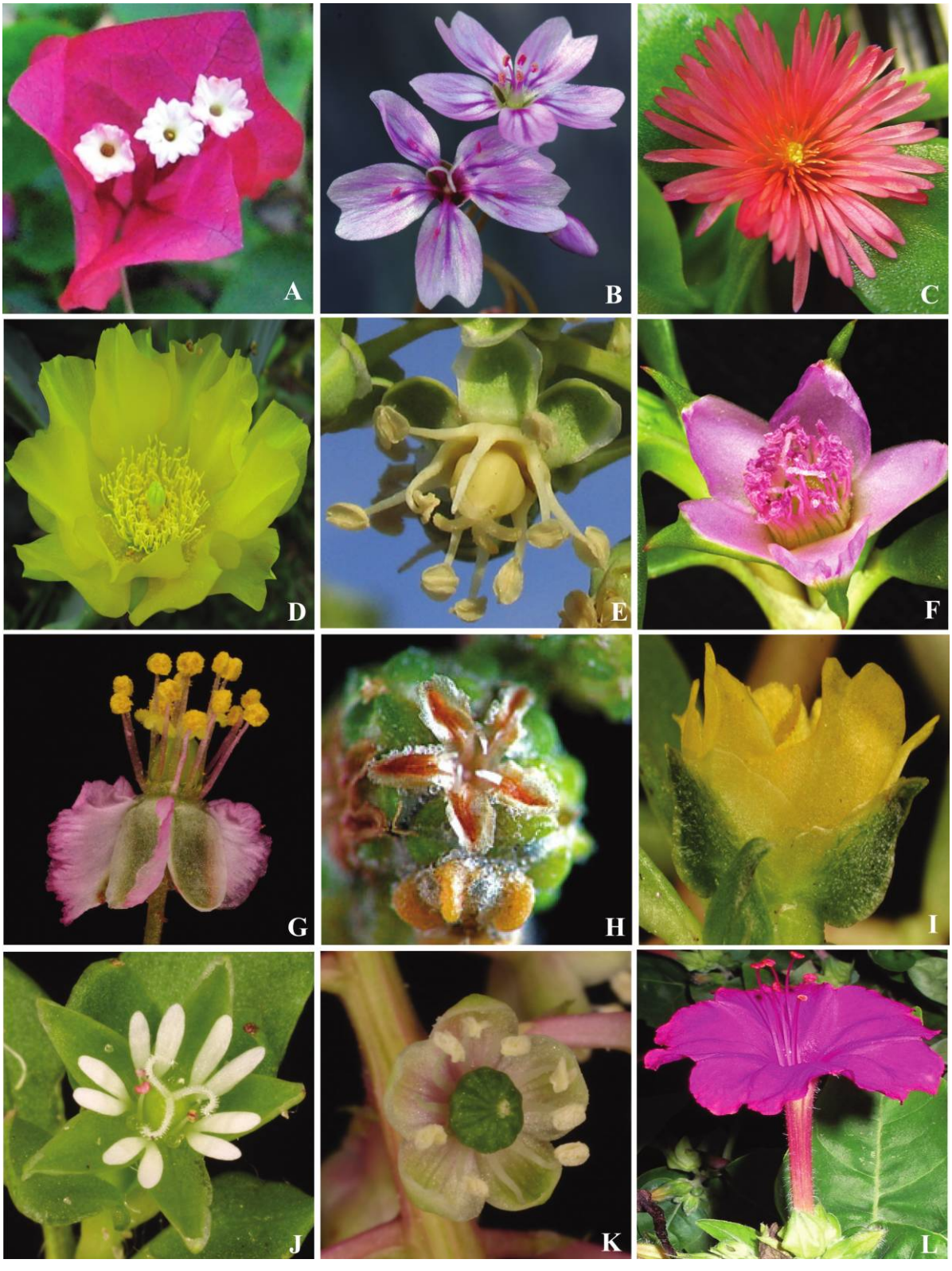

Fig. 4 Diverse forms of perianth in the core Caryophyllales. *A*, *Bougainvillea* sp. (Nyctaginaceae); *B*, *Claytonia* sp. (photo by Ron Wolf; Portulacaceae s.l.); *C*, *Aptenia cordifolia* (Aizoaceae); *D*, *Opuntia humifusa* (Cactaceae); *E*, *Stegnosperma* sp. (photo by Debra Valov; Stegnospermataceae); *F*, *Sesuvium portulacastrum* (Aizoaceae); *G*, *Hypertelis salsoloides* (Molluginaceae); *H*, *Chenopodium* sp. (photo by Brian Johnston; Amaranthaceae); *I*, *Portulaca oleracea* (photo by Kurt Neubig; Portulacaceae); *J*, *Stellaria media* (photo by Kurt Neubig; Caryophyllaceae); *K*, *Phytolacca americana* (photo by Kurt Neubig; Phytolaccaceae); *L*, *Mirabilis jalapa* (photo by Walter Judd; Nyctaginaceae).

tween the bracteoles and the sepals; however, there have been other interpretations as to the nature of these phyllomes.

Sharma (1954; reviewed in Milby 1980), who examined vascular anatomy in *Portulaca* and *Talinum*, concluded that the flowers are essentially dimerous, with the pentamerous petaloid perianth inferred as a derived condition. These alternatives will merit further developmental study as phylogenetic understanding within this group is clarified, but it is valuable to consider these different interpretations in light of the current phylogeny and perianth reconstruction analysis (fig. 3). The ancestral floral condition of the portulacaceous cohort is uniseriate pentamery; therefore, Sharma's (1954) interpretation suggests reduction to a dimerous state, followed by a reversal to a pentamerous condition. Irrespective of the developmental origin of these two phyllomes, in many species they cover the developing floral meristem very early in development and thus perform the function of a calyx in a differentiated perianth (Hofmann 1994). Subfunctionalization of perianth roles may have facilitated the high degree of petaloidy in the inner quincuncial perianth members of these families (fig. 4*B*, 4*I*, 4*L*): "the involucral phyllomes cover the inner bud very early and take over the function of the calyx. Therefore, the sepals [uniseriate pentamers] behave like petals" (Hofmann 1994, p. 138).

Within the portulacaceous cohort, a very different floral structure is found in Cactaceae. In contrast to the perianth of Portulacaceae s.l., Didiereaceae, and Basellaceae, Cactaceae exhibit a great increase in perianth parts. Increases in floral merism and generally modified floral form make it challenging to determine correspondence between perianth in Cactaeae and its closest relatives—in this study, *Portulaca* and *Talinum*. The perianth parts of Cactaceae are suggested to be bracteal rather than staminodial in their homology (Buxbaum 1950–1955, pp. 122, 123; Ronse De Craene 2007, 2008) and are arranged in a spiral phyllotaxy. The perianth may have arisen by inclusion and differentiation of supernumary bracts (Ronse De Craene 2008) or simply by formation of additional bracts. Differentiation of the perianth occurs with outer sepaloid parts and highly petaloid inner parts (fig. 4*D*). This high degree of differentiation, together with a spiral phyllotaxy, is unusual within the Caryophyllales; however, Ronse De Craene (2008) highlights that this combination of floral characters (large, spirally arising petals with a multistaminate androecium) occurs in several derived lineages in the core eudicots. Endress (2002) suggested that increases in numbers of stamens and/or carpels may result in increase in size of the flower, greater plasticity, and irregular petal development. This developmental interpretation is consistent with our reconstruction analyses, which do not argue for an independent origin of differentiated perianth in Cactacaeae; rather, an increase in meristem size and merosity of reproductive organs may be in part responsible for the unusual perianth in Cactaceae.

Petaloid Modification of the Androecium

Reconstruction analyses suggest that perianth differentiation through sterilization and petaloid modification of the outer members of a centrifugally initiating androecium has arisen a minimum of three times in Caryophyllales (fig. 3): clear examples occur in Aizoaceae (fig. 4*C*), Molluginaceae, and *Corbichonia* (not sampled in this study but shown to be a distinct lineage within the raphide clade; Cuénoud et al. 2002). In *Glinus*, *Corbichonia*, and Aizoaceae, the petaloid structures can be readily interpreted as differentiated staminodial structures (Ronse De Craene 2008). For example, within Aizoaceae subfamilies, Ruschioideae, and Mesembryanthemoideae, androecial development proceeds centrifugally, and the basipetal members become progressively more sterile and petaloid, with intermediates conceptually linking the outermost petals to the inner fertile stamens (fig. 4*C*). A similar situation has been described in *Glinus* in the Molluginaceae (Hofmann 1994) and in *Corbichonia* (Ronse De Craene 2007).

Petaloid members of the differentiated perianth in Caryophyllaceae, *Limeum*, *Stegnosperma*, and *Macarthuria* have also been attributed to the androecium (Hofmann 1973; Ronse De Craene 2007, 2008). The reconstruction analyses suggest that these differentiated perianths have occurred independently and thus merit further developmental study. The assessment of homology between the petaloid members of the differentiated perianth and the androecium is complicated by a high degree of variability in androecium organization, processes of reduction, and differences in phyllotaxy. However, several lines of evidence suggest an androecial origin of the petals in Caryophyllaceae (Rohweder 1967; reviewed in Ronse De Craene et al. 1998; Ronse De Craene 2007, 2008). Ronse De Craene et al. (1998) review the presence and absence of petals in 52 genera of Caryophyllaceae: nine genera lack petals, 11 genera have both species with petals and species without, while the remaining 32 genera in the survey possess petals. It remains unclear whether the absence of petals is ancestral in Caryophyllaceae or whether instances of petal loss have occurred. The most comprehensive molecular phylogeny of Caryophyllaceae to date (Fior et al. 2006) sampled only two genera with apetalous members (*Paronychia* and *Sagina*), but none of the entirely apetalous genera were sampled.

Differentiation of Homologous Perianth Parts

Despite the high degree of variation in floral structure found in different lineages of Caryophyllales, there are key common elements. Almost all lineages within the order possess five perianth members that are organized in a uniseriate quincuncial arrangement (with the exception of Cactaceae, which is multiseriate). Occasionally, one of the members in this series has been lost, to give a tetramerous perianth, e.g., in *Tetragonia* and *Aptenia* in Aizoaceae, Rivinoideae, and Didiereaceae, but these cases of tetramery are clearly derived from pentamerous ancestors. These uniseriate quincuncial perianth members are probably homologous, given their constancy, position in the flower, and common phyllotaxy. These putatively homologous organs have, however, undergone considerable differentiation in certain lineages, which often correlates with the emergence of a differentiated perianth. For example, in Aizoaceae, members of the early-diverging subfamilies Sesuvioideae and Aizooideae possess a quincuncial uniseriate perianth whose members are petaloid on the adaxial surface and sepaloid on the abaxial side. In the derived subfamilies Mesembryanthemoideae and Ruschioideae, the androecium is polyandrous, and the outer members of centrifugally initiating stamens are sterile, resulting in a differentiated perianth with androecial-derived petaloid organs. Concomitantly, the outer quincuncial uniseriate perianth loses

all petaloid characteristics and resembles only a calyx. In instances where differentiation of the perianth has been achieved through recruitment of involucral bracts and/or bracteoles (Portulacaceae and *Mirabilis*), the involucral organs act as a calyx, and the now-inner uniseriate quincuncial perianth members are considerably more showy and petaloid (cf. the showy petaloid perianth of the portulacaceous cohort [fig. 4*B*, 4*D*, 4*I*] with the diminutive simple perianth of some genera in Molluginaceae). Seemingly homologous perianth parts within Caryophyllales can be petaloid, e.g., Nyctaginaceae (fig. 4*A*, 4*L*) and Portulacaceae (fig. 4*I*); sepaloid, e.g., *Limeum*, *Stegnosperma* (fig. 4*E*), Molluginaceae, Ruschioideae, and Mesembryanthemoideae (fig. 4*C*), Caryophyllaceae (fig. 4*J*), and *Simmmondsia*; or chimeric, e.g., Sesuvioideae/Aizooideae (fig. 4*F*) and *Hypertelis* (fig. 4*G*).

Caryophyllales as a System for Floral Evo-Devo

Nine origins of a differentiated perianth, the concomitant evolution of petaloidy from either androecial or bracteal organs, and varying degrees of petaloid differentiation in homologous structures across the order make the Caryophyllales a valuable system for exploring the evolutionary developmental genetics of petaloidy in core eudicots. In the majority of core eudicots whose petal developmental genetics have been examined (e.g., in *Arabidopsis thaliana*, *Antirrhinum majus*, *Solanum lycopersicon*, *Nicotiana tabacum*, *Petunia hybrida*), differentiation of the petals is strongly influenced by MADS-box transcription factors: *APETALA3* (*AP3*) and *PISTILLATA* (*PI*; in *A. thaliana*) and their orthologues (Irish and Kramer 1998; Kramer and Irish 1999). In these core eudicot species, *AP3* and *PI* orthologues are expressed throughout the development of the petal, and their ubiquitous expression in the petal has been shown to be necessary for normal petal development in *A. thaliana* and *A. majus* (Bowman et al. 1989; Sommer et al. 1991; Zachgo et al. 1995). It seems apparent that these genes play a conserved role in petal identity in the core eudicots examined so far, yet core eudicot petals have also traditionally been considered to be homologous, stamen-derived organs: this homology has been invoked to explain such developmental genetic similarities (Irish and Kramer 1998). More recently, however, the assertion that petals in core eudicots are largely homologous and predominantly stamen derived has been questioned (Ronse De Craene 2007). Although in Caryophyllales the homology of the perianth parts to petals in other core eudicots is uncertain, it is clear that many lineages (Sesuvioideae, Nyctaginaceae, Portulacaceae, Cactaceae) possess petaloid organs that are bracteal rather than staminal in origin. Furthermore, the occurrences of stamen-derived petals within Caryophyllales (Caryophyllaceae, Aizoaceae, *Glinus*, and *Corbichonia*) are phylogenetically derived, independent events. These independent occurrences are valuable for further study because there are very few examples of petals within core eudicots that are unquestionably stamen derived (Ronse De Craene 2007). The pattern of perianth evolution in the Caryophyllales therefore presents a unique opportunity to address long-standing questions regarding differences and/or similarities in the developmental genetics of bracteopetals and andropetals (Ronse De Craene 2008); rearticulated by Ronse De Craene (2008), this question remains highly pertinent in studies of floral diversification. The improved phylogenetic understanding reported here provides opportunities for comparing bracteopetalous and andropetalous lineages that have arisen more recently than both basal angiosperms (traditionally considered to bear bracteopetals) and the early-diverging eudicot lineages (with their presumed andropetals). The Caryophyllales are a well-defined clade within core eudicots, but, in a sense, the patterns of perianth evolution discussed here recapitulate (on a smaller phylogenetic scale) the evolutionary trends traditionally thought to have taken place across the angiosperms as a whole (Bessey 1915; Takhtajan 1991). Therefore, despite uncertainty surrounding the precise correspondence of the caryophyllid perianth with the perianth of other eudicots, evo-devo investigations in the Caryophyllales may have far-reaching implications for our understanding of petal evolution and perianth differentiation. Is there latent developmental genetic homology underlying these derived and oft-seemingly dissimilar occurrences of perianth differentiation in Caryophyllales? What is the involvement of *AP3* and *PI* orthologues in these bracteopetals in Caryophyllales? How do expression and function of *AP3* and *PI* orthologues in caryophyllid bracteopetals compare with their expression and function in the derived instances of andropetals? How do expression and function of *AP3* and *PI* orthologues compare in the different occurrences of andropetals? Evolutionary developmental approaches to these questions are currently under way (Brockington et al. 2007) and may shed light on the evolutionary origins and homology of these diverse perianth forms in relation to the perianth in other core eudicots.

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Literature Cited

Albert VA, SE Williams, MW Chase 1992 Carnivorous plants: phylogeny and structural evolution. Science 257:1491–1495.

APG (Angiosperm Phylogeny Group) II 2003 An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Bot J Linn Soc 141:399–436.

Applequist WL, RS Wallace 2001 Phylogeny of the portulacaceous cohort based on *ndhF* sequence data. Syst Bot 26:406–419.

Behnke HD 1994 Sieve element plastids: their significance for the evolution and systematics of the order. Pages 87–121 *in* HD Behnke, TJ Mabry, eds. Caryophyllales: evolution and systematics. Springer, Berlin.

——— 1997 Sarcobataceae: a new family of Caryophyllales. Taxon 46:495–507.

Bessey CE 1915 The phylogenetic taxonomy of flowering plants. Ann Mo Bot Gard 2:108–164.

Birkinshaw C, R Edmond, C Hong-Wa, C Rajeriarison, F Randriantafika, G Schatz 2004 Red lists for Malagasy plants. II. Asteropeiaceae. Missouri Botanical Garden, St. Louis.

Bittrich V 1993 Halophytaceae. Pages 320–321 *in* K Kubitzki, JG Rohwer, V Bittrich, eds. The families and genera of flowering plants. Vol 2. Springer, Berlin.

Blackwell WH, MJ Powell 1981 A preliminary note on pollination in the Chenopodiaceae. Ann Mo Bot Gard 68:524–526.

Bollback JP 2006 SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7:88.

Bowman JL, DR Smyth, EM Meyerowitz 1989 Genes directing flower development in *Arabidopsis*. Plant Cell 1:37–52.

Braun A 1864 Uebersicht der Natürlichen Systems nach der Anordnung Derselben. Pages 22–67 *in* P Ascherson, ed. Flora der Provinz Brandenburg, der Altmark und des Herzogthums Magdeburg. Hirschwald, Berlin.

Brockington SF, M Frohlich, PJ Rudall, DE Soltis, PS Soltis 2007 Differing patterns of MADS-box gene expression associated with shifts in petaloidy within Aizoaceae (Caryophyllales). Page 191 *in* Program and abstract book. Plant Biology and Botany Congress, Chicago.

Brockington SF, E Mavrodiev, J Ramdial, A Dhingra, PS Soltis, DE Soltis 2008 Keep the DNA rolling: multiple displacement amplification of archival plant DNA extracts. Taxon 53:944–948.

Buxbaum F 1950–1955 Morphology of cacti. Abbey Garden, Pasadena, CA.

Carolin R 1987 A review of the family Portulacaceae. Aust J Bot 35: 383–412.

Clement JS, TJ Mabry 1996 Pigment evolution in the Caryophyllales: a systematic overview. Bot Acta 109:360–367.

Couvreur TLP 2008 Revealing the secrets of African Annonaceae: systematics, evolution and biogeography of the syncarpous genera *Isolona* and *Monodora*. PhD thesis. Wageningen University.

Couvreur TLP, JE Richardson, MSM Sosef, RHJ Erkens, LW Chatrou 2008 Evolution of syncarpy and other morphological characters in African Annonaceae: a posterior mapping approach. Mol Phylogenet Evol 42:302–318.

Cronquist A 1981 An integrated system of classification of flowering plants. Columbia University Press, New York.

——— 1988 The evolution and classification of flowering plants. New York Botanical Garden, Bronx.

Cronquist A, RF Thorne 1994 Nomenclatural and taxonomic history. Pages 87–12 in HD Behnke, TJ Mabry, eds. Caryophyllales: evolution and systematics. Springer, Berlin.

Cuénoud P, V Savolainen, LW Chatrou, M Powell, RJ Grayer, MW Chase 2002 Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. Am J Bot 89:132–144.

Dahlgren R 1975 A system of classification of the angiosperms to be used to demonstrate the distribution of characters. Bot Not 128: 119–147.

Dhingra A, KM Folta 2005 ASAP: amplification, sequencing and annotation of plastomes. BMC Genomics 6:176.

Douglas NA, PS Manos 2007 Molecular phylogeny of Nyctaginaceae: taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America. Am J Bot 94:856–872.

Downie SR, DS Katz-Downie, KJ Cho 1997 Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF2280 homolog sequences. Am J Bot 84:253–273.

Downie SR, JD Palmer 1994 A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. Syst Bot 19:487.

Doyle J, J Doyle 1987 Genomic plant DNA preparation from fresh tissue-CTAB method. Phytochem Bull 19:11.

Ehrendorfer F 1976 Closing remarks: systematics and evolution of centrospermous families. Plant Syst Evol 126:99–106.

Eichler A 1875–1878 Blüthendiagramme. 1, 2. Engelmann, Leipzig.

Endress PK 1994 Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.

——— 2002 Morphology and angiosperm systematics in the molecular era. Bot Rev 68:545–570.

Fay MF, KM Cameron, GT Prance, MD Lledo, MW Chase 1997 Familial relationships of *Rhabdodendron* (Rhabdodendraceae) plastid *rbcL* sequences indicate a caryophyllid placement. Kew Bull 52:923–932.

Felsenstein J 1978 Cases in which parsimony or compatibility methods will be positively misleading. Syst Zool 27:401–410.

——— 1985 Confidence-limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Fior S, PO Karis, G Casazza, L Minuto, F Sala 2006 Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast *matK* and nuclear rDNA ITS sequences. Am J Bot 93: 399–411.

Friedman J, SCH Barrett 2008 A phylogenetic analysis of the evolution of wind pollination in the angiosperms. Int J Plant Sci 169:49–58.

Giannasi DE, G Zurawski, G Learn, MT Clegg 1992 Evolutionary relationships of the Caryophyllidae based on comparative *rbcL* sequences. Syst Bot 17:1–15.

Hershkovitz MA 1993 Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on Phylogeny of the portulacaceous Alliance. Ann Mo Bot Gard 80: 333–365.

Hershkovitz MA, EA Zimmer 1997 On the evolutionary origins of the cacti. Taxon 46:217–232.

Hilu KW, T Borsch, K Muller, DE Soltis, PS Soltis, V Savolainen, MW Chase, et al 2003 Angiosperm phylogeny based on *matK* sequence information. Am J Bot 90:1758–1776.

Hofmann U 1973 Morphologische Untersushungen zur Umgrenzung und Gliederung der Aizoaceen. Bot Jahrb Syst 93:247–324.

——— 1994 Flower morphology and ontogeny. Pages 123–166 *in* HD Behnke, TJ Mabry, eds. Caryophyllales: evolution and systematics. Springer, Berlin.

Huelsenbeck JP, R Nielsen, JP Bollback 2003 Stochastic mapping of the morphological characters. Syst Biol 52:131–158.

Huelsenbeck JP, F Ronquist 2001 MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Hunziker JH, R Pozner, A Escobar 2000 Chromosome number in *Halophytum ameghinoi* (Halophytaceae). Plant Syst Evol 221:125–127.

Irish VF, EM Kramer 1998 Genetic and molecular analysis of angiosperm flower development. Adv Bot Res 28:197–230.

Jansen RK, C Kaittanis, C Saski, SB Lee, J Tomkins, AJ Alverson, H Daniell 2006 Phylogenetic analyses of *Vitis* (Vitaceae) based on complete chloroplast genome sequences: effects of taxon sampling and phylogenetic methods on resolving relationships among rosids. BMC Evol Biol 6:32.

Jaramillo MA, EM Kramer 2007 The role of developmental genetics

in understanding homology and morphological evolution in plants. Int J Plant Sci 168:61–72.

Judd WS, CS Campbell, EA Kellogg, PF Stevens 1999 Plant systematics: a phylogenetic approach. Sinauer, Sunderland, MA.

Kramer EM, VF Irish 1999 Evolution of genetic mechanisms controlling petal development. Nature 399:144–148.

Kubitzki K, C Bayer, eds 2003 The families and genera of vascular plants. Vol 5. Springer, Berlin.

——— 2007 The families and genera of vascular plants. Vol 9. Springer, Berlin.

Kubitzki K, JG Rohwer, V Bittrich, eds 1993 The families and genera of vascular plants. Vol 2. Springer, Berlin.

Kuhn U 1993 Chenopodiaceae. Pages 253–280 *in* K Kubitzki, J Rohwer, V Bittrich, eds. The families and genera of vascular plants. Vol 2. Springer, Berlin.

Maddison W, D Maddison 2008 Mesquite: a modular system for evolutionary analysis, version 2.5. http://mesquiteproject.org.

Milby TH 1980 Studies in the floral anatomy of *Claytonia* (Portulacaceae). Am J Bot 67:1046–1050.

Moore MJ, CD Bell, PS Soltis, DE Soltis 2007 Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proc Natl Acad Sci USA 104:19363–19368.

Morton CM, KG Karol, MW Chase 1997 Taxonomic affinities of *Physena* (Physenaceae) and *Asteropeia* (Theaceae). Bot Rev 63:231–239.

Nelson B, GT Prance 1984 Observations on the pollination of *Rhabdodendron macrophyllum* (Spruce ex Benth.) Huber. Acta Amazon 14:411–426.

Nyffeler R 2007 The closest relatives of cacti: insights from phylogenetic analyses of chloroplast and mitochondrial sequences with special emphasis on relationships in the tribe Anacampseroteae. Am J Bot 94:89–101.

Nylander J 2004 MrModeltest, version 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Prance GT 2003 Rhabdodendraceae. Pages 339–341 *in* K Kubitzki, C Bayer, eds. The families and genera of vascular plants. Vol 5. Springer, Berlin.

Rambaut A, A Drummond 2003 Tracer, version 1. http://tree.bio.ed.ac.uk/software/tracer/.

Renner SS, L Beenken, GW Grimm, A Kocyan, RE Ricklefs 2007 The evolution of dioecy, heterodichogamy, and labile sex expression in *Acer*. Evolution 61:2701–2719.

Rettig JH, HD Wilson, JR Manhart 1992 Phylogeny of the Caryophyllales: gene sequence data. Taxon 41:201–209.

Rohweder O 1967 Centrospermenstudien. 3. Blütenntwicklüng und Blütenblau bei Silenoideen (Caryophyllaceae). Bot Jahrb Syst 86: 130–185.

Rohweder O, K Huber 1974 Centropsermenstudien. 7. Beobachtungen und Anmerkungen zur Morpholgie und Entwicklungsgeschichte einiger Nyctaginaceae. Bot Jahrb Syst 90:447–468.

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

Ronse De Craene LP 2007 Are petals sterile stamens or bracts? the origin and evolution of petals in the core eudicots. Ann Bot 100: 621–630.

——— 2008 Homology and evolution of petals in the core eudicots. Syst Bot 33:301–325.

Ronse De Craene LP, EF Smets, P Vanvinckenroye 1998 Pseudodiplostemony and its implications for the evolution of the androecium in the Caryophyllaceae. J Plant Res 111:25–43.

Savolainen V, MW Chase, SB Hoot, CM Morton, DE Soltis, C Bayer, MF Fay, AY De Bruijn, S Sullivan, YL Qiu 2000 Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. Syst Biol 49:306–362.

Schmitz-Linneweber C, RM Maier, JP Alcaraz, A Cottet, RG Herrmann, R Mache 2001 The plastid chromosome of spinach (*Spinacia oleracea*): complete nucleotide sequence and gene organization. Plant Mol Biol 45:307–315.

Sharma HP 1954 Studies in the order Centrospermales. I. Vascular anatomy of certain species of Portulacaceae. J Indian Bot Soc 33: 98–111.

——— 1963 Contributions to the morphology of the Nyctaginaceae. 2. Floral anatomy of some species. Proc Indian Acad Sci 57:149–163.

Soltis DE, PS Soltis, MW Chase, ME Mort, DC Albach, M Zanis, V Savolainen, et al 2000 Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. Bot J Linn Soc 133:381–461.

Soltis PS, DE Soltis, MW Chase 1999 Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402:402–404.

Sommer H, W Nacken, P Beltran, P Huijser, H Pape, R Hansen, P Flor, H Saedler, Z Schwarzsommer 1991 Properties of *deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*. Development 113:169–175.

Swofford D 2000 PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, MA.

Takhtajan A 1980 Outline of the classification of flowering plants (Magnoliophyta). Bot Rev 46:225–359.

——— 1991 Evolutionary trends in flowering plants. Columbia University Press, New York.

Thompson JD, TJ Gibson, F Plewniak, F Jeanmougin, DG Higgins 1997 The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882.

Thorne R 1976 A phylogenetic classification of the Angiospermae. Evol Biol 9:35–106.

——— 1992 An updated classification of the flowering plants. Aliso 13:365–389.

Vanvinckenroye P, E Cresens, LP Ronse De Craene, E Smets 1993 A comparative floral developmental study in *Pisonia*, *Bougainvillea* and *Mirabilis* (Nyctaginaceae) with special emphasis on the gynoecium and floral nectaries. Bull Jard Bot Natl Belg 62:69–96.

Wikstrom N, V Savolainen, MW Chase 2001 Evolution of the angiosperms: calibrating the family tree. Proc R Soc B 268:2211–2220.

Williams SE, VA Albert, MW Chase 1994 Relationships of Droseraceae: a cladistic analysis of *rbcL* sequence and morphological data. Am J Bot 81:1027–1037.

Wyman SK, RK Jansen, JL Boore 2004 Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20:3252–3255.

Zachgo S, ED Silva, P Motte, W Trobner, H Saedler, Z Schwarzsommer 1995 Functional-analysis of the *Antirrhinum* floral homeotic *deficiens* gene in-vivo and in-vitro by using a temperature-sensitive mutant. Development 121:2861–2875.

Zwickl D 2000 Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD diss. University of Texas, Austin.